Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 2961 - 2979
Received 15 December 2020; Accepted 05 January 2021.

A STUDY ON PREVALENCE OF BACTERIAL ISOLATES CAUSING SEPTICEMIA AMONG HOSPITALIZED PATIENTS IN A TERTIARY CARE HOSPITAL

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ABSTRACT

The present study on prevalence of bacterial isolates causing Septicemia among Hospitalized patients in a tertiary care hospital was conducted in the department of microbiology, SreeBalaji Medical College and Hospital, Chennai. From July 2014 to July 2015. A total of 120 blood samples (under SIRS criteria) were collected in the age group between 16 to 65 years. In this study, increased incidence of septicemia was seen among patients with symptoms of Fever (100%), Anemia (50%) and Respiratory Distress & Urine symptoms (23.33%). Blood culture was found to be positive in 48 (40%) of clinically suspected cases of septicemia. The organisms isolated were Coagulase negative staphylococcus (CONS) 12(25%), Staphylococcus aureus 10 (20.83%), Escherichia coli 10 (20.83%), Pseudomonas species 6 (12.5%) Salmonella typhi 1 (2.08%) and Acinetobacter species 1(2.08%). Most of the Gram-positive organism were susceptible to Vancomycin& Linezolid and most of the Gram-negative organism were susceptible to Amikacin, imipenem&piperacillin/tazobactam. In this study, patients with septicemia were found to be associated with high morbidity & mortality.

Introduction

Septicemia is one of the major causes of mortality and morbidity in patient attending tertiary care hospital. Therefore, knowing the causative agents is necessary for prevention and treatment. It arises from infections of various sites of the body such as Intravenous lines, skin, lungs, abdomen and urinary tract. According to CDC (Centers for Disease control) [1], a patients presenting with fever (temperature >38oc) which is not connected with any other causes and whose blood cultures are positive for a bacteria are considered to have septicemia. If the first blood culture which is obtained before or within 48 hours of hospitalization is positive, it is defined as community acquired bacteremia (CAB). If signs and symptoms appear after 48 hours following hospital admission, the patients are considered to have hospital acquired septicemia.

Individuals with bacteremia may develop septicemia, in which multiplying bacteria release toxins into bloodstream causing fever, chills, malaise with difficulty in breathing, increased heart rate and confusion [2, 3]. The circulating microorganism leads to life threatening condition like multiple organ failure, shock, DIC and Death. Septicemia with primary diseases admitted in tertiary care hospital is meningitis, Pneumonia, Infective Endocarditis and Urogenital-sepsis. Secondary infections can occur due to reasons like Urinary catheter insertion related sepsis, surgical sites infection like Intra-abdominal abscesses in patient who had abdominal surgery, vascular catheter insertion and infection arising out of hospital acquired or ventilator associated pneumonia [4].

Early signs of septicaemia are increased heart rate, high cardiac output, high blood sugar level, decreased urine output, low blood pressure and dysfunctions of blood coagulation [5]. A critically ill patient admitted in the tertiary care hospital can be managed with maintenance of intravenous fluid, Electrolyte balance, adequate nutrition, administration of antibiotic, high- flow of oxygen, close monitoring of blood pressure and urine output, promoting vital organ support like hemodialysis in kidney failure, mechanical ventilation in lung dysfunction and transfusion of

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blood products & doing important investigations like lactate, haemoglobin determination and blood culture [3].

In the United States, the incidence of septicaemia in the hospital setting was approximately 3 in 1,000 people [6]. Another study reported that septicaemia was the second most common reason for readmission within 30 days [7]. The most common bacteria isolated from patients with septicaemia are Gram-positive cocci (*Staphylococcus aureus and Coagulase negative Staphylococcus)* and Gram-negative bacilli (*Escherichia coli, Klebsiellaspp and Pseudomonas aeruginosa*). *CONS* which was earlier considered as skin commensal, has gained clinical importance and now recognized as pathogen [4, 8].

The most important laboratory test performed for diagnosis of blood stream infections is blood culture. Blood culture is considered as the gold standard for the detection of septicaemia [4]. Blood culture helps in detecting early threat to the patient's life and an urgent need for appropriate antibiotic and other therapy. Blood culture is mainly done for important clinical situations like septicaemia, bacteraemia and PUO (pyrexia of unknown origin). Blood culture examination for the detection of Blood stream infection in patients attending tertiary care hospital helps in treating and reducing the morbidity & mortality due to septicemia.

The probable [2] contaminants are growth of coagulase negative staphylococcus and Corynebacteriumspp occur only one of some cultures. The probable pathogen [2, 9] growth of same organisms in repeated cultures, and isolation of pathogenic bacteria from blood culture of patients suspected to be septicaemia. Septicaemia is thought of as a life-threatening condition that must be dealt with emergency.

Hence, this study was taken in our institution to evaluate the prevalence of septicaemia in our hospital setup in relation to their source of infection and to analyses the antibiotic susceptibility pattern of the organisms isolated.

MATERIAL AND METHODS

To study the prevalence of bacterial isolates causing septicemia in hospitalized patients.

