Original Research Article

Bacteriological Profile of Blood Culture from Adult Sepsis Patients from a Rural Based Tertiary Care and Teaching Hospital, Piparia, Vadodara, India

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In the last decade a number of tests were developed and used for early detection of sepsis yet blood culture remains a gold standard in establishing diagnosis of sepsis according to 1992-2001 definition which gives an emphasis on the role of microbes in the pathogenesis of sepsis. Thus this study was aimed at isolating and identifying bacterial and/or fungal agents from blood culture of the patients diagnosed clinically as having sepsis. A total of 673 blood cultures were performed from 743 patients and in the remaining patients microbes other than bacteria/fungi were found as cause of sepsis and so blood cultures were not performed in them. A total of 50.96% (343/673) samples did not yield any bacterial/fungal growth whereas the rest 49.03% (330/673) showed growth with 339 isolates. The majority samples had single bacterial isolate i.e. 292 (88.48%) whereas 12 (3.64%) had polymicrobial i.e. >1 type of bacteria or bacteria plus fungus together from a single sample and 26 (7.90%) samples had only fungal isolates. Also, of the 339 isolates, 51.32% (174/339) were Gram negative bacilli, 38.64% (131/339) were Gram positive bacteria and 10.02% (34/339) *Candida species*. *Klebsiella spp.* - 19.46% (66/339) was the most frequent isolate from blood culture followed by *Staphylococcus aureus*- 15.04% (51/339), *CoNS*- 14.45% (49/339), *E. coli* 14.15% (48/339), *Candida species* 10.02% (34/339), *Enterococcus species* 7.37% (25/339), *Acinetobacter species* 7.96% (27/339), Salmonella species 4.42% (15/339), *Pseudomonas spp.* 4.13% (14/339) and a small percentage of each of *Streptococcus pyogenes* (4/339), *Citrobacter freundii* (2/339), *Proteus spp.* (n=1), an unidentified GNB (1/339) and contaminant GPR (2/339).

Introduction

The last decade witnessed the development and use of number of biomarker based tests for early detection of sepsis like procalcitonin, interleukin-6 (IL-6) etc. yet the blood culture remained a gold standard in establishing diagnosis of sepsis fulfilling the 1992-2001 definition which gave an emphasis on the role of microbes in the pathogenesis of sepsis

using the term "suspected or proven infection" in the definition (Vincent, 2002; Nagoba *et al.,* 2005; Bone *et al.,* 1992).

Bacteria are a common cause of sepsis around the world and blood culture helps to isolate these and identify them as a cause of sepsis. Gram negative sepsis predominates over gram positive sepsis and also the proportion of *Candida species* has considerably increased in

many institutions due to prolonged treatment with multiple antibiotics, the use of indwelling intravascular devices, and prolonged neutropenia in patients with cancer have been demonstrated to be independent risk factors for the acquisition of nosocomial candidemia (Reinhart *et al.,* 2005).

This study was aimed at determining the bacteriological profile of clinically diagnosed sepsis patients in our setup.

The objectives of this study were to perform blood culture in clinically diagnosed sepsis adult patients and to isolate and identify the blood culture isolates obtained using standard biochemical tests.

Materials and Methods

This study was conducted in Clinical Microbiology Laboratory in Dhiraj Hospital under the Department of Microbiology from September 2012 to December 2015.

After obtaining ethical approval from the Institutional Ethical Committee.

A total of 743 patients with clinically diagnosed sepsis, with a proven/suspected infection and who fulfilled the inclusion criteria were included in the study.

Inclusion Criteria (Levy *et al.,* 2003):

Adult patients (age >18 years) and having 2 or more of the following:

Temperature: >38^{0}C or <36^{0}C
Tachypnea: >20 breaths/minute
Tachycardia: Heart rate >90 beats/minute
Leukocyte count: >12,000/µl or <4000/µl

Methods are described under the following headings: (Collee *et al.,* 14th edn.; Betty A. Forbes *et al.,* 12th edn.; Koneman *et al.,* 6th edn).

Collection

For blood culture, blood samples were collected before administering antibiotics at our hospital. However, for patients referred from private hospitals, already treated with antibiotics, were still considered for blood culture and blood samples were collected before starting antibiotic in our setup.

