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The Study of Bacteremias By Blood Cultures Using Bactec® 9050 System and The Follow Up of Isolates in Medical Microbiology Laboratory

KEYWORDS: Blood culture system BACTEC® 9050, Bacteremia , Antibiotic Sensitivity

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ABSTRACT *The study was done for 100 blood samples received in the laboratory from patients suffering from haematological cancers solid tumours.The specimens were processed using BACTEC® 9050 (Becton Deckinson Systems) culture systems. A total of 22 (22%) positive cultures were studied. Gram- positive, Gram negative isolates were 9 (9 %), 13 (13%) respectively. Staphylococcus aureus showed 100% sensitivity towards Gentamycin, Amikacin, Netromycine, Amoxyclave. Coagulase Negative Staphylococcus (CONS) showed 100% sensitivity towards Ampicillin and Vancomycin and 100% resistance towards Tetracycline, Ceptoxime, Ofloxacin. Enterococci showed 100% sensitivity towards Ampicillin, Ciprofloxacin, Tetracycline, Bacitracin, Vancomycin, Ceftizoxamine and Amoxyclave whereas 100% resistant towards most of the antibiotics studied. Escherichia coli was 100% resistant to Ceptoxime and 80% resistant to all the other antibiotics tested, Salmonella typhi was found tob be 100% sensitive towards Ciprofloxacin only. Klebsiella showed 100% sensitivity towards Amikacin only. Pseudomonas aeruginosa was 100% sensitive for Pefloxacin , Cefoperazone only.*

Introduction

Microorganisms present in the circulating blood,whether continuously or transiently, are a threat to every organ in the body.The costly detection and identification of blood borne pathogens is one of the most important function of the microbiology laboratory. Positive blood cultures may help provide a clinical diagnosis as well as aspecific etiologic diagnosis. Pathogens of all four major groups Bacteria, Fungi, Viruses and Parasites may be found in circulating blood during the course of disease[1,2].

The leading causes of disease and death in most countries is infectious disease.The invasion of the blood stream by Microbes may lead to serious immediate consequences and finally death. This is the reason that detection of microorganisms in a patient's blood has great therapeutic and prognostic significance [3]. Blood culture remains central to this quest for identifying bacterial causes of bacteremia and septicemia. Despite recent advanced developments like molecular techniques for microbial diagnosis, blood culture still remains the most practical and reliable method for the diagnosis of infections in the blood stream. Besides eliminating cross contamination of cultures during repeated subcultures.Instrumentation used for blood cultures provides rapid, accurate andcost- effective treatment [4].

The BACTEC® 9050 instrument is designed for the rapid detection of bacteria and fungi in clinical culture of blood. The BACTEC® 9050 series of blood culture systems are fluorogenic, automated, non-invasive with a capacity of 50 bottles.The BACTEC® 9050 instrument performs self diagnostics and loads its operating instructions. Then the instrument begins automated testing. A row of light emitting diodes(LEDs) behind the vials illuminate activating the vials fluorescent sensors. The instrument photodetectors then take the readings. A test cycle is completed every ten minutes. Microorganisms present in the positive blood cultures metabolize nutrients (^{14}C labelled glucose, aminiacids and alcohols) in the culture medium releasing CO_2 into the medium. A dye present in the sensor reacts with CO_2. This modulates the amount of light that is absorbed by a fluorescent material in the sensor. The instrument photodetector measures the level of fluorescence, which corresponds to the amount of CO_2 released by organisms.Then the measurement is interpreted by the system according to the pre-programmed positively parameters.[4,5].

The aim of this study was to study bacteremias by blood cultures and isolate bacteria using blood culture vials in BACTEC® 9050 system and study the antibiotic susceptibility of the isolates found in the positive blood culture vials.

MATERIALS AND METHODS

This study was conducted in the department of Medical Microbiology of The Gujarat Cancer and Research Institute, Ahmedabad. The study group consisted of patients suffering from haematological cancers and solid tumours.