MATERIALS:

- Sterile blood culture blood
- Sterile swabs
- Bunsen burner
- Lighter
- Cotton
- Loop wire
- Straight wire
- Hanging drop slide
- Petri dish plates
- Test tubes
- Compound microscope
- Gram staining kit
- Gram staining slide
- Cover slip
- Antibiotic discs
- Disposal tray

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Media used

- Biphasic BHI (Brain heart infusion) broth
- Nutrient agar
- MacConkey agar
- Blood agar
- 0.5 McFarland' s standard medium
- Mueller-Hinton agar(MHA)

Biochemical test

- Indole test,
- Urease test,
- Citrate utilization test,
- Triple sugar iron agar,
- Methyl Red(MR) test,
- Voges-proskuer test,
- Sugar fermentation (glucose, lactose, sucrose, maltose, mannitol, arabinose),
- Tube and slide catalase,
- Tube and slide coagulase,
- Oxidase disc test

Antibiotics used

The following drugs used are Gentamicin (30µg), Erythromycin (15µg), Ciprofloxacin (5µg), Cotrimoxazole (25µg), Ampicillin (10µg), Linezolid (30µg), Vancomycin (30µg), Amikacin (30 µg) piperacillin/Tazobactam (100/10µg), Imipenem (10µg), Cefotaxime (30 µg), Ceftazidime (30µg), Cefipime (30µg). Amoxycillin/Clavulanic acid (30µg).

- Zone of inhibition was measured in mm.

Inclusion criteria

- Patient age group above16 years upto 65 years
- Known cases of sepsis or clinical suspected cases of sepsis
- Signs of SIRS

Exclusion criteria

- No clinical suspected cases of sepsis
- No history of Prior antibiotic administration

METHODOLOGY

Permission to conduct this study was obtained from the institutional ethical committee. The known cases of sepsis or clinical suspected cases of sepsis with signs of SIRS admitted in SreeBalaji Medical College and Hospital, Chrompet, Chennai are included in our study. The study was conducted from July 2014 to July 2015. A total of 120 blood samples were collected from both sexes in age group between 16 to 65 years. Informed consent was obtained from all the patients. Past medical history was obtained and complete clinical examination was carried out from all the patients. Blood samples were collected and gram staining and culture were done in

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Microbiology laboratory. Organisms identified after biochemical reactions were taken up to study the prevalence of septicaemia. The patients were followed regularly until the patients discharged from the hospital or after admission. The patients were selected as per as inclusion criteria. A detailed history was taken and complete clinical examination was carried out from all the patients.

Collection of blood samples:

A Blood samples was collected under aseptic precautions. The venipuncture site on the patient's skin was disinfected by applying 70% isopropyl alcohol in water with 1-2% chlorhexidine for 1 min and allowed to dry. Then a fresh sterile needle was fitted and the required volume was inoculated into each blood culture bottle.

Processing

For culture maximum 5-10ml of blood should be collected and half of the sample should be inoculated into biphasic BHI (Brain heart infusion) broth [10]. Blood culture bottles were incubated at 37°C for 24 hrs. First Subcultures were done at the appearance of generalized turbidity or gas production at 24 hrs, second subcultures irrespective of turbidity or gas production is done at 48 hrs and final subculture were done at the end of 5-7th day.

Identification of organisms

The standard loop technique of streaking was used for nutrient agar, MacConkey agar and blood agar. The plates were then incubated at 37°c overnight in an incubator. The organism thus isolated were identified on the basis of as follows.

Gram stain – to identify whether Gram-positive or Gram-negative bacteria

- Hanging drop done to find out whether it is motile and non-motile organism
- Standard biochemical reactions such as slide and tube catalase test, slide and tube coagulase test, indo le test, citrate utilization test, urease test, triple sugar iron (TSI) test, MR test, VP test, nitrate reduction, oxidase tests, carbohydrate fermentation tests. Were done to identify the biochemical characteristics of the isolated organisms.
-

ANTIMICROBIAL SUSCEPTIBILITY TESTS:

Antimicrobial susceptibility testing of the bacterial isolates were performed by using Kibry-Bauer diffusion methods with commercially available discs on Mueller Hinton agar plates. The diameter of zone of inhibition of growth was measured and interpreted according to the CLSI [12].

Storage of Antimicrobial Disc

The antibiotic discs were refrigerated at 4-8oc or kept frozen at -14oc. β-Lactam antibiotics were stored frozen. Disc container was removed from refrigerator one or two hours before use and brought to room temperature. Cartridge of discs has been removed from its sealed package and it was placed in a tightly sealed container. Turbidity Standard for Inoculum Preparation. To standardize the inoculum density for susceptibility test, a Barium Sulfate (BaSO4) turbidity standard equivalent to a 0.5 McFarland standard was used.

Preparation of inoculums

About 4-5 isolated colonies were picked up and put in 4-5ml of peptone water broth and incubated at 37c for 2-6 hours to attain 0.5ml of McFarland's standard which corresponds to 150 million organism/ml. More turbid broth then added some more amount of peptone water broth

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and adjusted to 0.5 McFarland's standard by comparing against a card with white background and contrasting black lines. This was carried out within 15 minutes of adjusting the turbidity.

