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Original Research Paper

A Comparative Study of The Drug Resistance Profile of The Blood Culture Isolates of Two Spatially Separated Neonatal Intensive Care Units of Ajmer (Rajasthan)

Medical Science

KEYWORDS : Neonatal sepsis, antibiotic resistance, Blood culture

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ABSTRACT

In India alone, of the 25 million babies who are born every year, one million die, accounting for 25%of the mortality around the world. Neonatal sepsis is responsible for about 30-50% of the total neonatal deaths in developing countries. It is estimated that up to 20% of neonates develop sepsis and approximately 1% die of sepsis related causes. Present study is conducted to determine the Bacteriological Profile & Susceptibility pattern of pathogens causing Neonatal sepsis.

Introduction

Septicemia in neonates refers to generalized bacterial infection documented by a positive blood culture in the first 4 weeks of life & is one of the four leading causes of neonatal mortality in India (Singh M. et al. 1991)18.Blood culture is the gold standard test in the diagnosis and treatment of neonatal septicemia (Arpita Jigar Shah et al., 2012)2.

Prior to antibiotic era, the mortality from septicemia was 90% but it has declined to 24-58% after antibiotics came in use (Kaushik et al., 1998)16. Multiple antibiotic resistance among cases of Neonatal sepsis (Neonatal septicemia) are currently one of the greatest challenges to the effective management of infections (Arpita Jigar Shah et al., 2012)16. The rapid emergence of antibiotic resistant bacteria is a major threat to public health (ECDC, 2007; WHO, 2007)11, 12. Each & every hospital must have its own local antibiogram mentioning empirical therapy options (Arpita Jigar Shah et al.,2012)16.

So, the present study is conducted to determine the Antibiotic Susceptibility pattern of bacterial isolates causing Neonatal sepsis & to provide it to the Pediatrician for a better patient management.

Material and methods

This study is Comparative study of blood culture isolates & their antibiograms based on a prospective analysis of 164 cases admitted to the Nurseries of J.L.N Hospital & Rajkiya mahila chikatsalya (R.M.C),Ajmer from 1st January 2013 to 30th June 2013. Blood culture was done for all neonates suspected to have septicemia. Blood culture sample included a single sample collected from a peripheral vein or artery under aseptic conditions. The local site was cleansed with 70% alcohol and povidone iodine (1%), followed by 70% alcohol again. Blood cultures were done in a brain heart infusion biphasic medium. Approximately, 3 ml of blood was inoculated into the brain heart infusion broth and incubated at 37°C. Subcultures were done on sheep blood agar and MacConkey agar at the earliest visual detection of turbidity or blindly on days 1, 4, and 7 if the bottles did not show turbidity. Isolate was identified by their characteristic appearance on their respective media, Gram staining and confirmed by the pattern of biochemical reactions using the standard method8. Members of the family enterobacteriaceae were identified by indole production, H2S production, citrate utilization, motility test, urease test, oxidase, carbohydrate utilization tests, and other tests. For Gram-positive bacteria, coagulase, catalase, bacitracin and optochin susceptibility tests and other tests were used. Blood culture broth that showed no microbial growth within seven days was reported as culture negative, only after result of routine subculture on blood, MacConkey, and chocolate agar. Antimicrobial susceptibility testing was performed for all blood culture isolates by Kirby–Bauer disc diffusion method as recommended in the National Committee for Clinical Laboratory Standards (NCCLS) guidelines.

The drugs for disc diffusion testing were in the following concentrations: Ampicillin (10 µg), amoxiclav (20/10 µg), cephalexin (30 µg), cefuroxime (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), (30 µg), penicillin (10 units), tetracycline (30 µg), co-trimoxazole (1·25 µg trimethoprim/23·75 µg sulfamethoxazole), amikacin (30 µg), ofloxacin (5 µg), cefoperazone (75 µg), netilmicin (30 µg), imipenem (10 µg), piperacillin/tazobactam (100/10 µg), azithromycin (15 µg), and linezolid (30 µg). The discs were obtained from Himedia (India) Laboratories.

RESULTS

A total of 164 newborns were analyzed for clinical sepsis of both the NICU. A total of 100 neonates from J.L.N & 64 from R.M.C clinically suspected of septicemia were studied and out of them 50% (J.L.N-50), 75% (R.M.C-48) were blood culture positive.

Male newborns were more affected as compared to female newborns. Ratio of septicemia in Male: female was 2.1:1 at J.L.N & 3:1 at R.M.C. Late-onset sepsis cases were found to be two times higher than early-onset sepsis. Out of 98 cases, 45 (45%) had early-onset sepsis and 54(55%) had late-onset sepsis.

Detailed etiology of the 98 isolates is provided in Table 1. These included comparative analysis of Gram-negative bacilli at J.L.N & R.M.C(34% & 50%) and Table 2 Gram-positive cocci at J.L.N & R.M.C (66% & 50%). Klebsiella spp. and coagulase-negative staphylococci (CONS) were the most common Gram-negative and Gram-positive organisms.