Hence 5-7ml of blood sample was collected by venepuncture taking all aseptic precautions. For this a tourniquet was tied around the arm tightly and the patient was asked to make fist. The vein was palpated and located. Isopropyl alcohol (70%) or Povidone Iodine (10%) was applied with a help of sterile cotton over the cubital aspect of the forearm. Depending on the case, the samples were collected in BACTEC bottles (from BD Diagnostics - 30 ml of broth) or in commercially available conventional Monophasic Brain Heart Infusion (BHI) Broth bottles (HiMedia-70ml broth) and also in the Biphasic Medium (HiMedia) containing BHI broth 40ml and BHI slope 20ml. The BACTEC bottles (Figure 1) after collection were placed in BACTEC 9050 machine (Figure 2) while the Monophasic and Biphasic BHI (brain heart infusion) broth bottles (Figure 3 and 4) were placed in incubator at 37^{0}C for 24 hours before processing.

Processing

The BACTEC bottles were removed only after the machine showed the sign of "positive/growth" or "negative/no growth" on its screen whereas in case of conventional monophasic and biphasic medium containing BHI broth subcultures were done on the next day.

Thus after the bottles were removed from machine/incubator direct smears were prepared and stained by Gram stain to detect

presence of organism in the bottles and the reports of the smears were communicated to the clinician as early as possible. Thereafter the samples were sub-cultured on Mac Conkey's agar, Blood agar and also on Saboraud's Dextrose Agar (SDA) (all media from HiMedia) if fungal elements were seen on direct smear. Inoculated plates were incubated for 18-24 hours at 37^0C. The plates were then observed for the formation of colonies. In case of biphasic medium, the bottle was tilted to allow the broth containing blood to flow over the surface of the slope and then kept in upright position in the incubator.

For the bottles showing no growth the BACTEC machine indicated negative result on the 5th day. The specimens from these bottles were also subcultured to ensure that no organism is missed. After 24 hours if no growth was found on the plates, it was reported as negative i.e. "No Organism Isolated" finally on the 6th day. In case of monophasic and biphasic medium bottles, 3 subcultures were done on alternate days before declaring as negative i.e. "No organism isolated."

Identification

All plates showing growth, either from BACTEC or conventional BHI bottles with monophasic or biphasic medium slopes, were subjected to identification using standard methods. The colony characters were observed and utilised for identification. The gram stained smears from colonies were observed for presumptive identification of colonies as gram negative bacilli colonies or gram positive cocci colonies.

For identification of gram positive cocci isolates, catalase, coagulase, mannitol salt agar test, bile esculin and bacitracin sensitivity were used whereas for gram negative isolates, catalase, oxidase, indole, methyl red, Voges-

Proskauer, citrate, urease, phenlypyruvic acid, motility, triple sugar iron test and sugar fermentation tests were carried out according to the standard methods (Collee *et al.,* 14th edn.; Betty A. Forbes *et al.,* 12th edn.; Koneman *et al.,* 6th edn.).

Results and Discussion

A total of 743 patients diagnosed clinically as sepsis were included in the study from September 2012 to December 2015 (3 years 3 months). Of the total 743 patients, 61.50% (457/743) were male and 38.49% (286/743) were female patients. The mean age of the patients was 48.02 years.

A total of 673 blood cultures were performed and in rest the causes other than bacteria/fungi were established and so blood cultures were not performed. Of the total 673 blood samples cultured, 50.96% (343/673) samples did not yield any bacterial/fungal growth whereas the rest 49.03% (330/673) showed growth.

A total of 339 isolates were obtained from 330 (49.18%) blood culture positive samples. The majority samples had single bacterial isolate i.e. 292 (88.48%) whereas 12 (3.64%) had polymicrobial i.e.>1 type of bacteria or bacteria plus fungus together from a single sample and 26 (7.90%) samples had only fungal isolates as shown in Chart 1.

Of the 339 isolates, 51.32% (174/339) were Gram negative bacilli, 38.64% (131/339) were Gram positive bacteria and 10.02% (34/339) *Candida species*. Predominantly Gram negative bacteria were isolated. In our study *Klebseilla spp.* - 19.46% (66/339) was the most frequent isolate from blood culture followed by *Staphylococcus aureus* - 15.04% (51/339), *CoNS*- 14.45% (49/339), *E. coli* 14.15% (48/339), *Candida species* 10.02% (34/339), *Enterococcus species* 7.37% (25/339), *Acinetobacter species* 7.96%

(27/339), *Pseudomonas spp.* 4.13% (14/339) and a small percentage of each of *Streptoccus pyogenes* (4/339), *Citrobacter freundii* (2/339), *Proteus spp.* (n=1), an unidentified GNB (1/339) and contaminant GPR (2/339) as shown (in the Chart 2) below. The blood culture isolates also included 15 *Salmonella species* contributing to 4.42% of the total number of blood culture isolates. Amongst Salmonella species (n=15), *S. typhi* were more common i.e. n=12 followed by *S. paratyphi* A

(n=2) and *S. paratyphi* B (n=12).

