Research Article

Study of causative agent & their susceptibility pattern in sepsis in young infants below 3 months of age

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Abstract

Introduction: Pathogens causing blood stream infections and their antibiotic susceptibility patterns constantly change over time & it is essential to monitor the epidemiology of infections to design appropriate antibiotic policy. **Methodology:** Blood culture reports of Children below 3 months of age admitted to our hospital over a period of 4 years were analysed to find the causative agents of sepsis & their antibiotic susceptibilities. All data were collected in validated preformatted proforma sheet & analysed using appropriate statistical methods. **Results**: Among 1401 blood cultures, culture positive growth was observed in 226 cases (16.1 %). In our study, Klebsiella pneumoniae was the commonest isolated in 23.4 % of blood cultures. Acinetobacter baumanii was the next commonest organism isolated in 13.7 % followed by MRSA growth in 11.9 %, MSSA in 4.8 %, E.coli in 8.8 %, Enterococcus faecalis in 7.5 %, B.cepacia in 6.6 %, P. aeruginosa in 5.3% & Enterobacter in 4.4 % of blood cultures. About 49% of K. pneumoniae were susceptible to Carbapenam & 60% of E.coli were susceptible to Amikacin & Tobramycin. All Enterobacter were sensitive to Imipenam, Meropenam, Ertapenam & Amikacin & 77% of A.baumanii were sensitive to Carbapenam. Yeasts were isolated in 8.4 %, the commonest being Candida tropicalis. **Conclusion:** It is essential to closely monitor the bacterial flora and their antibiotic sensitivity pattern to evolve rational antibiotic policy which is suitable for each unit. Guidelines on the reduction of emergence of drug resistance must be provided and instituted within the units.

Key words: Sepsis, Antibiotic Sensitivity, Blood stream infections

Introduction

Sepsis is an important issue with a high morbidity and mortality rate in spite of new advances in antibiotic therapy. Identifying the causative agents and their antibiotic sensitivity helps the physician to choose the most appropriate antibiotic therapy. Pathogens causing infections and their antibiotic susceptibility patterns may change over time [1,2,3] & differ between countries [4]. Blood cultures remain the mainstay of investigation of potential sepsis in infants, despite recent advances in the molecular diagnosis of bacterial and fungal sepsis [5]. But in real clinical situations, laboratory report on culture and antibiotic sensitivity may not be available till 48 hours. Moreover inappropriate use of antibiotics has been implicated in the development of multi drug resistant bacteria in hospitals [6]. It is therefore essential to monitor the epidemiology of infections of the locality & design appropriate antibiotic policy.

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Objectives

This study was aimed to find out the etiological agent and antibiotic susceptibilities in infants with culture positive sepsis.

Methodology

Study Site and Patient Selection: The study was conducted at Sunrise children's hospital, Hyderabad, India and Department of clinical microbiology, Global Hospitals, Hyderabad, India from December 2010 to March 2015. Children less than 3 months of age were included in the study. The study protocol was reviewed and approved by the institutional ethics committee.

Microbiologic Methods: Single blood cultures were obtained from young infants with suspected sepsis before initiating antimicrobial therapy. Trained technical staff at the study centre collected blood using sterile technique and inoculated it into BACTEC PEDS PLUS/F medium. Samples were transported to the laboratory in not more than one hour and processed. Blood culture bottles were

loaded onto the BACTEC 9050 and BACTEC 9120 system (Becton, Dickinson and Company, Sparks, Maryland USA) [7,8,9]. Positive blood cultures as flagged off the instrument were subjected to Gram stained smear and explanted on Columbia blood agar with 5% sheep blood, MacConkey agar, chocolate agar and Sabouraud Dextrose agar. Blood isolates were identified using conventional biochemical tests [10]. Antimicrobial susceptibility testing for bacterial isolates was carried out using disc-diffusion technique and interpreted by British society for antimicrobial chemotherapy (BSAC) guidelines incorporating changes as per the version used [11,12]. All isolates from blood were correlated with the clinical information on the young infants.

Statistics: Descriptive statistical measures were used.

Results

Table 1: Year wise Culture Positivity Rate, Polymicrobial Growth & Contamination Rate

	2010	2011	2012	2013	2014	2015	Total
Total	54	22	297	544	398	86	1401
Positivity rate	12(22.2%)	4(18.1%)	63(21.2%)	84(15.4%)	56(14%)	7(8.1%)	226(16.1%)
Polymicrobial	Nil	Nil	Nil	2	1	1	4
Contamination	Nil	1	15(5%)	20(3.6%)	26(6.5%)	3(3.4%)	65(4.6%)

A total of 1401 blood cultures were sent during the study period out of which culture positive growth was observed in 226 cases (16.1 %). Polymicrobial growth was observed in 4 cases & the contamination rate was 4.6 % in our study.

