ORIGINAL ARTICLE

Bacterial Isolates and their Antibiotic Sensitivity Pattern in Clinically Suspected Cases of Fever of Unknown Origin

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Abstract

The present study was conducted on 100 suspected cases of fever of unknown origin to identify the prevalence of predominant bacterial microorganisms and their drug sensitivity pattern. The blood samples were subjected to conventional blood culture and BACTEC 9050 culture system. Out of 100 suspected cases, culture positivity was seen in 46% cases with 80.43% pathogenic bacterial isolates comprising of 54.05% gram positive and 45.94% gram negative isolates. Predominant gram positive isolates were coagulase negative Staphylococcus 35% followed by 30% Staphylococcus aureus with sensitivity to vancomycin (100%) and resistance to ampicillin, cloxacillin & cefalexin. Gram negative isolates were Salmonella typhi (29.41%) followed by E coli (17.64%) showing sensitivity to piperacillin/tazobactum and cefoperazone/sulbactum (90%) each and resistance to amoxicillin. BACTEC 9050 was observed to be sensitive(100%) as compared to conventional blood culture(67.56%) for cultural isolation of pathogenic organisms in clinical specimens.

Key Words

BACTEC 9050- Becton Dickinson Microbiology Systems, Fever of Unknown Origin

Introduction

Pyrexia of unknown origin (PUO) remains one of the major diagnostic challenges for the clinician. Sir William Osler truly said "Humanity has but three great enemies; Fever, Famine and War; of these, by far, the greatest, by far, the most terrible is Fever."(1) Fever of unknown origin (FUO) identifies a syndrome of fever that does not resolve spontaneously, in which the cause remains elusive after an extensive diagnostic workup. (2) The differential diagnosis for FUO comprises over 200 disorders and is among the longest of any condition in medicine.(3) Infections (46.4%) are still the most common cause of FUO of which enteric fever is the commonest (63.9%), followed by malarial fever(19.4%) and tuberculosis fever(11.1%).(4) In FUO, there is no diagnostic gold standard against which other diagnostic tests may be measured. In many cases the cause for failure of diagnosis is lack of a protocol for investigation. If the investigation is based on the clinical findings, most of the cases can be easily diagnosed. A meticulous history, a thorough physical examination, discriminative use of investigative procedures and constant reassessment of all the parameters reveal the cause of the patients fever.(5) Despite recent developed techniques, like nucleic acid probes, PCR and other molecular techniques for microbiological diagnosis, blood culture still remains the most practical and reliable method in the diagnosis of bloodstream infections.(6)

Blood cultures provide the best yield for microbiological diagnosis, with sensitivity ranging from 53% to 90%.(7) Conventional method includes culture for two weeks to detect slow growing organisms and on special media if necessary. The BACTEC 9000 series of blood culture systems are fluorogenic, automated, non-invasive blood culture system designed for processing three to five blood cultures per day. (8) Identification of bacterial isolates and their antibiotic sensitivity pattern can provide useful therapeutic input for management of such infections, which forms the basis of present study

Material and Methods

This cross-sectional study was carried out during the

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period January 2011 to December 2011 at M.M.Institute of Medical Sciences and Research , Mullana. Blood samples from 100 suspected cases of fever of unknown origin (FUO) attending the OPD and indoor were included in the study. Diagnosed cases of fever were excluded.

10 ml of blood was collected aseptically from adult patients and was divided equally into BACTEC blood culture vial (aerobic) and conventional blood culture bottle containing 50ml of brain heart infusion broth (Dilution 1:10). (9) For paediatric patients, 2 ml of blood was collected and equally transferred into the BACTEC™ PEDS PLUS/F vial and conventional blood culture bottle containing 10ml of brain heart infusion broth.(10) The inoculated BACTEC vials and conventional blood culture bottles were transported to the laboratory immediately.