Drawing a Blood Specimen:

Approximately 4-5 ml of blood was collected by aseptic procedures usually from the patients veins. Before collecting the blood the area was cleaned thoroughly with 70% ethanol and allowed to dry to prevent possible haemolysis of the blood.

Incubation of the blood cultures in the laboratory:

The blood samples were inoculated into blood culture bottles and were placed in the BACTEC 9050 blood culture instrument for 24 hrs soon after collection of the blood. Positive cultures were flagged by an indicator light, an audible alarm and are dsplayed on the LCD screen on the front of the instrument. Negative cultures were kept for seven days and even then if it shows no growth in the blood culture bottle is said to be sterile (BACTEC® 9050 System user's manual).

Culturing of Microorganisms:

A loopful blood sample from the positive blood culture was streaked on Blood agar, MacConkey agar, Choclate agar and Sabouraud agar slants.The culture plates were incubated at 37^0C for 24 hrs. Next day the colony characteristics, gram staining , biochemical test such as Oxidase, catalase, Coagulase, Urease, IMViC and TSI and antimicrobial sensitivity test were performed for the identification of the organisms.

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Result

1.Clinical diagnosis of the patient:

From the total 100 patients (TableNo:1) 55% were suffering from haematological cancers whereas 45% were victims of solid tumours.

Table No1: Clinical diagnosis of the patient

Clinical Diagnosis	Total	Percentage (%)
I.Haematological Cancers		
1.ALL	28	28
2.AML	14	14
3.CML	6	6
4.NHL	5	5
5.RMS	2	2
II.Solid Tumours		
1.Germ Cell Tumor	2	2
2.Breast Cancer	4	4
3.Febrile Neutorpia	5	5
4.Peripheral Line	4	4
5.Anorexia	1	1
6.Lymphoma	3	3
7.Neuroblastoma	1	1
8.8.Procytopenia	1	1
9.Chlorio-carcinom	1	1
10.M.mycloma	1	1
11.Aplastic Anaemia	2	2
12.Ewing,s Sarcoma	3	3
13.Oesophagus cancer	1	1
14.Oesteo Srcoma	1	1
15.Post Oesophagus	1	1
16.Fever Neutropia	4	4
17.Mucer Mycosis	1	1
18.Blastic crisis	1	1
19.Small Bowel fistula	1	1
20.Oral cancer	1	1
21.Enteric fever	2	2
22.Miscellenous	4	4
Total	100	

2.Gram Staining and Cultural Characteristics:

9% organism were Gram positve cocci whereas 13% organism were Gram negative bacilli.The morphological and cultural characteristics of the gram positve cocci or gram negative bacilli grown on macconkey agar medium and blood agar medium Table no:2

Table No 2 : Gram Staining and Cultural Characteristics

The Cultural Characteristics shown by the organism isolated on Macconkey and blood agar media							
Organism	Size	Shape	Colour	Margin	Elevation	Opacity	Consistency
Gram -ve bacilli							
E.coli	1mm	Circular	Pink	complete	Slightly raised	Opaque	Soft
Klebsiella sp	1mm	Round	Pink	complete	Slightly raised	Translucent	Mucoid
Salmonella typhi	0.5-1mm	Circular	Colour less	irregular	Flat	Opaque	Soft
Pseudomonas aeruginosa	1mm	Circular	Colour less	Entire	Flat	Opaque	Soft
Gram+ve Cocci							
Staphylococcus aureus	3-4 mm	Circular	Golden yellow	Entire	Convex	Opaque	Soft
Coagulase Negative Staphylococcus (CONS)	3-4 mm	Circular	Golden yellow	Entire	Convex	Opaque	Soft

3.Biochemical Test:-

The result of biochemical test such as catalase,coagulase,urease , IMVIiC, TSI are summarized in Table No 3