\Inoculation of MHA plates [13]

A sterile cotton swab was dipped into the medium. The swab was rotated several times and pressed firmly on the inside wall of the tube. So that excess broth from the swab was removed. A dried surfaced Mueller Hinton agar plate was taken. Inoculation was done by streaking the swab over the entire sterile agar surface. This procedure was done by streaking two more times rotating the plate approximately 60° each time ensure an even distribution of inoculum and finally the rim of agar was swabbed. Application of discs to Inoculated agar plates [14]

The predetermined battery of antimicrobial discs was placed on agar plates and given mild pressure to ensure complete contact with the agar plates. Discs were distributed evenly so that they were not closer than 24mm from center to center. Finally, plates were inverted and incubated at 37°C for 16-18 hours.

Reading plates and interpretation of results

After 16-18 hours of incubation, plates were examined. The plates which were satisfactorily streaked with proper inoculum showed uniformly circular zones of inhibition and confluent lawn of growth. If individual colonies were apparent, the test was repeated because probably the inoculum was too light. The diameter of the zone of complete inhibition were measured using a sliding calipers which was held on the back of the inverted Petri dish.

The petri dish plate was held a few inches above a black, non- reflecting background and illuminated with reflected light. The zone margin was taken as area showing no obvious, visible growth that can be detected with the unaided eye. The tiny colonies, which was detected only with a magnifying lens at the edge of the zone of inhibition growth was ignored. Discrete colonies growing within a clear zone of inhibition was sub-cultured, re-identified and retested. The size of the zones of inhibition were interpreted by referring to the CLSI standards and reported as susceptible, intermediate or resistant to the agents that have been tested. [15-17]

Controls used with each batch:

1. *Escherichia coli* ATCC 25922
2. *Pseudomonas aeruginosa* ATCC 27853
3. *Staohylococcusaureus* ATCC 25923

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Figure 1: Biochemical reaction for the identification of *Escherichia coli*

Figure 2: Biochemical reactions for the identification of *Klebsiellapneumonia*

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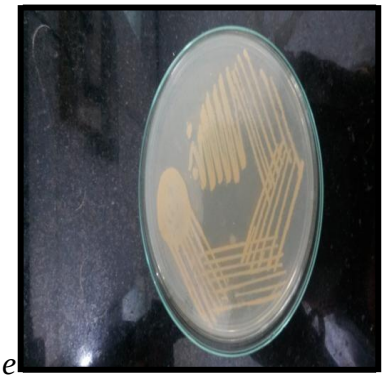

Figure 3: Nutrient agar showing golden Yellowcolonies of *S. aureus*

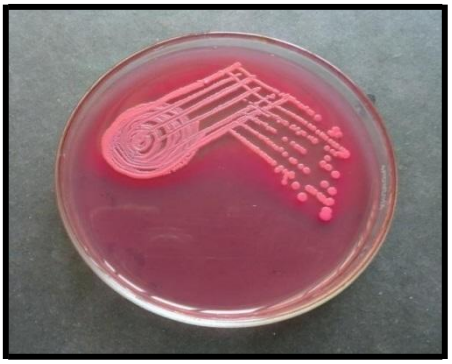

Figure 4: MacConkey plate showing colonies of *E. coli*

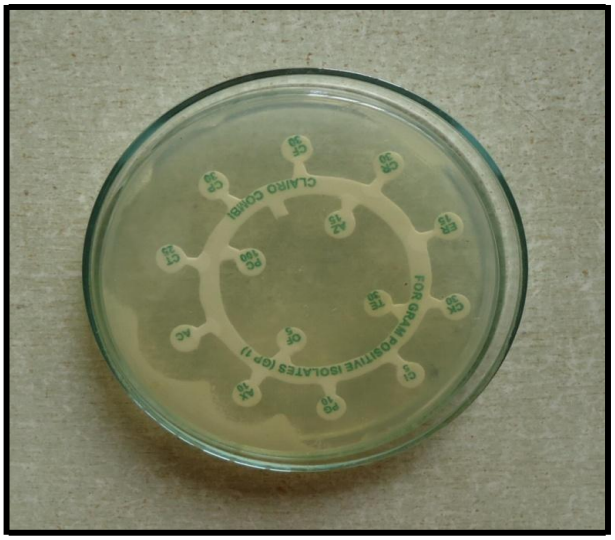

Figure 5: MHA showing antibiotic susceptibility pattern

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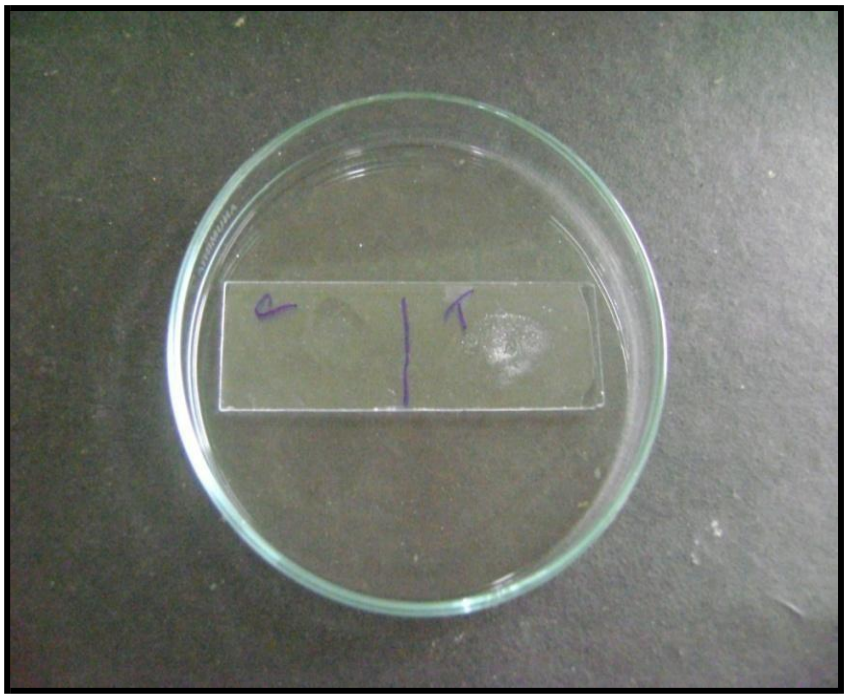