The BACTEC bottle was placed into the BACTEC 9050. On receiving positive signals, bottles were removed and an aliquot of the broth was gram stained and subcultured onto the Blood agar, Mac Conkey's agar and Chocolate agar. Organism were identified by battery of biochemical reaction and all bottles were incubated for a minimum of 7 days before labelling as negative as per the manufacturer's protocol.8All negative bottles were subcultured to chocolate agar plates and incubated aerobically at the end of the incubation period.Conventional technique was done by inoculating blood into blood culture bottles containing brain heart infusion broth. The bottles were incubated at 37 0 C and were shaken periodically. On 3rd, 5th, and 7th day, subcultures were done on Blood agar, Mac Conkey's agar and Chocolate agar. Futher processing was done by standard laboratory procedures.(11) The negative blood sample was discarded after 7 days of incubation. Antibiotic sensitivity testing was performed by modified Kirby-Bauer disc diffusion method as per CLSI recommendations. (12)

Results

Out of the 100 samples of fever of unknown origin (FUO), 46 (46%) cases were culture positive and 54 (54%) were sterile by either of the two methods (*Table-1*). Out of 46 isolates, 37(80.43%) isolates were pathogenic while remaining 9(19.56%) were non pathogenic isolates (*Table-2*). Amongst the pathogenic isolates, 20 isolates (54.05%) were gram positive while 17 isolates (45.94%) were gram negative (*Table-3*). Out of 20 gram positive cases, 7(35%) were coagulase negative Staphylococcus followed by 6 (30%) Staphylococcus aureus, 3(15%) Streptococcus pneumonia, Enterococcus 2 (10%) and Candida 2(10%). (*Table 4*) . *Table 5* shows antibiotic sensitivity pattern of gram positive bacterial isolates. Isolates were sensitive to Vancomycin (100%), Ceftriaxone (94%), Gentamycin (83%), Erythromycin(77%) and Linozolide (56%). coagulase negative Staphylococcus and Staphylococcus aureus were resistant to the commonly used antibiotics, including ampicillin, cloxacillin & cefelexin. None of the gram-positive isolates were resistant to vancomycin. Out of 17 gram negative cases, 5 (29.41%) were Salmonella typhi followed by E coli 3(17.64%), Pseudomonas 3(17.64%), Klebsiella 3(17.64%), Acinetobacter 2(11.76%), and Citrobacter 1(5.88%) (*Table 6*).

Table 1. Rate of Culture Positivity in Suspected Patients of Fever of Unknown Origin

Total (N)	Culture Positive N(%)	Sterile N(%)
100	46 (46)	54 (54)

Table.2 Distribution of Blood Culture Isolates on The Basis of Pathogenicity

Total (N)	Pathogenic Isolates N (%)	Non Pathogenic Isolates N (%)
46	37 (80.43)	9 (19.56)

Table.3 Distribution Of Pathogenic Organisms In Blood Culture

Total (N)	Gram Positive N (%)	Gram Negative N(%)
37	20(54.05)	17(45.94)

Table 7 shows antibiotic sensitivity pattern of gram negative bacterial isolates. Gram-negative organisms were resistant to commonly used antibiotics such as amoxicillin. Maximum sensitivity was shown by amikacin 76% , ciprofloxacin 70-80% and cefriaxone 70%. Newer combinations of antibiotics like piperacillin/tazobactum & cefoperazone/sulbactum were sensitive in more than 90% of cases In the present study males constituted majority (65.22%) of the patients from rural background (65.22%). No definite correlation between occupation and culture positivity was observed .Maximum patients (52.17%) were found in the younger age group of less than 20 years followed by 31-40 years (21.73%), 21- 30 years (13.04%) , 31- 40 years (8.69%) and > 50 years of age (4.34%). (*Table-8*). Majority of the isolates were coagulase negative Staphylococcus (100%) and Staphylococcus aureus (86%) (*Table 9*).Maximum pathogenic isolates were recovered by BACTEC 9050 37/37(100%) as compared to conventional blood culture 25/37 (67.56%).

Discussion

Patients with Fever of unknown origin (FUO) are elusive and challenging clinical cases. Prompt diagnosis

Table 4. Distribution of Pathogenic Gram Positive Organisms

Total (n)	Coagulase negative staphylococcus n (%)	Staphylococcus aureus n (%)	Streptococcus pneumonia n (%)	Enterococcus n (%)	Candida n (%)
20	7(35)	6(30)	3(15)	2(10)	2(10)