Table No 3: Biochemical Test

Sr.No	Organism	Oxidase	Urease	Indole	Methyl Red	Voges Proskaur	Citrate	Triple Sugar Iron Agar			
								Slope	Butt	H_2S	Gas
1	E.coli	-	-	+	+	-	-	Y	Y	-	+
2	Klebsiella sp	-	+	-	-	+	+	Y	Y	-	+
3	Salmonella typhi	-	-	-	+	-	-	R	R	Weak	-
4	Pseudomonas aeruginosa	+	D	-	-	-	+	R	R	-	-

Where : **R –** Red (Pink), Alkaline, **Y –** Yellow, Acid,**D –** Different strains give different results,

+ -- Positive Reaction, - -- Negative Reaction

4.Type of Organism Isolated

On the basis of biochemical test it was clear that the gram positive cocci isolated were 9% (9/100). *Staphylococcus aureus* were 5% (5/100). *Coagulase Negative Staphylococci* were 3% (3/100) and *Enterococci* were 1% (1/100). The Gram negative bacilli isolated were13% (13/100). Out of which *E.coli* were 5% (5/100), *Salmonella typhi* were 2% (2/100), *Klebsiella* were 3% (3/100).

Gram-positive bacteria, Gram-negative bacteria and fungal isolation rates were 403 (61.52%), 242 (36.94%) and 10 (1.52%), respectively. Among Gram-positive bacteria, 196 (29.92%) were determined to be *Coagulase-negative Staphylococci*. Among Gram-negative bacteria, 166 (25.34%) were the members of *Enterobacteriaceae* [4]. Out of 2921 blood culture specimens, 465 (16%) yielded growth. Out of these, 245 (53%) isolates were Gram positive, 209 (45%) Gram negatives while 11 (2%) were yeast. Among Gram positive isolates, 208 (85%) were *Staphylococcus spp*. Amongst Gram negative group, 115 (55%) isolates were members of *enterobacteriaceae* family and *E. coli* was the leading pathogen, while 94 (45%) were non-

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fermenters (NF). Among these, Acinetobacter spp. and Pseudomonas aeruginosa were common pathogens[6]. Positive cultures were obtained in 493 (20.5%) cases. Among culture positive isolates, Gram-negative bacteria accounted for 67.5% cases; most common being Pseudomonas spp. (16%) followed by Salmonella typhi and S. paratyphi A (14.2%). Of the pathogenic Gram-positive isolates, Staphylococcus aureus (8.3%) was the predominant isolate followed by Enterococcus faecalis (3.7%)[7]. The five most common isolates were Escherichia coli, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* [8]. Out of 728 cases, 346 (47.5%) were positive on blood culture. The most frequent offender was *Klebsiella spp.* (24.5%) followed by *Enterobacter spp.* (22.8%). There was an overall predominance of gram negative organisms. *Coagulase negative staphylococci* (CONS) were more frequently isolated (16.5%) than *Staphylococcus aureus* (14%)[9]. Positive blood cultures were obtained in 9.2% of cases of which Gram-positive bacteria accounted for 58.3% of cases with *staph. aureus* predominance; gram negative bacteria accounted for 40.2% with *enterobactereciea* predominence; and 1.5% were fungal isolates [10].

5. Antibiotic Sensitivity Test

Staphylococcus aureus showed 100% sensitivity towards Gentamycin, Amikacin, Netromycine, Amoxyclave. 80% sensitivity towards Ampicillin, Pefloxacin, Ciprofloxacin, Lincomycin, Clindamycin, Vancomycin and 20-60% toards the other tested antibiotics.*Coagulase Negative Staphylococcus* (CONS) showed 100% sensitivity towards Ampicillin and Vancomycin. 33.3 to 66.6 % sensitivity towards the others. It also showed 100% resistance towards Tetracycline, Ceptoxime, Ofloxacin. *Enterococci* showed 100% sensitivity towards Ampicillin, Ciprofloxacin, Tetracycline, Bacitracin, Vancomycin, Ceftizoxamine and Amoxyclave Whereas 100% resistance towards Gentamycin, Amikacin, Pefloxacin, Lincomycin, Clindamycin, Cephazoline, Cefoxitine, Cefoperazone, Netromycine, Cephaloridine, Ceftazidine, Ceptoxime, Ofloxacin