Table 2: Year wise Causative Agents of Sepsis - Bacterial

Organisms :	2010	2011	2012	2013	2014	2015	TOTAL
Klebsiella pneumoniae	2 (16.6%)	Nil	17(26.9%)	17(20.2%)	14(25%)	3(42.8%)	53(23.4%)
Carbapenem Resistant K. pneumoniae	Nil	Nil	6	11	7	2	26/53(49%)
ESBL K. pneumoniae	2	Nil	10	3	5	1	21
K. oxytoca	Nil	Nil	Nil	1	Nil	Nil	1
E. coli	3(25%)	Nil	8(12.6%)	5(5.9%)	4 (7.1%)	Nil	20(8.8%)
Carbapenem Resistant E. coli	1	Nil	2	2	2	Nil	7(35%)
Ertapenem – Resistant	Nil	Nil	5	1	Nil	Nil	6
Enterobacter species	Nil	Nil	9(14.2%)	1(1.1%)	Nil	Nil	10 (4.4%)
P. aeruginosa	1(8.3%)	Nil	5(7.9%)	3(3.5%)	3(5.3%)	Nil	12 (5.3%)
Carbapenem Resistant P. aeruginosa	1	Nil	Nil	Nil	Nil	Nil	1
A. baumanii	1(8.3%)	Nil	6(9.5 %)	17(20.2%)	7(12.5%)	Nil	31(13.7%)
Carbapenem Resistant A. baumanii	1	Nil	6	14	4	Nil	25/31(80.6%)
B. cepacia	Nil	Nil	8(12.6%)	4(4.7%)	2(3.5%)	1(14.2%)	15(6.6%)
MSSA	2	1	1	4	3	Nil	11
MRSA	1	1	4	10	10	1	27
MLSB type resistance	1	2	2	4	5	Nil	14
Enterococcus faecalis	2	Nil	1	10	4	Nil	17
HLAR Type	1	Nil	Nil	6	2	Nil	9

resistance							
E. faecium	1	Nil	Nil	1	2	2	6
VRE Van A phenotype	Nil	Nil	Nil	1	Nil	2	3
Corynebacterium	Nil	Nil	1	Nil	Nil	Nil	1
S. pneumomniae	Nil	Nil	1	1	Nil	Nil	2
H. parainfluenzae/ influenza	Nil	Nil	Nil	Nil	1	Nil	1

In our study, Klebsiella pneumoniae was the most common organism isolated in 23.4 % of blood cultures. Acinetobacter baumanii was the next commonest organism isolated in 13.7 % of blood cultures. We observed MRSA growth in 11.9 % & MSSA in 4.8 % of blood cultures. E.coli was isolated in 8.8 % of blood cultures. We observed that Enterococcus faecalis was isolated in 7.5 %, B.cepacia in 6.6 %, P. aeruginosa in 5.3% & Enterobacter in 4.4 % of blood cultures.

Table 3 – Yearwise Causative Agents of Sepsis - Yeasts

	2010	**2011**	**2012**	**2013**	**2014**	**2015**	**TOTAL**
YEASTS	**Nil**	**2 (9%)**	**2 (3.1%)**	**10 (11.9%)**	**4 (7.1%)**	**1 (14.2%)**	**19 (8.4%)**
Candida tropicalis	Nil	2	Nil	1	1	1	5
Candida albicans	Nil	Nil	Nil	1	1	Nil	2
Candida parapsilosis	Nil	Nil	Nil	2	1	Nil	3
Candida glabrata	Nil	Nil	Nil	2	1	Nil	3
Candida famata	Nil	Nil	1	Nil	Nil	Nil	1
Candida krusei	Nil	Nil	Nil	1	Nil	Nil	1
Candida guillermondii	Nil	Nil	Nil	3	Nil	Nil	3
Rhodotorula rubra	Nil	Nil	1	Nil	Nil	Nil	1
OTHER ORGANISMS							
E. meningoseptica	Nil	Nil	Nil	1	1	Nil	2
NF GNB	Nil	Nil	1	Nil	Nil	Nil	1
S. paratyphi A	Nil	Nil	Nil	1	Nil	Nil	1

Yeasts were isolated in 8.4 % of blood cultures. The most common yeast isolated was Candida tropicalis.