Figure 6: Slide coagulase test

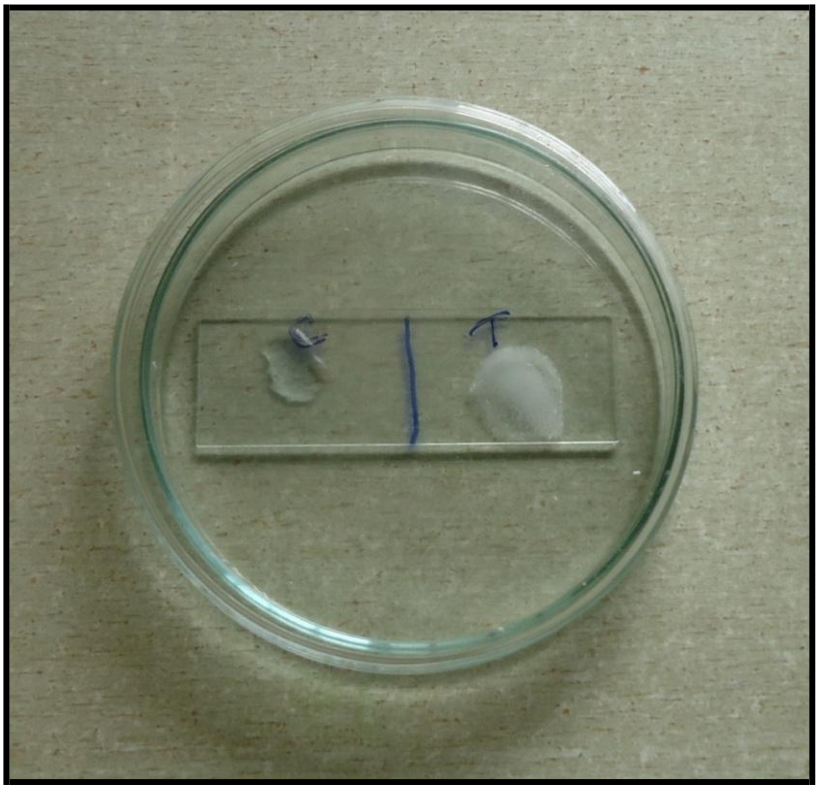

Figure 7: Slide catalase test

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RESULTS

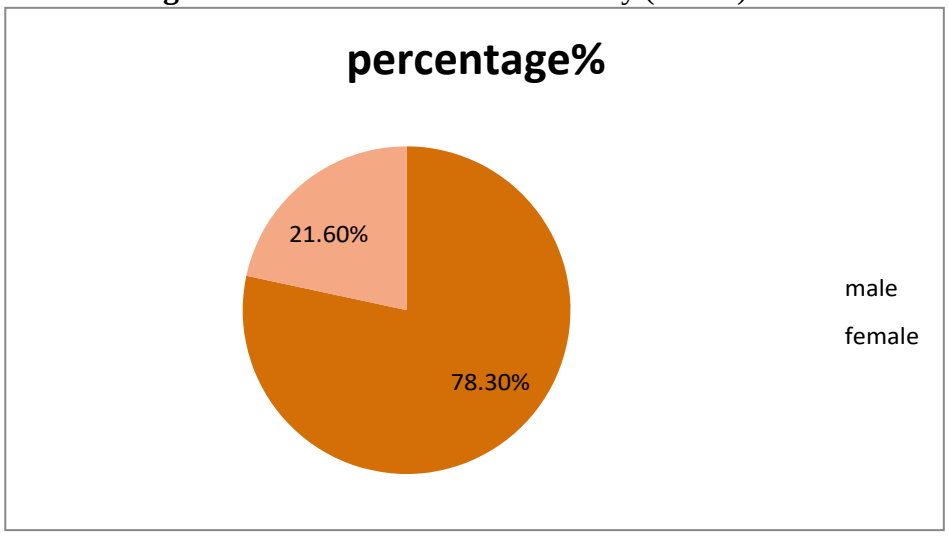
A total 120 blood samples were collected from clinical diagnosis of septicaemia patient attending SBMCH. Blood culture and antimicrobial susceptibility testing was done for all the samples.

Table 1: Age distribution in the study (n=120)

Age	**No of cases**		**Total**	**Percentage %**
	Male	**Female**		
16-20	7	2	9	6.92
21-30	14	3	17	13.07
31-40	25	5	30	23.07
41-50	30	9	39	30
51-60	12	6	18	13.84
>61	6	1	7	5.38
Total	**94**	**26**	**120**	**100**

Majority of the male cases belong to the age group of 41 to 50 years (30%) and the next commonest age group is 31 to 40 years (23.07%). Males are more when compared to female in this study.