Escherichia coli was 100% resistant to Ceptoxime and 80% resistant to all the other antibiotics tested. *Salmonella typhi* was found tob be 100% sensitive towards Ciprofloxacin only. The organism was 100% resistant towards Cephazoline, Cefoxitine, Ceftizoxamine, Cefoperazone, Netromycine, Cephaloridine, Ceftazidine, Ofloxacin, Amoxyclave and 50% sensitivity towards the rest. *Klebsiella* showed 100% sensitivity towards Amikacin only. It showed 100% resistance towards Lincomycin, Clindamycin, Vancomycin, Cephazoline, Cefoxitine, Ceftizoxamine, Amoxyclave, Cephaloridine and 33.3 to 66.6% sensitivity towards the rest. *Pseudomonas aeruginosa* was 100% sensitive for Pefloxacin , Cefoperazone. 100 % resistant towards Tetracycline,Lincomycin, Clindamycin, Vancomycin, Cephazoline, Cefoxitine and Cephaloridine whereas 33.3 to 66.6 % sensitive towards the other antibiotics.

Antimicrobial susceptibility of staphylococci revealed that 100% isolates were susceptible to vancomycin and linezolid. The organisms of family *enterobacteriaceae* revealed better susceptibility to amikacin (68.7%) and imipenem (64.3%). The NF group showed better in vitro susceptibility to tazobactam/piperacillin (65%). Vancomycin and linezolid in case of Gram positive and amikacin and tazobactam/piperacillin against Gram negative organisms revealed better in vitro efficacy [6]. Maximum Gram-negative isolates were sensitive to cefoperazone-sulbactam combination (81%). Vancomycin sensitivity was reported in 100% *Staph. aureus* and 83.3% *Enterococcus faecalis*.[7].The most sensitive drugs for Gram-positive isolates were vancomycin, teicoplanin, daptomycin, linezolid, and tigecycline and for Gram-negative were carbapenems, colistin, aminoglycosides, and tigecycline [10].

Table No4 :-Antibiotic Sensitivity of Gram Positive Bacteria

Sr. No	Antibiotic Group	***Staphylococcus aureus* (n=5)**		***Coagulase Negative Staphylococcus* (n=3)**		***Enterococci* (n=5)**	
		%Sensi tive	%Resistant	%Sensitive	%Resis tant	%Sensi tive	%Resistant
I	PENICILLIN						
	1.Ampicillin	80	20	100	--	100	--
II	AMINOGLYCOSIDES						
	1.Gentamycin (GM)	100	--	66.6	33.3	--	100
	2.Amikacin (AK)	100	--	66.6	33.3	--	100
III	QUINALONES						
	1.Pefloxacin (PF)	80	20	66.6	33.3	--	100
	2.Ciprofloxacin (CP)	80	20	33.3	66.6	100	--
IV	VANCOMYCIN						
	1.Tetracycline (TE)	60	40	--	100	100	--
	2.Bacitracin (BA)	40	60	33.3	66.6	100	--
	3.Lincomycin(LM)	80	20	66.6	33.3	--	100
	4.Clindamycin (CD)	80	20	33.3	66.6	--	100
	5.Vancomycin (Va)	80	20	100	--	100	--
V	CEPHALOSPORINE						

