



Bacterial profile of Blood Stream infections and Antibiotic resistance pattern of Isolates

?????????

Abstract

Blood samples from 2542 clinically diagnosed cases of septicemia were processed. Out of these 946 (76.55%) were from pediatric deptt and rest from other deptts. Growth was obtained in 509(20.02%) cases. Candida spp were isolated from 23 (4.57) cases Out of 486 bacterial isolates 52.67 % were gram positive bacteria whereas 47.33% were gram negative bacilli. Staph aureus 133 (27.37%) was the predominant organisms followed by CONS 98 (20.1%). Amongst gram negative organisms Enterobacter 69 (14.19 %) was the most predominant followed by Esch Coli 45 (9.27 %) Pseudomonas 37 (7.62 %) and Acinetobacter spp 34 (6.69 %). Amongst gram positive organisms maximum resistance was seen with Ampicillin (74.61%) and Erythromycin (69.67 %). Most of the gram negative bacilli were MDR (71%). Maximum resistance was observed with Ampicillin (86.1%) cephalixin (68.07%) and piperacillin (57.71%). Most successful drugs were Amikacin, gentamicin and cefotaxime. 34.35% of the isolates were ESBL producers.

Keywords

Blood Stream Infections, Antibiotic Resistance pattern, ESBL.

Introduction

Microorganisms present in the circulating blood whether continuously intermittently are threat to every organ in the body. Approximately 200,000 cases of bacteraemia and fungemia occur annually with mortality rates ranging from 20-50 % (1). Therefore early diagnosis and appropriate treatment of these infections can make the difference between life and death.

Since early 1950s, there is striking increase in incidence of bacteraemia caused by members of Enterobacteriaceae and other gram negative bacilli (2-5) Escheriachia coli which was reported to be common in the past 2.4 is being replaced by other multi drug resistant bacteria like Klebsiella, Enterobacter,

Salmonella, Citrobacter, Pseudomonas, Acinetobacter etc (5,6). The infection caused by MDR organism are more likely to prolong the hospital stay, increase the risk of death and require treatment with more expensive antibiotics.

The retrospective study was therefore undertaken to determine the bacterial flora of the blood stream infections and their antibiotic resistance pattern.

Material and Methods

A total of 2542 samples received in the Bacteriological lab in the deptt of Microbiology from various hospitals attached to Govt Medical College, Amritsar from July 2003- march 2006 were analyzed from the record and the relevant findings were noted

From the

Correspondence to :



Blood Culture: Venous blood 5ml from adults and 2ml from children was obtained aseptically and inoculated into brain heart infusion broth . In suspected cases of enteric fever, bile broth was used and incubated at 370 C for 24 hours . Blind Subculture was done on to fresh 5% sheep blood agar and MacConkey's agar. A negative result was followed up by examining the broth daily and doing a final subculture at the end of 7th day or at the appearance of turbidity , gas production or the presence of microcolonies over the clot which ever was earlier. Where ever necessary second blood culture was taken. In SABE three consecutive blood samples were taken. Organisms were identified by cultural characters, Morphology and standard biochemical tests(7). Antibiotic susceptibility testing was performed by the

Kirby Bauer disc diffusion method as per NCCLS recommendations (8) ESBL production was detected by double disc potentiation technique (9).

Results

Out of 2542 cases studied 2292 (89.97 %) were indoor patients while 250 (10.03 %) were from various out patient departments . Maximum blood samples 1946 (76.55 %) were received from pediatric ward with history of Septicemia and 43 (1.9 %) from Pediatric OPD with PUO. 37 (1.46 %) from surgery ward with H/O wound infection and PUO. 516 (20.29 %) were from various other departments (table 1).

Out of 2542 samples growth was obtained in 509 samples (20.02 %) . 486 (19.12 %) showed bacterial

	Hospitalized Patients	% age	Positive Culture	% age	Out patients	%age	Positive Culture	% age
Pediatrics	1946	76.55	439	86.25	43	1.68	8	1.58
Medicine	264	10.38	31	06.09	146	5.74	16	3.149
Gynecology	11	0.44	4	0.78	0	0	0	0
Surgery	37	1.46	5	0.98	5	0.19	0	0
Others *	34	1.36	3	0.59	56	2.2	3	0.59