As shown in the Table 1, our findings in terms of culture positive findings (49.0%) are compared to studies from other regions of India are higher. This could be because of the coordinated efforts of the consultants in ICU and the Clinical Microbiology Laboratory, proper sample collection and timely processing of the samples.

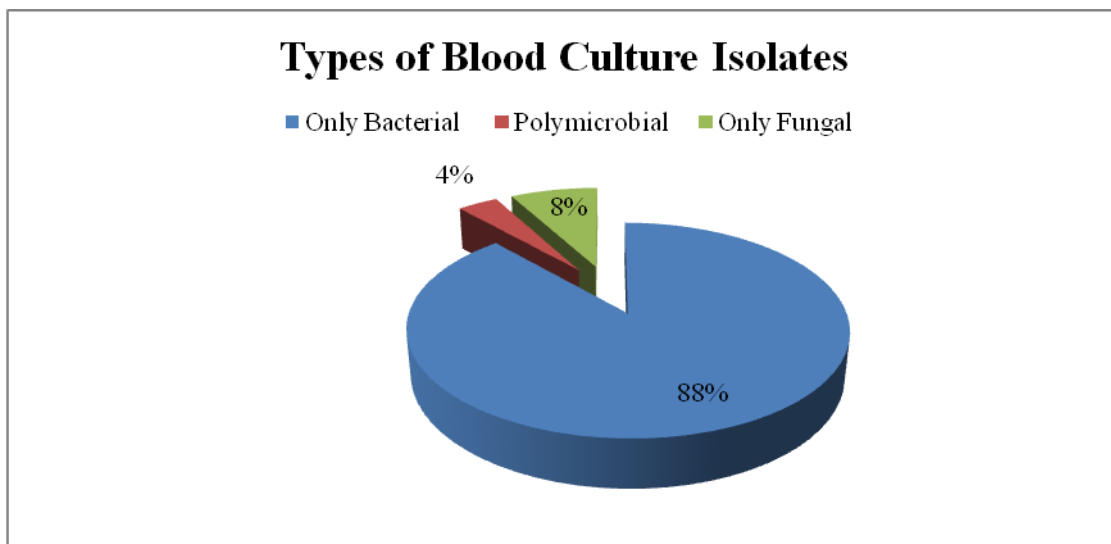**Chart.1** Types of Isolates obtained from blood culture positive specimens (n=330)

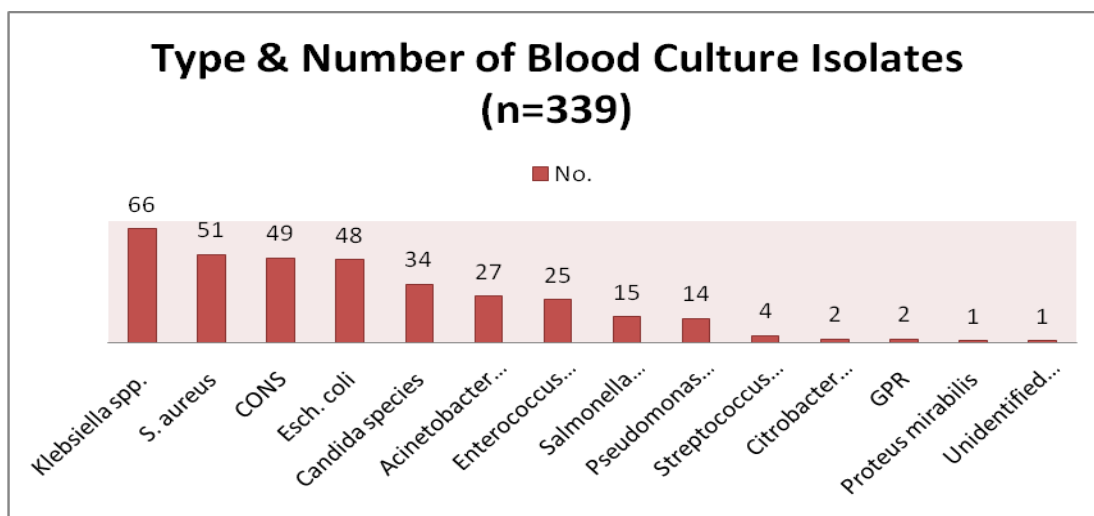**Chart.2** Number & Type of Isolates (n=339) Obtained from Blood Culture Positive Specimens (n=330)