Figure 8: Sex distribution in this study (n=120)



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78% of cases were males and 21% of cases were females. Males outnumbered females in this study

Table 2: Clinical Symptoms (n=120)

S. No	Clinical feature	Cases (n=120)	%
1.	Fever	120	10
2.	Respiratory Distress and Urinary symptoms	28	23.33
3.	TB infection	20	16.66
4.	Anemia	60	50
5.	Skin lesion	10	8.33
6.	Ascites and Pedal oedma	15	12.5
7.	Neurological symptoms*	8	6.66

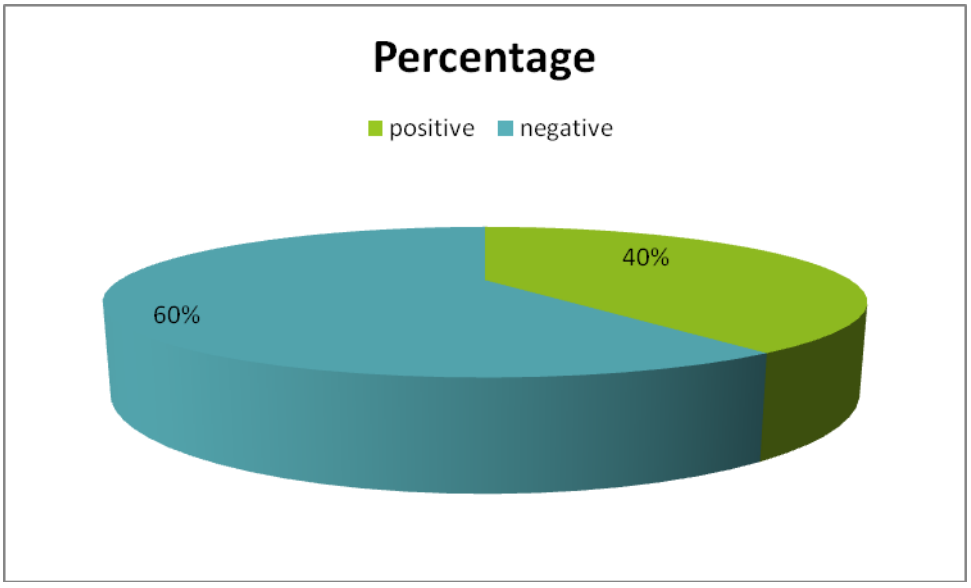
* Includes Headache, Hemiparesis, Hemiplegia.

Fever (100%) was present in all cases. Anemia (50%) was the second presenting symptoms in patients with septicemia. Respiratory Distress and Urinary Symptoms (23.33%), TB infection (16.66%), were next commones t symptoms in the descending order.

Table 3: Results of blood cultures (n=120)

Blood culture	No. of cases	%
Positive	48	40
Negative	72	60

Figure 9: Results of blood cultures



Blood culture was found to be positive in 48 (40%) cases.

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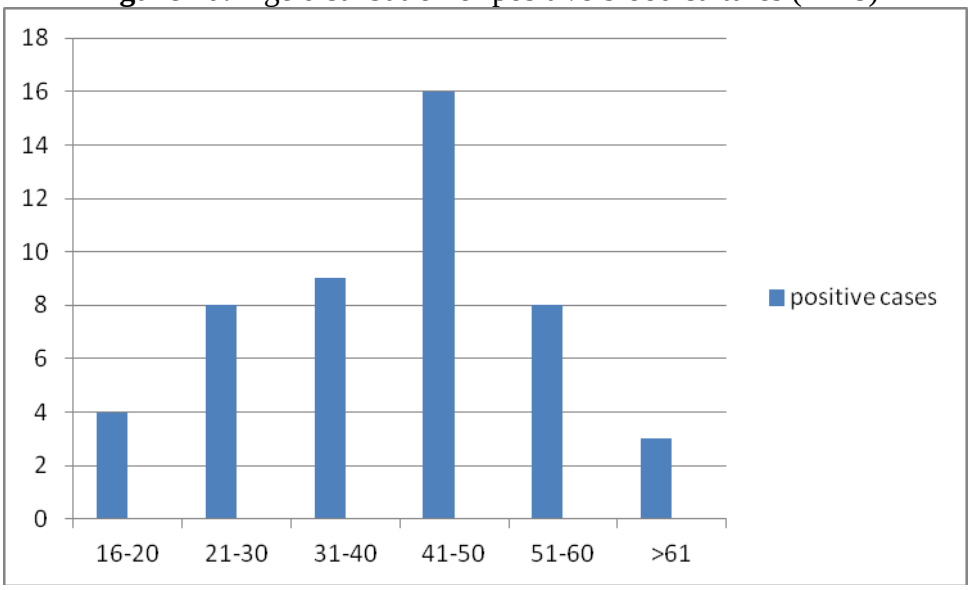
Table 4: Age distribution of blood culture positive cases (n=48)

Age	No. of cases		Total	Percentage%
	Male	Female		
16-20	3	1	4	8.33
21-30	6	2	8	16.66
31-40	**7**	2	9	18.75
41-50	12	4	16	33.33
51-60	6	2	8	16.66
>61	2	1	3	6.25
Total	**36**	**12**	**48**	**100**

Figure 10: Age distribution of positive blood cultures (n=48)



In Septicemia positive cases 36(75%) were males and 12(25%) were females. Most common age group associate with septicemia was 41 to 50 years (33.33%) and the next common age group was 31 to 40 years (18.75%).