Organisms	No of Isolates	%age
Gram Positive bacteria		
Staphylococcus aureus	133	27.37
Coagulase negative Staphylococcus	98	20.16
Enterococci	15	03.08
Streptococcus pyogenes	6	01.24
Others *	4	00.83
Gram Negative bacteria		
Enterobacter	69	14.19
Escherichia coli	45	09.27
Pseudomonas aeruginosa	37	07.62
Acinetobacter Spp	34	06.99
Klebsiella Pneumoniae	28	05.76
Others**	17	03.49



Antibiotic Resistance pattern of Gram positive bacteria*

Organisms	A 10µg	CT 10µg	CS 30µg	CF 1µg	AK 30µg	G 10µg	PC 30µg	GF 5µg	E 15µg	LZ 30µg
Staph Pyogenes (n=133)	92(69.17)	48(36.10)	64(48.13)	57(42.86)	20(15.04)	49(36.85)	72(54.14)	61(45.87)	85(63.90)	117(87.97)
CONS (n=98)	78(79.59)	32(32.65)	53(54.08)	40(40.82)	14(14.29)	46(46.94)	56(57.14)	62(63.27)	68(69.39)	80(81.63)
Enterococcus (n=15)	14(93.33)	10(66.67)	13(86.67)	10(66.67)	9(60)	11(73.33)	13(86.67)	8(53.33)	13(86.67)	13(86.67)
Stepto Pyogenes (n=6)	5(83.33)	1(16.67)	3(50)	2(33.33)	1(16.67)	3(50)	2(33.33)	3(50)	5(83.33)	0
SteptoViridans (n=3)	1(33.33)	1(33.33)	1(33.33)	1(33.33)	1(33.33)	1(33.33)	1(33.33)	2(66.67)	2(66.67)	2(66.67)
Micro cocci (n=1)	1(100)	0	1(100)	1(100)	0	1(100)	0	-	1(100)	1(100)

Antibiotic Resistance Pattern of gram Negative Bacteria*

Organisms	A 10µg	CT 10µg	CS 30µg	CF 1µg	AK 30µg	G 10µg	PC 30µg	GF 5µg
nterobacter (=69)	56(81.16)	24(34.78)	43(62.39)	44(63.76)	19(27.54)	23(33.33)	41(59.42)	27(39.13)
sh Coli (=45)	40(88.89)	21(46.67)	32(71.11)	26(57.78)	9(20)	13(28.89)	33(73.33)	18(40)
seudomonas (=37)	36(97.30)	14(37.84)	31(83.78)	10(27.03)	11(29.73)	14(37.84)	21(56.76)	13(35.14)
cinetobacter (=34)	31(91.18)	12(35.29)	24(70.59)	7(20.59)	5(14.71)	11(32.75)	14(41.18)	28(82.35)
lebsiella n=38)	24(85.71)	8(28.57)	20(70.43)	4(14.29)	7(25)	6(21.43)	18(64.29)	13(46.43)
itrobacter (=5)	7(100)	0(0)	5(71.43)	1(14.29)	0(0)	0(0)	2(28.57)	0(0)
almonella ypi (=5)	2(40)	0(0)	1(20)	0(0)	0(0)	1(20)	3(60)	0(0)
roteus irabilis (=2)	1(50)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
lkaligenes ecalis (=2)	2(100)	0	1(50)	0	0	0	1(50)	1(50)
paratyphi (=1)	1(100)	0	1(100)	0	0	0	-	-



growth and *Candida Spp* was isolated from 23 (4.51%) of the cases. Out of the 486 isolates 256 (52.67%) were gram positive bacteria while 230 (47.33%) were gram negative bacilli. *Staph aureus* was the predominant organism 27.37% followed by CONS 20.16%. Distribution of gram negative organism is shown in the table (2).

The antimicrobial resistance pattern of gram positive and gram negative organisms is shown in the table 3&4 respectively. ESBL production was observed in 34.35% of the isolates.