Table. 5 Antibiotic Sensitivity Pattern of Gram Positive Bacterial Isolates

Antibiotics	Coagulase negative Staphylococcus n(%)	Staphylococcus aureus n(%)	Streptococcus pneumonia n(%)	Enterococcus n(%)
Nitrofurantoin	3(42.85)	4 (66.66)	-	2(100)
Linozolide	4(57.14)	4(66.66)	1(33.33)	1(50)
Gentamicin	7(100)	5(83.33)	1(33.33)	2(100)
Vancomycin	7(100)	6(100)	3(100)	2(100)
Erythromycin	6(85.71)	5(83.33)	2(66.66)	1(50)
Ciprofloxin	3(42.85)	3(50)	-	-
Imipenum	2(28.57)	2(33.33)	-	-
Cloxacillin		-	2(66.66)	-
Ampicillin		-	3(100)	-
Amikacin	2(28.57)	1(16.66)	-	-
Ceftrixone	7(100)	6(100)	3(100)	1(50)
Cefelexin	-	-	-	1(50)
Cefuroxime		6(100)	-	1(50)
Chloramphenicol		1(16.66)	2(66.66)	1(50)

Table.6 Distribution of Pathogenic Gram Negative Organisms

Total	S.typhi n(%)	Ecoli n(%)	Pseudomonas n (%)	Klebsiella n (%)	Acinetobacter n (%)	Citrobacter n (%)
17	5 (29.41)	3 (17.64)	3 (17.64)	3 (17.64)	2 (11.76)	1(5.88)

Table.7 Antibiotic Sensitivity Pattern of Gram Negative Bacterial Isolates

Antibiotics	S.typhi n(%)	E coli n(%)	Pseudomonas n(%)	Klebsiella n(%)	Acinetobacter n(%)	Citro Bacter n(%)
Gentamicin	1(20)	3(100)	1 (33.33)	2 (66.66)	2(100)	1 (100)
Ampicillin	2(40)	1(33.33)	-	-	-	-
Ceftriaxone	5 (100)	3 (100)	1(33.33)	1(33.33)	2(100)	-
Amikacin	2(40)	3(100)	2(66.66)	3(100)	2(100)	1(100)
Piperacillin-tazobactum	4(80)	3(100)	3 (100)	2(66.66)	2(100)	1(100)
Ciprofloxin	5(100)	3(100)	2(66.66)	2(66.66)	2(100)	-
Amoxicillin	-	-	-	-	-	-
Cefoperazone/Sulbactum	5 (100)	3 (100)	3 (100)	2 (66.66)	1 (66.66)	1 (100)
Imipenam	-	2(66.66)	3(100)	2(66.66)	-	-
Chloramphenicol	-	2(66.66)	-	1(33.33)	-	-
Nitrofurantoin	2(40)	3(100)	-	-	-	1(100)
Cefotaxime	-	-	2(66.66)	-	2(100)	-
Cotrimoxazole	2(40)	-	1(33.33)	-	-	-

Table.8 Distribution of Culture Positive Cases According to Gender, Age and Residence

Parameters	Culture Positive (N=46)	(%)
Gender		
Male	30	65.22
Female	16	34.78
Age		
<20	24	52.17
21-30	6	13.04
31-40	10	21.73
41-50	4	8.69
>50	2	4.34
Social Status		
Urban	16	34.78
Rural	30	65.22

Table.9 Distribution of Pathogenic Isolates by Conventional Blood Culture & Bactec 9050

Pathogenic Isolates (N)	Conventional Blood Culture (N)	Bactec 9050 (N)
Coagulase Negative Staphylococcus (7)	6	7
Staphylococcus Aureus (6)	4	6
Streptococcus Pneumonia (3)	2	3
Enterococcus (2)	1	2
Candida (2)	2	2
Salmonella Typhi (4)	2	4
E.Coli (4)	2	4
Pseudomonas (3)	2	3
Klebsiella (3)	2	3
Acinetobacter (2)	1	2
Citrobacter (1)	1	1