Table 4– Antimicrobial Susceptibility Pattern of Common Bacterial Agents

	No	**Sensitivity pattern**
P. aeruginosa	12	Sensitive TO ALL
B. cepacia	15	SensitiveTO ALL
S. aureus	38	MRSA -26, MSSA-9, MLSBC -8, MLSBI-3, CIP -3, G-13, AK-22, CD-18,LINCO-21
E. faecalis	17	VRE VAN A-1, HLAR-8 IM-8, AMP-5
E. faecium	6	VRE VAN A-1, HLAR-2 IM -1, VRE VAN B-2
Corynebacterium	1	Sensitive TO VA
S. pneumoniae	2	SensitiveTO ALL
H. parainfluenzae	1	Sensitive to CEFOTAXIME, CEFTRIAXONE, MEROPENEM
E. meningoseptica	2	Sensitive to VA
NF GNB	1	Sensitive to COL
S. paratyphiA	1	Sensitive to TO ALL

Table 5– Antimicrobial Susceptibility Pattern of Common Bacterial Agents

		ESBL	AMP	CR	MBL +	IM	MEM	ETP	QL	G	AK	NT	TB	TZP	TIM	TGC
K. pneumoniae	53	19 35.8 %	0	26 49 %	24 45.2 %	25 47.1 %	25 47.1 %	25 47.1 %	11 20.7 %	18 33.9 %	25 47.1 %	19 35.8 %	19 35.8 %	22 41.5 %	12 41.5 %	18 33.9 %
K.	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	0
E. coli	20	9 45 %	0	6 30 %	6 30 %	11 55 %	11 55 %	5 25 %	3 15 %	9 45 %	12 60 %	11 55 %	12 60 %	5 25 %	4 20 %	1 5 %
Enterobac	10	1 10 %	8 80 %	0	0	10 100 %	10 100 %	10 100 %	9 90 %	8 80 %	10 100 %	8 80 %	9 90 %	9 90 %	1 10 %	7 70 %
A. baumanii	31	0	0	24 77 %	22 70.9 %	6 19.3 %	6 19.3 %	0	4 12.9 %	10 32.2 %	11 35.4 %	13 41.9 %	12 38.7 %	2 6.4 %	0	3 9.6 %

About 49% of K. pneumoniae were susceptible to Carbapenam; 60% of E.coli were susceptible to Amikacin & Tobramycin. All Enterobacter were sensitive to Imipenam, Meropenam, Ertapenam & Amikacin; 77% of A.baumanii were sensitive to Carbapenam.

Discussion

In our study the culture positivity rate was 16.1 %. Contamination rate was 4.6 %. Klebsiella pneumoniae was the most common organism isolated in 23.4 % of blood cultures. Other authors have observed similar results in their study with Klebsilla as most common organism [4, 13-21]

We noted that A. baumanii was the next commonest organism isolated in 13.7 % of blood cultures. Abdullah Al-Taiar et al noted that Non-fermenting Gram-negative bacteria like Acinetobacter & Pseudomonas are emerging as important pathogens in Asian neonatal care units[19]. We observed MRSA growth in 11.9 % & MSSA in 4.8 % of blood cultures. Other authors have observed similar results in their study with Staphylococcus aureus as most common organism [22-26].

In our study E.coli was isolated in 8.8 % of blood cultures. R Yousefimashouf et al [27] in his study noted that the most common species of neonatal sepsis was Escherichia coli. We observed that Enterococcus faecalis was isolated in 7.5 %, B. cepacia 6.6 %, P. aeruginosa in 5.3% & Enterobacter in 4.4 % of blood cultures.

Susceptibility: In our study about 47.1% Klebsiella pneumoniae were susceptible to Imipenam as compared to 70% in a study by Zaki M et al [28]. Other studies have also noted that imipenem remained strongly active against Klebsiella [29-31]. We noted that about 41.5% Klebisiella were sensitive to piperacillin-tazobactam as compared to 81.4% by Ariffin N et al[29] & Al Jarousha AM et al[30]. In our study about 35.8% were extended spectrum beta-lactamase producer type; Zaki M et al found 42% of Klebsiellas to be ESBL producers [28]. Tsering DC et al & Zakariya BP et al noted that 48-58% of Klebsiella as ESBL-producing strains [32,33].

We observed that 33.9% Klebsiella pneumoniae were sensitive to Gentamicin. The sensitivity pattern to gentamicin was observed to be 21% by Akindele JA et al [34], 31.2% by Kirsty Le Doare et al [13], around 50% by Y. Bell et al [16], Abdullah Al-Taiar et al [19] & Zaki M et al [28]. It was as high asn70% in other studies [35-38].

In our study about 20.7% were sensitive to Quinolones. Other studies also observed low resistance of klebsiella to ofloxacin and levofloxacin [25,39-42]. We noted that none of the Klebsiella isolated were susceptibile to Ampicillin. Resistance to ampicillin was 58.0% in other studies [35 -39] & 94% by Kirsty Le Doare et al [13]. Osrin D et al [23] also noted that Klebsiella pneumoniae are most often not susceptible to Ampicillin.