Table 1

Positive blood cultures in hospitalized patients & outpatients

Table 2

Distribution of Bacterial Pathogens

- * *Streptococcus viridans, micrococcus*.
- ** *Citrobacter freundii, S. typhi, Alkaligenes faecalis, proteus mirabilis, S. paratyphi*

Table 3

*Antibiotic Resistance pattern of Gram positive bacteria**

Table 4

*Antibiotic Resistance Pattern of gram Negative Bacteria**

*- Concentration per disc

A-Ampicillin CT-Cefotaxime CS-Cephalexin

CF-Ciprofloxacin AK-Amikacin

G-Gentamicin PC-Piperacillin GF-Gatiflox

Discussion

In the present study blood culture positively was seen in 509 (20.02%) cases whereas Khanall0 Sharma11 and Roy et al 12 has reported 44%, 33.9% and 16.4% respectively. In India, variation might be due to the fact that most of the patients are given the antibiotics before they come to the tertiary care hospital & other reason is that in most of the cases self medication is very common as the medicines are available at the counter. In the concurrent study the incidence of gram positive organisms was 52.67% while 47.33% isolates were gram negative bacilli. It is in accordance with the study of other workers 11. Khanal et al10 have reported incidence of *Streptococcus* & *Staphylococcus* to be 25% and gram negative bacilli 15%. But in most of the studies gram negative organisms have taken over the gram positive

organisms especially in the hospital settings 2,5 In our study *S.aureus* was isolated in 27.37% of cases & CONS in 20.16% of the cases. However, Roy12 et al have reported CONS 16.5% and *S.aureus* 14% in neonatal septicemia. 9% incidence of both CONS & *S.aureus* have been reported by Surinder et al13. In the present study *Candida* isolates were seen in 23 (4.73%) of the cases. This is consistent with the study of Narain et al14 where as in other studies the incidence is much higher15. Amongst the gram negative organisms *Enterobacter* isolation was 14.19% whereas Roy et al2 reported *Enterobacter* in 22.9% of cases. *Enterobacter spp* are emerging as an important nosocomial pathogen. Similar concern regarding *Enterobacter* sepsis was expressed in a report from Karachi in 199616.

Esch. coli was isolated from 9.27% of the cases. While surinder et al 12 has reported it to be 14.4%. *Pseudomonas*, one of the important nonfermenter was isolated in 7.62% of cases where as it has been reported to be 5.9% in the other studies13. 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 & treatment purposes. *Acinetobacter* was isolated in 6.29% of the cases which has been reported earlier from our centre as an emerging cause of late onset septicemia in 12.13% of the cases17. *Klebsiella pneumoniae* isolation was 5.76% which is in contrast to other studies who have reported it to be 25.8% 13. *Citrobacter* was isolated in 3.04% in consistent with the study of Surinder K et al13 who has reported it to be 4.6%. Difference in the incidence in different institutes is because of different setup and the type of antibiotics used in those hospitals.

Amongst the gram positive organisms maximum resistance was seen with Ampicillin 74.61% and Erythromycin 69.67%. An increased Ampicillin resistance of 64%, 87%, was also reported by Guha et al (1978) 4 and Karki et al 18 respectively in their studies.

Enterococci Sp displayed markedly high level of drug resistance to most of the commonly used antibiotics with 93.33% resistance to Ampicillin, 86.67% resistance was noted to Erythromycin, linezolid, piperacillin &



Cephalexin. In another study 50-60 % isolates were resistant to all the antibiotics tested 13.

Most of the gram negative bacilli (71%) were multi drug resistant. Other workers also have reported majority of gram negative isolates in their study as multidrug resistant. 11 Maximum resistance was seen against Ampicillin (89.96%), Cephalexin (68.07%) and piperacillin (57.71%). It is consistent with the findings of Surinder et al 13 . 27.54%, 20%, 29.73%, 14.71% & 25% resistance to Amikacin was shown by Enterobacter sps , Esch. Coli , Pseudomonas , Acinetobacter & klebsiella sps respectively 34.35 % of the isolates were ESBL produces while other workers have reported it to be 6.67% 20 & 18 % 21 . The much higher incidence of ESBL production in our institute might be because of injudicious use of antibiotic.

So it is concluded that Septicemia is an important cause of morbidity and mortality . The causative agents of septicemia are acquired in hospital .The retrospective study conducted showed both gram positive and gram negative bacteria were responsible for blood stream infections . Most of the strains were multi drug resistant. To bring down the incidence of nosocomial infections rational and judicious use of antibiotics is essential according to the antibiotic resistance pattern of that institution . Moreover, there is need for strict aseptic precautions on the part of health care workers.