Table 5: Organisms isolated by Blood Culture in Septicaemia Patients (n=48)

Organisms	**No. of cases (n=48)**	**%**
GRAM POSITIVE COCCI	**22**	**45.83**
Coagulase Negative Staphylococcus	12	25
Staphylococcus aureus	10	20.83

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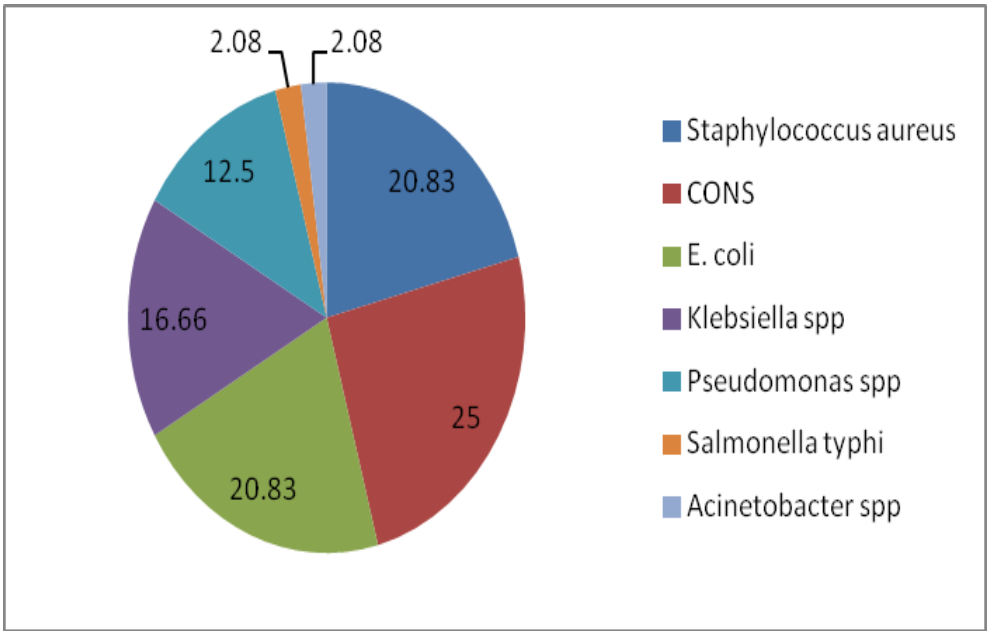
GRAM NEGATIVE BACTERIA	**26**	**54.16**
Escherichia coli	10	20.83
Klebsiella species	8	16.66
Pseudomonas species	6	12.5
Salmonella typhi	1	2.08
*Acinetobacter*spp	1	2.08

Figure 11: Organisms isolated by Blood Culture in Septicaemia Patients



Total Culture positive cases were 48 (40%). Gram-positive cocci were 22 (45.83%) Gram-negative bacilli were 26 (54.16%).

Table 6: Antimicrobial Susceptibility of Gram-positive organism

Antibiotics	**Cons sensitive (n=12)**	**Cons resistant (n=12)**	**Staphylococcus Auerus sensitive (n=10)**	**Staphylococcus Aureus Resistant (n=10)**

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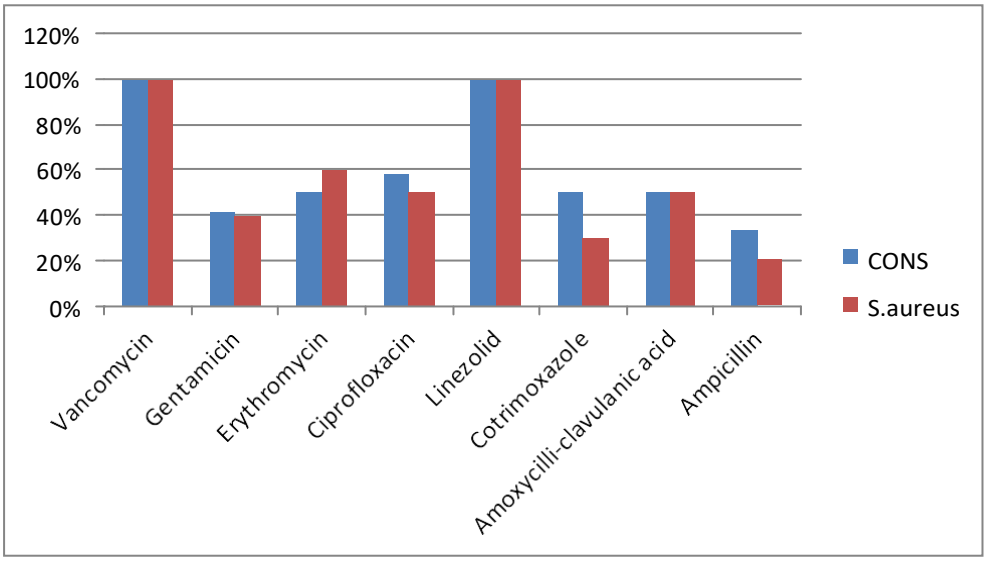
Vancomycin	100%	0	100%	0
Gentamycin	41.66%	58.33%	40%	60%
Erythromycin	50%	50%	60%	40%
Ciprofloxacin	58.33%	41.66%	50%	50%
Linezolid	100%	0	100%	0%
Cotrimoxazole	50%	50%	30%	70%
Amoxycillin-Clavulanic acid	50%	50%	50%	50%
Ampicillin	33.33%	66.66%	20%	80%