and effective treatment is necessary to prevent death and complications from septicaemia. Timely detection and identification of blood borne pathogen is one of the most important functions of microbiology laboratory. In the present study, blood culture positivity was seen in 46% cases with 37% pathogenic isolates comprising of 54.05% gram positive and 45.94% gram negative bacteria. These results were consistent with the study done by Jung *et al* (5) and Gopi *et al* (13) who reported that out of clinically significant microbial growth 61.52% were gram positive and 36.94% were gram negative bacteria.13 In present study, maximum isolates of gram positive bacteria were coagulase negative Staphylococcus (35%) followed by Staphylococcus aureus (30%), Streptococcus pneumonia (15%), Enterococcus (10%) and Candida (10%). While gram negative bacteria comprised mainly of Salmonella typhi (29.41%) followed by E coli (17.64%), Pseudomonas (17.64%), Klebsiella (17.64%), Acinetobacter(11.76%)and Citrobacter (5.88%). These findings were in agreement with earlier studies by Gopi *et al* (13) who reported that among(61.52%) gram positive bacteria, coagulase negative Staphylococcus (29.92%) while gram negative bacteria (36.94%) comprised mainly of Enterobacteriacea (25.34%) i.e. Salmonella typhi (14.35%), E coli (2.90%), Klebsiella (1.06%), Citrobacter (1.37%) and Acinetobacter (5.80%).

However contrary to present study, Durmaz *et al* (6) reported more Gram negative isolates from such FUO cases.Gram positive bacterial isolates showed maximum sensitivity towards Vancomycin (100%), Ceftriaxone (94%), Gentamycin (83%), Erythromycin (77%) and Linozolide (56%). Coagulase negative Staphylococcus and Staphylococcus aureus were resistant to the commonly used antibiotics, including Ampicillin, cloxacillin & cefelexin. Vancomycin still remains the most sensitive drug for S.aureus which correlates with the findings of Roy *et al* (14). Majority of gram-negative organisms were resistant to commonly used antibiotic amoxicillin. Present study also showed that piperacillin/tazobactum & cefoperazone/ sulbactum were the two most effective antibiotics against gram-negative organisms. These findings were in agreement with the study done by Kairavi

et al (15). Males constituted majority (65.22%) of the patients and maximum (65.22%) were from rural background. No definite correlation between occupation and culture positivity was observed. These findings were similar with Asmaa *et al* (16) who reported (66.66%) male and (33.33%) female with male to female ratio of 1.99:1.The increase member of male patients over female in this study might be due to occupational exposure to animals. Male are the active and main earning member of the most of the family still now, so they are more privileged to visit physician chamber for treatment.(17) Maximum patients (52.17%)were found in the younger age group of less than 20 years followed by (21.73%) in 31-40 years, (13.04%) in 21- 30 years, (8.69%) in 31- 40 years and (4.34%) were > 50 years of age. A study by Asmaa *et al* (16) reported most of the clinically suspected cases (43.30%) in the age group between 1-15 years having very close correlation with present study. This study shows the recovery rate of pathogenic bacterial isolates by conventional blood culture system and BACTEC 9050. The highest rate of pathogenic isolates were recovered by BACTEC 9050 (100%) as compared to conventional blood culture (67.56%). Maximum detected by BACTEC 9050 were coagulase negative Staphylococcus and Staphylococcus aureus. These results were consistent with the previous study which reported that 13 of 16 patients showed bacteraemia (81.2%) and 11(85%) patients were detected by BACTEC 9050 system and 2 (15%) patients by conventional blood culture methods. (18) Furthermore, the mean time to detection of significant pathogens was significantly less with the blood culture system than with conventional media.

Conclusion

Present study revealed that 46% were culture positive and 54% were sterile by either of the two methods i.e. conventional blood culture and BACTEC 9050. Highest rate of pathogenic isolates were recovered by BACTEC 9050 (100%) as compared to conventional blood culture (67.56%) indicating sensitivity and accuracy of BACTEC 9050 for culturing the microorganism in clinical specimens. 80.43% isolates were pathogenic with (54.05%) Gram positive and (45.94%) Gram negative isolates. Maximum gram positive isolates were coagulase negative Staphylococcus (35%) and Staphylococcus aureus (30%) showing antibiotic sensitivity towards vancomycin (100%) and ceftriaxone (94%) and resistance to commonly used antibiotics such as ampicillin, cloxacillin & cefelexin. Gram negative bacteria comprised mainly of Salmonella typhi (29.41%) and E coli (17.64%) with maximum antibiotic sensitivity to piperacillin/tazobactum & cefoperazone/ sulbactum and resistance to commonly used antibiotics like amoxicillin. Therefore, drug susceptibility test is essential before prescribing any antimicrobials as it would hasten the administration of appropriate antimicrobial therapy thereby decreasing morbidity and mortality due to complications of septicaemia as well as preventing spread of infection in the community.

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