References

1. Forbes BA, Sahm DF and Weissfeld AS .Bailey and Scott's Diagnostic Microbiology : A textbook for isolation and identification of pathogenic microorganisms , 11th ed., the Mosby Company , St. Louis 2002 : 378 -422.
2. Karpuch J , Azzizi I and Beer S. Bacteracemia among hospitalized children- a four year prospective study. *J Infect* (1984) 9: 139
3. Winchester PD, Todd Jk and Rose MH , Bacteraemia in Hospital children , *American J Dis Child* (1977)131 : 753.
4. Guha DK, Jaspal D, Das KMS , Guha RA , Khatri RL and Sri Kumar R , Outcome of neonatal septicemia : A clinical and bacteriological profile. *Indian Pediatr*(1978) 15: 423.
5. Chugh K, Aggarwal DB , Kaul VK and Aryase . Bacteriological profiles of neonatal septicemia . *Indian J Pediatr*. (1988) 55:961.
6. Chatuvedi P, Agarwal M and Narang P Analysis of blood culture isolates from neonates of a rural hospital . *Indian Pediatr*.
7. Colle JG Miles RS, Watt b. Test for identification of bacteria In : Mackie and McCartney Practical Medical Microbiology. Collee JG, Fraser AG , Marimon BP , Simmon A; 14th Edn , *Churchill Livingstone, New York* 1996 ; 2:131-149.
8. National Committee for Clinical Laboratory Standard . Performance standards for antimicrobial disc susceptibility tests: Approved standard , 7th ed Villanova Pa . national Committee for Clinical Laboratory Standards 2000 ; 20 (1) : M2-A7.
9. Jarlier V, Nicolas MH, Fournier G , Phillipon A. ESBL's conferring transferable resistance to newer beta -lactam agents in Enterobacteriaceae, Hospital prevalence and susceptibility pattern. *Rev Infect Dis* 1988 ; 10 ;867-78.
10. Khanal B, Harish BN, Sethuraman KR , Srinivasan S. Infective endocarditis : Report of prospective study in an Indian hospital *Trop Doct* 2002 ; 32:83-5 .
11. Sharma M, Goel N, Chaudhary U Aggarwal R, Arora DR. Bacteraemia in children. *Indian J Pediatr* 2002; 69:1029-32
12. Roy I, Jain A , Kumar M, Agarwal SK: Bacteriology of Neonatal Septicaemia in a Tertiary Care Hospital of Northern India. *Indian Journal of Medical Microbiology*, (2002) 20 (3) : 156 -159.
13. Kumar Surinder, Rizvi Meher, Vidhani Shalini , Sharma VK. Changing face of Septicaemia and increasing drug resistance in blood isolates. *Indian J Pathol Microbial* 2004, Vol 47, No 3 : 441-446
14. Narain S, Shastri JS, Mathur M, Mehta PR. Neonatal Systemic candidiasis in a tertiary care centre. *Indian Journal of Medical Microbiology*, (2003) 21(1):56-58
15. Chakrabarti A, Chander J , Kasturi P , Panigrahi D, Candidaemia: a 10 -year study in an Indian teaching hospital. *Mycoses*1992;35:47-51.
16. Roy A Maiti PK , Adhya S ,Bhattacharya A , Chakraborty G, Ghosh E, et al . neonatal candidemia . *Indian J Padiatr* 1993 ; 60; 799-801 .
17. Arora U, Jaitwani J . Acinetobacter Spp - An Emerging Pathogen in Neonatal Septicemia in Amritsar. *Indian Journal of Medical Microbiology*, (2003)21(1) : 56-58.
18. Bhutta ZA. Enterobacter sepsis in the new born- a growing problem in Karachi. *Hospital Infect* 1996 ; 34 : 211-216.
19. Karki BM, Parija SC: Analysis of blood culture isolates from hospitalized neonates in Nepal. *Southeastv Asian J Trop. Med. Public Health* 1999;30(3); 546-548 .
20. Aamer Ali Shah, Fariha Hasan , Safia Ahmed & Abdul Hameed : Prevalence of extended -Spectrum β -lactamases in nosocomial and outpatients (Ambulatory). *Pak J Med Sci*. 2003 , 19(3) 187-191 .
21. Jarlier V Nicolas MH, Fournier G & Phillippon A : Extended - spectrum β - lactamases conferring transferable resistance to newer β lactam agents in Enterobacteriaceae : hospital prevalence and susceptibility patterns. *Rev Infect Dis* 1988: 10:867 -878.