In our study among E coli the sensitivity to Amikacin was 60% as compared to 83% in a study by Shahsanam G et al [43]; the sensitivity to Tobramycin was 60% as compared to 75% in a study by Stefania Vergnano et al [44]; the sensitivity to Imipenam was 55% as compared to 83% in a study by Shahsanam G et al [43]; the sensitivity to Gentamicin was 45% as compared to 90% in a study by Y.Bell et al [16] & Stefania Vergnano et al [44]. We noted that the sensitivity to Quinolones was 15% as compared to 73% in a study by Vergnano et al [44] & 83% by Shahsanam G et al[43]. In our study the susceptibility to Ampicillin was 0% as compared to 18% in a study by Margot Anderson et al[22]. Abdullah Al-Taiar et al[19], Hill PC et al[35], Nantanda R et al [36], Nwadioha SI et al [37] & Onipede AO et al [38] noted over 50% resistance rates to ampicillin. Osrin D et al [5] noted that E. coli are most often not susceptible to ampicillin. In our study the sensitivity of Acinetobacter to piperacillin-tazobactam was 6.4%. However Ariffin N et al [29] & Al Jarousha AM et al [30] noted higher sensitivity upto 90% in their studies. Similarly the sensitivity to imipenam was 19.3% in contrast to 95% by Ariffin N et al [29]& Al Jarousha AM et al [30].

In our study, Pseudomonas was sensitive to all antibiotics. Shahsanam G et al also observed that Pseudomonas was sensitive to Imipenem/ Meropenem in 100% cases & to Ciprofloxacin / Levofloxacin in 87.5% cases [43]. Ariffin N et al [29] & Al Jarousha AM et al [30] noted that Pseudomonas demonstrated, moderate resistance to piperacillin-tazobactam (11.0%, 9.5%), and low resistance to imipenem (4.2%, 0%) respectively. In our study, among Staphylococcus aureus, 68.42% were MRSA 23.68% were MSSA.The sensitivity to Gentamicin was 34.12% as compared to 56.4% in a study by Tinuade A Ogunlesi et al [25]

Conclusion

Sepsis is the most common cause of infant mortality in developing countries, accounting for 30–50% of all deaths each year. It is essential to closely monitor the bacterial flora and the antibiotic sensitivity pattern of pathogens to evolve rational antibiotic policy which is suitable for each unit. Low in vitro sensitivity of the leading microbes to commonly used drugs is challenging. Guidelines on the reduction of emergence of drug resistance must be provided and instituted within the units.

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Abbreviations of Organisms

A BAUMANNII: *Acinetobacter baumannii*
B.CEPACIA: *Burkholderia cepacia*
E.FAECALIS: *Enterococcus faecalis*
E.FAECIUM: *Enterococcus faecium*
E.MENINGOSEPTICA: *Elizabethkingia meningoseptica*
H PARAINFLUENZAE: *Haemophilus parainfluenzae*
K.PNEUMONIAE*: Klebsiella pneumoniae*
K.OXYTOCA: *Klebsiella oxytoca*
MRSA: Methicillin resistant *Staphylococcus aureus*
MSSA: Methicillin susceptible *Staphylococcus aureus*
NFGNB: Nonfermenting gram negative bacilli
P.AERUGINOSA: *Pseudomonas aeruginosa*
S.AUREUS: *Staphylococcus aureus*
S.PARATYPHI-A: *Salmonella paratyphi A*
S.PNEUMONIAE: *Streptococcus pneumoniae*
VRE: Vancomycin resistant Enterococcus

Abbreviations of Antimicrobial Agents

AK: amikacin
AMP: ampicillin
CD: clindamycin
CIP: ciprofloxacin
COL: colistin
ETP: ertapenem
G: gentamicin
IM: imipenem
LINCO: lincomycin
MEM: meropenem
NT: netilmicin
QL: quinolones
S-TO ALL: susceptible to all antibiotics
TB: tobramycin
TIM: ticarcillin-clavulanic acid

TGC: tigecycline
TZP: piperacillin-tazobactam
VA: vancomycin

Mechanisms of Resistance

CR: carbapenem resistant
ESBL : extended spectrum beta-lactamase producer
HLAR: High level aminoglycoside resistance
MBL +: Metallobeta-lactamase producer
MLS_Bc phenotype: macrolide –lincosamide - streptogramin B constitutive resistance
MLS_Bi phenotype: macrolide- lincosamide-streptogramin B inducible resistance

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