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	Ist Generation						
	1.Cephazoline (CZ)	60	40	66.6	33.3	--	100
	IInd Generation						
	1.Cefoxitine(CA)	60	40	66.6	33.3	--	100
	IIIrd Generation						
	1.Ceftizoxamine (Ci)	60	40	33.3	66.6	100	--
	2.Cefoperazone (Cs)	40	60	66.6	33.3	--	100
VI	MISCELLENOUS						
	1.Netromycine (Nt)	100	--	66.6	33.3	--	100
	2.Amoxyclave (AC)	100	--	66.6	33.3	100	--
	3.Cephaloridine (Cr)	60	40	66.6	33.3	--	100
	4.Ceftazidine (Ca0	40	60	33.3	66.6	--	100
	5.Ceptoxime (Cf)	20	80	--	100	--	100
	6. Ofloxacin (OF)	40	60	--	100	--	100

Table No 5 :-Antibiotic Sensitivity of Gram Negativee Bacteria

Sr. No	Antibiotic Group	*Escherichia coli* (n=5)		*Salmonella typhi* (n=2)		*klebsiella* (n=3)		*Pseudomonas* (n=3)	
		% Sensitive	%Resistant	% Sensitive	%Resistant	%Sensitive	%Resistant	%Sensitive	%Resistant
I	PENICILLIN								
	1.Ampicillin	20	80	50	50	33.3	66.6	33.3	66.6
II	AMINOGLYCOSIDES								
	1.Gentamycin (GM)	20	80	50	50	33.3	66.6	66.6	33.3
	2.Amikacin (AK)	80	20	50	50	100	--	33.3	66.6
III	QUINALONES								
	1.Pefloxacin (PF)	20	80	50	50	66.6	33.3	100	--
	2.Ciprofloxacin (CP)	20	80	100	--	66.6	33.3	66.6	33.3
IV	VANCOMYCIN								
	1.Tetracycline (TE)	20	80	50	50	33.3	66.6	--	100
	2.Bacitracin (BA)	20	80	50	50	33.3	66.6	66.6	33.3
	3.Lincomycin(LM)	20	80	50	50	--	100	--	100
	4.Clindamycin (CD)	--	100	50	50	--	100	--	100
	5.Vancomycin (Va)	--	100	50	50	--	100	--	100
V	CEPHALOSPORINE								
	Ist Generation								
	1.Cephazoline (CZ)	20	80	--	100	--	100	--	100
	IInd Generation								
	1.Cefoxitin(CA)	20	80	--	100	--	100	--	100
	IIIrd Generation								
	1.Ceftizoxamine (Ci)	20	80	--	100	--	100	66.6	33.3
	2.Cefoperazone (Cs)	20	80	--	100	33.3	66.6	100	--
VI	MISCELLENOUS								
	1.Netromycine (Nt)	20	80	--	100	33.3	66.6	66.6	33.3
	2.Amoxyclave (AC)	20	80	--	100	--	100	33.3	66.6
	3.Cephaloridine (Cr)	20	80	--	100	--	100	--	100
	4.Ceftazidine (Ca0	20	80	--	100	66.6	33.3	66.6	33.3
	5.Ceptoxime (Cf)	--	100	50	50	66.6	33.3	33.3	66.6
	6. Ofloxacin (OF)	20	80	--	100	33.3	66.6	--	100

Conclusion

The blood culture were advised for the pyrexia or pyrexia of unknown origin,for the various underlying cancers or solid tumours.Because of gross immunosuppression in the haematological malagnancies as the total WBC count goes down and the patient becomes prone to opportunistic infection. It is seen that the blood culture were advised in this than when compared to solid tumour.

The bacteremia due to both gram postive and gram negative bacteria were 22%. It is clearly seen that the bacteremia due to gram negative bacteria were more than the gram positive bacteria.Thus it is seen that there is a changing pattern of the emerging gram negative bacterial infection causing bacteremia.Since all the gram negative bacilli can show a resistance ranging from 40-100% for various antibiotics, it is essential to take preventive measures for

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the hospital prevalent gram negative bacteria to gain access into the critically ill patients also that these bacteria are multidrug resistant a proper antibiotic policy will help to save the development of bacterial resistance to the antibiotics.

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