Table.1 Comparison of blood culture outcomes and isolate types with other studies

Author	Place	Positive Blood Culture Outcome (%) and Isolate types (%)	Frequent Isolates (%)
Kante Meenakshi *et al.,* **2014.**	Andhra Pradesh, South India	Outcome - 17% Gram negative – 23/34 (67.64%) Gram positive – 11/34 (32.36%)	*Pseudomonas aeruginosa* (6.6%) *S. aureus* (4.5%) *Acinetobacter spp.* (6.6%) *Klebsiella pneumoniae* (2%) *CoNS* (1%)
Abrahamsen *et al.,* **2013.**	Tamil Nadu, South India	Outcome - 7% Gram negative – 42.85% Gram positive –19.04%	*E. coli* (14.3%) *S. typhi/paratyphi* (14.3%) *S. aureus* (9.5%) *Klebsiella spp.* (4.8%) *Enterococcus spp.* (4.8%) *Strep. viridians* (4.8%) Unidentified GNB (4.8%) *Pseudomonas spp.* (0%) *Acinetobacter spp.* (0%) Other (38.1%) Fungi (4.8%)
Sahoo *et al.,* **2016.**	Odisha, East India	Outcome -26% Gram negative – 69.23% Gram positive – 30.76%	*E. coli* (34.6%) *Klebsiella spp.* (26.9%) *S. aureus* (23.1%) *A. baumanii* (7.7%) *CoNS* (7.7%)
Gupta *et al.,* **2016.**	New Delhi, North India	Outcome -16.5% Gram negative – 58.34% Gram positive – 41.65%	*E. coli* (22.4%) *Klebsiella spp.* (19.7%) *S. aurues*(18.3%) *CoNS* (17.4%) *Pseudomonas spp.* (8.40%) *Acinetobacter spp.* (5.20%) *Enterococcus spp.* (4.8%) *Streptococcus spp.* (4.8%) *S. typhi* (1%) *Citrobacter spp.* (0.9%) *Proteus spp.* (0.35%)
Sonawane J *et al.,* **2016.**	Navi Mumbai, West India	Outcome 10.29% Gram negative – 83.22% Gram positive – 16.78%	*Klebsiella spp.* (22.38%) *Pseudomonas spp.* (20.98%) *Acinetobacter spp.* (17.48%) *E. coli* (11.19%) *S. typhi* (7.70%) *Enterococcus spp.* (7.70%) *S. aureus* (6.99%)

			Enterobacter spp. (3.50%) *Strep. spp.* (2.10%)
Oza S *et al.,* 2016.	Surendranagar, Gujarat	Outcome 18.62% Gram negative – 67.40% Gram positive – 32.60%	*K. pneumoniae* (34.78%) CoNS (15.22%) *Pseudomonas spp.* (10.87%) *E. coli* (8.69%) *S. typhi* (4.35%) *Enterococcus spp.* (4.35%) *Acinetobacter spp.* (4.35%) *Unidentified GNB* (4.35%) *Strep. spp.* (2.17%)
Sundarmono *et al.,* 2017 (SEA Infectious Diseases Clinical Research Network)	South East Asia Region	Outcome 12% (in adults)	*E. coli* (5%) *S. typhi* (0.2%) *Acinetobacter spp.* (1%) *Strep. suis* (1%) *Burkholderia pseudomallei* (<1%)
Lee *et al.,* 2007.	North Carolina, USA	Outcome 73.1% (in first culture)	*S. aureus* (20.70%) *CoNS* (14.10%) *Enterococcus spp.* (4.66%) Strep. spp. (5.46%) *K. pneumoniae* (9.55%) *E. coli* (7.62%) *Pseudomonas spp.* (4.43%) Yeasts (8.64%)
Present Study	Vadodara, Gujarat	Outcome 49.03% Gram negative – 51.03% Gram positive – 38.64%	*Klebsiella spp.* (19.47) *S. aureus* (15.04) *CoNS* (14.45) *E. coli* (14.16) *Candida spp.* (10.03) *Enterococcus spp.* (7.37%) *Acinetobacter spp.* (7.96) *Pseudomonas spp.* (4.13) *Salmonella spp.* (4.42) *Strep. pyogenes* (1.17%) *Citrobacter freundii* (0.58%) Gram positive bacilli (0.58%) *Proteus mirabilis* (0.29%) Unidentified GNB (0.29%) *Candida spp.* (10.32%)

Fig.1 BACTEC Aerobic Culture Bottle

Fig.2 BACTEC 9050

Fig.3 Biphasic Blood Culture Bottle

Fig.4 Biphasic Blood Culture Bottle

Septicaemia due to Gram negative bacteria predominated over Gram positive and these findings are comparable to those with other studies from India as well as other countries (Saravu *et al.,* 2015; Divatia *et al.,* 2016; Kante *et al.,* 2014; Sahoo *et al.,* 2016; Gupta *et al.,* 2016; Sonawane *et al.,* 2016; Oza *et al.,* 2016; Jacob *et al.,* 2009) but contrasting findings have been reported by Lee *et al.,* (2007) and Kumalo *et al.,* (2016) who

reported Gram positive cocci predominance with 52.33% and 53.3% respectively.