Figure 12: Antibiotic sensitive pattern of CONS and *S. aureus*



All gram positive cocci showed 100% sensitivity for Vancomycin and Linezolid.

Table 7: Antimicrobial Susceptibility of Gram-negative organism

Antibiotics	***E. coli* (n= 10)**		***Klebsiella* sp. (n= 8)**		***Pseudomonassp.* (= 6)**		***Salmonella typhi* (n= 1)**		**Acinetobacter sp. (n= 1)**	
	S%	**R%**	**S %**	**R%**	**S%**	**R%**	**S%**	**R%**	**S%**	**R%**
Amikacin	90	10	87. 5	12. 5	66. 6	33. 3	100	0	100	0
Ciprofloxacin	60	40	75	25	66. 6	33. 3	100	0	100	0

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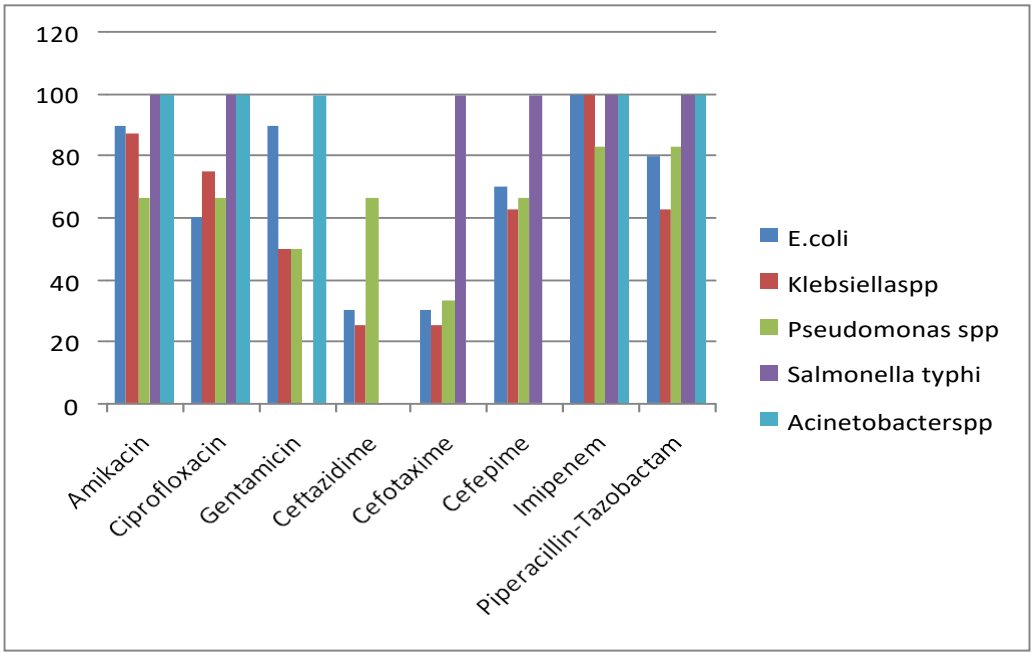
Gentamicin	90	10	50	50	50	50	0	100	100	0
Imipenem	100	0	100	0	83. 3	16. 6	100	0	100	0
Ceftazidime	30	70	25	75	33. 3	66. 6	0	100	0	100
Cefotaxime	30	70	25	75	66. 6	33. 3	100	0	0	100
Cefepime	70	30	62. 5	37. 5	83. 3	16. 6	100	0	0	100
Piperacillin-tazobactam	80	20	62. 5	37. 5	83. 3	16. 6	100	0	100	0

All gram negative organism except Pseudomonas aeruginosa showed 100% sensitivity for Imipenem.

Figure 13: Antibiotic sensitive pattern of Gram-negative organism



DISCUSSION

Septicaemia is one of the most common blood stream infections in the world. It is a major cause of morbidity and mortality among patients admitted in tertiary care hospital. Many studies have been undertaken to determine the organisms responsible for sepsis. Results have varied in different parts of the world. The cause of infection is multifactorial and depends on pathogens related, source of infection, underlying risk factors and proper treatment. Hence the present study was undertaken to determine the septicaemia, and their source of infection and the antimicrobial susceptibility pattern of the isolates from blood culture in our tertiary care hospital. In this study, total 120 blood culture samples were taken out of which 48(40%) were blood culture positive for growth. The rate (40%) of bacterial isolation in the blood culture in this study was relatively low

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compared to previous studies (68.4%) (MAJDA QURESHI AND FAR) [20]. other study has been reported by Sharama et al, also gave the prevalence of Bacteremia was (56%) [21].