In our study *Klebseilla spp.* was the most frequent isolate from blood culture followed by *Staphylococcus aureus*, *CoNS* and *E. coli*. These findings are similar to Gupta *et al.,* (2016) and Sonawane *et al.,* (2016). Whereas in the other studies, *Klebsiella pneumoniae* (Oza *et al.,* 2016), *Staphylococcus aureus*

(Lee *et al.,* 2007 and Kumalo *et al.,* 2016), *E. coli* (Sahoo *et al.,* 2016 and Gupta *et al.,* 2016) and non-typhoidal *Salmonella* (Jacob *et al.,* 2009) have been reported as the most frequent isolates from blood culture samples.

In our study we found 4.42% (15/339) *Salmonella species* obtained from blood cultures of patients. Our findings in regards to isolation of *Salmonella species* are more than 1% and 0.2% reported by Abrahamsen *et al.,* 2013 and South East Asia Region study by Sudarmono *et al.,* (2017) respectively but lesser than 8% reported by Chrispal *et al.,* (2010). Ugas *et al.,* (2016) reports a case of septic shock with acute respiratory distress syndrome due to *Salmnella typhi* from Oklahoma, USA, which was found to be susceptible to ceftriaxone, cotrimoxazole and ciprofloxacin. Another case report from New Delhi, India by Randhawa *et al.,* (2007), reports *Salmonella paratyphi A*, susceptible to Cefotaxime, Ciprofloxacin, Gentamicin, Amikacin and Cotrimoxazole which is similar to the findings of our.

CoNS was the third most common blood culture isolate i.e. (n=49) 14.45% as well as the second most common gram positive cocci. Only single blood cultures were performed except for patients who had suspected endocarditis, where more than one blood cultures were obtained from different sites. Most of the studies consider it as a contaminant (Sudarmono *et al.,* 2017) but as these have been isolated from critically ill patients admitted in ICU with clinically diagnosed sepsis these isolates were considered to be pathogenic rather than contamination. S Singh *et al.,* 2016 used correlation with clinical parameters like temperature >38^{0}C or <36^{0}C, systolic blood pressure of <90mm/Hg, total leukocyte count >12,000/μl or <4000/μl and presence of >10% immature bands for establishing the significance of *CoNS* isolated from single cultures. In our study these clinical parameters are already fulfilled under sepsis definition. Thus with the same concept these were all considered significant. Also Oza *et al.,* (2016) and Kumalo *et al.,* (2016) report 13.3% *CoNS* being the second most common isolates from blood culture. Kumalo *et al.,* (2016) suggests that though in past they were considered as non-pathogenic but currently due to increased use of indwelling medical devices they are on the rise.

Though the 1992-2001 definition gave importance to bacteria/fungi in the pathogenesis of sepsis, in February 2016 the Third International Consensus Definitions for Sepsis and Septic Shock defined sepsis as 'life-threatening organ dysfunction caused by a dysregulated host response to infection" and the organ dysfunction in turn should be identified by bedside qSOFA (Quick Sequential Organ Failure Assessment) score of 2 points or more i.e. altered mental status, systolic blood pressure ≤100 mm Hg or respiratory rate ≥22/min (Singer M *et al.,* 2016). Thus the 2016 definition does not make any reference to the role of infection or microbes in sepsis. However, it is important to know the type of bacteria which helps to its pathogenesis, also helps clinicians choose right treatment when antimicrobial susceptibility results are made available to them and ways to prevent infections due to such microbes from spreading in the hospital. Overall the gram negative bacteria were commonly found as the cause of sepsis in our setup with *Klebsiella* spp. been the most common isolate and amongst gram positive cocci *Staphylococcus aureus* was the most common isolate. Moreover, though other bacteria like *Streptococcus pyogenes*, *Citrobacter freundii* and *Proteus mirabilis* were isolated in smaller percentage, yet they were found to be important cause of a serious condition like sepsis.

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