In the concurrent analysis the incidence of bacterial isolation was (40%) this correlated with the study of Jordivalles et al, 2009 [22] in which the prevalence rate of blood stream infection was 30-40% cases. Similar study by Rello et al 2009 [23] also the prevalence of blood stream infection was 30%. While in other studies the incidence of microbial recovery is comparatively low (20.2%) [24] (22%) [25] (24.5%) [26].

In the present study, majority of the patients admitted in tertiary care hospital with clinical signs of sepsis (under SIRS criteria) were in the age group of 41 -50 years (33.33%)This results (Table 4) correlated with the study of prowler et al 2007 [27] in which majority of the patients were from 49-73 yrs and the mean age is 61 yrs. our study showed (Table 4) higher rate of prevalence of Septicemia in males (75%) compared to females (25%). Donowitz et al [28], also reported similar results in which male (64.8%) outnumbered females.

Fever was presenting symptom in all 100% of cases in this study. Since the patients with septicemia presenting clinically had other conditions like Anemia (50%) Respiratory distress& Urinary symptoms were seen in 23.33% of cases, TB infection in 16.66% and Ascites and Pedal edema (12.5%) were consistently present in majority of cases as in (Table 2). In the present study, Gram-negative organisms constituted the major group of isolates (54.16%) compared to Gram-positive organisms (45.83%) This correlates with the study done by Anbumani et al [29], 2008 where GNB accounted for 54.34% & GPC accounted for 45.7%. Similar study done by Elouenassann et al, where GNB accounted 51% while 49% isolates were gram positive.

In culture positive cases were more commonly Gram negative organisms (54.16%) with *Escherichia coli* being the most common isolate (20.83%) followed by Klebsiella species (16.66%), *Pseudomonas aeruginosa* (12.5%), Salmonella species (2.08%), Acinetobacterspp (2.08%). Many study done in India [29-32] reported *Escherichia coli* and *Klebsiella* species the most prevalent isolates. In this study Pseudomonas aeruginosa, one of the important non-fermenter was isolated in (12.5%) of cases, this is correlates with the other study of (10.7%) [27] and (9.8%) [33] Salmonella species were reported (2.08%) respectively in present study. It is in accordance with the study of (Karuikis et al;2006) (3.5%) [34]. The incidence of gram positive organism has been 45.8% in our study this is correlates with the other study done by Rao et al [35] Among gram positive organism, Coagulase negative staphylococcus (CONS) was predominant (25%) than the Staphylococcus aureus (20.83%). Jamal et al [36] &Ogston et al [37] 2009 reported Coagulase negative staphylococcus to be the most common isolates (46%) which correlated well with this study.

From 48 septicaemia positive cases CONS was the most common pathogen isolated in 12 (25%) followed by *Staphylococcus aureus* (20.83%), *Escherichia coli* (20.83%), Klebsiella species (16.66%), Pseudomonas species (12.5%), Salmonella typhi (2.08%), Acinetobacter (2.08%). The antibiotic sensitivity patterns of gram positive organism's vancomycin showed to highest antimicrobial activity in this study (100%) this was in accordance with other studies (PavaniNimmala et al) (100%) [38], (87%) and (86.1%) [38]. The present study Linezolid showed highly effective drug (100%) against gram positive organism this can be compared to the results of other study (100%) [38].

The commonest bacteria isolated in this study, CONS showed 100% sensitivity to Vancomycin, 100% sensitivity to Linezolid, 58.33% to ciprofloxacin, 50% sensitivity to Erythromycin. The second common isolate among Gram positive cocci was *Staphylococcus aureus* which showed 100% sensitivity to Vancomycin, 100% to Linezolid, 60% to Erythromycin, 50% to

ciprofloxacin. Ampicillin and Gentamycin showed highly resistant drug against gram positive organism.

In this present analysis among the various antimicrobial drugs tested, Amikacin was observed to be effective against gram negative organisms especially *Escherichia coli* (90%) in this is in correlate with the other studies (73.5%) and (78.9%) [40]. All gram negative organism except Pseudomonas species showed 100% sensitivity for Imipenem. This study showed highly sensitivity drug for (Non fermenter) bacteria including pseudomonas, salmonella species and Acintobacter was Ciprofloxacin. In the current study Cefipime and Piperacillin/ tazobactam were highly effective drug against gram negative organism. Ceftazidime showed low rates of sensitivity against Gram negative bacteria.

CONCLUSION

Septicemia is a leading cause of mortality and morbidity in our country. Males are more commonly affected than females. Most of the positive cultures were found to be between the age group of 41 to 50 years. Coagulase negative staphylococci, Staphylococcus aureus, Escherichia coli, Klebsiella species, pseudomonas species, Salmonella typhi and Acinetobacter species are the common organisms isolated by blood culture. Antibiotic sensitivity pattern was observed. The Gram positive organisms were susceptible to Vancomycin and Linezolid and Gram negative organisms were susceptible to Amikacin, Imipenem and piperacillin/ tazobactam. Early diagnosis & appropriate antibiotic therapy will reduce the mortality and morbidity.

Funding: No funding sources

Ethical approval: The study was approved by the Institutional Ethics Committee

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

The encouragement and support from Bharath University, Chennai is gratefully acknowledged. For provided the laboratory facilities to carry out the research work.

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