The antibiotic susceptibility pattern in Gram-negative and Gram-positive isolates of both NICU. (Tables 3and 4)

Methicillin resistance was seen in only 11.76% of S.aureus (MRSA) and 11.7% of CONS (MRSE) at J.L.N nursery. While at R.M.C 8.33 %(MRSA), 29.16 %(MRSE). In our comparative study vancomycin was found to be sensitive in 100% of gram positive sepsis cases at both the nurseries. Extended beta lactmase producing gram negative organism was seen in only 52.9 %(J.L.N), 25 %(R.M.C) for predomi-

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nant organism. The newer agent imipenem was found sensitive in 100% cases in gram negative sepsis.(Tables 5)

Table-1
Comparative Analysis: Different gram negative organisms isolated

GRAM NEGATIVE ORGANISM	Frequency of isolation-no.(%)	
	J.L.N	R.M.C
Klebsiella spp.	16(94.1)	18(75)
Enterobacter spp.	NIL	3(12.5)
Esch.coli	1(5.8)	1(4.1)
Pseudomonas spp.	NIL	1(4.1)
Citrobacter spp.	NIL	1(4.1)
TOTAL	17(34)	24(50)

Table-2
Comparative Analysis: Different gram positive organisms isolated

GRAM POSITIVE ORGANISM	Frequency of isolation- no.(%)	
	J.L.N	R.M.C
CONS	12(35.29)	14(58.32)
Enterococcus spp.	12(35.29)	6(25)
Staphylococcus aureus	9(26.4)	4(16.66)
Candida spp.	1(2)	NIL
TOTAL	34(66)	24(50)

Table 3
Comparative Analysis antibiotic sensitivity pattern of gram negative organism

ORGANISM			ANTIBIOTIC USED												
	AMP	AMC	AT	CPM	CAZ	CAC	C	CIP	COT	GEN	IMP	PI	TE		
Klebsiella spp.	NO.	J.L.N	15	11	13	15	15	12	4	9	13	12	0	9	10
		R.M.C	18	17	18	18	18	14	3	14	13	15	0	13	7
	%	J.L.N	100	73.3	86.6	100	100	80	26.7	60	86.6	80	0	60	66.6
		R.M.C	100	94.4	100	100	100	77.7	16.7	77.7	72.2	83.3	0	72.2	38.8
E. aerogenes	NO.	J.L.N	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
		R.M.C	3	3	3	3	3	2	2	2	2	1	0	3	0
	%	J.L.N	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
		R.M.C	100	100	100	100	100	66.6	66.6	66.6	66.6	33.3	0	100	0
E.coli	NO.	J.L.N	1	1	0	1	1	0	0	1	1	1	0	1	1
		R.M.C	1	1	0	1	1	0	0	1	0	0	0	1	1
	%	J.L.N	100	100	0	100	100	0	0	100	100	100	0	100	100
		R.M.C	100	100	0	100	100	0	0	100	0	0	0	100	100
P s . aruginosa	NO.	J.L.N	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
		R.M.C	1	1	1	1	1	1	1	1	1	1	0	1	1
	%	J.L.N	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
		R.M.C	100	100	100	100	100	100	100	100	100	100	0	100	100
Citrobacter koseri	NO.	J.L.N	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
		R.M.C	1	1	1	1	1	1	1	1	1	1	0	1	1
	%	J.L.N	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
		R.M.C	100	100	100	100	100	100	100	100	100	100	0	100	100

Table 4
Comparative Analysis: Antibiotic sensitivity pattern of gram positive organism

ORGANISM			ANTIBIOTIC USED												
	AMP	AMC	CN	CIP	COT	CD	E	GEN	LZ	OX	P	TE	VA		
Enterococcus	NO.	J.L.N	11	11	11	9	9	7	7	7	0	9	11	4	0
		R.M.C	6	4	6	6	6	4	4	4	0	6	6	1	0
	%	J.L.N	91.6	91.6	91.6	75	75	58.3	58.3	58.3	0	75	91.6	33.3	0
		R.M.C	100	66.6	100	100	100	66.6	66.6	66.6	0	100	100	16.6	0
CONS	NO.	J.L.N	12	7	10	8	7	6	10	4	0	4	10	0	0
		R.M.C	13	10	8	9	10	8	11	6	0	10	14	2	0
	%	J.L.N	100	58.3	83.3	66.6	58.3	50	83.3	33.3	0	33.3	83.3	0	0
		R.M.C	92.8	71.4	57.1	64.2	71.4	57.14	78.5	42.9	0	71.4	100	0	0
S.aureus	NO.	J.L.N	6	2	4	6	7	5	7	7	0	7	7	2	0
		R.M.C	2	3	2	2	3	2	3	0	0	3	3	1	0
	%	J.L.N	66.7	22.2	44.4	66.6	77.7	55.5	77.7	77.7	0	77.7	77.7	22.2	0
		R.M.C	66.6	100	66.6	66.6	100	66.6	100	0	0	100	100	33.3	0

DISCUSSION

The uncertainty surrounding the clinical approach to treatment of neonatal septicemia can be minimized by periodic epidemiological surveys of etiological agents and their antibiotic sensitivity patterns leading to recognition of the most frequently encountered pathogens in a particular geographical area. (TABLE-5)

Out of the 164 clinically suspected cases of sepsis from two nurseries added in our study, 98 were culture positive with a blood culture positivity rate of 59%. The incidence of Gram-negative and Gram-positive organisms was 55.7% and 44.3%, respectively. Male newborns were more affected as compared to female newborns,which agrees with previous reports. This might be because of the importance given to the male infants and also because of more number of male infants born compared to female infants born. A negative blood culture does not exclude sepsis and about 26% of all neonatal sepsis could be due to anaerobes.

The pathogens most often implicated in neonatal sepsis in developing countries differ from those seen in developed countries. Overall, Gram-negative organisms are more common and are mainly represented by Klebsiella, Escherichia coli & Pseudomonas. Out of the Gram-positive organisms, Staphylococcus aureus, CONS, Streptococcus pneumonia, and S. pyogenes are most commonly isolated, but in our study Gram-negative and Gram-positive septicemia was encountered in J.L.N are 34% and 66% & 50% and 50% at R.M.C of the culture-positive cases, which is comparable to a study conducted by other authors.(TABLE-5)

(TABLE-5)
Blood culture isolates in other studies

AUTHOR NAME	PERIOD	PREDOMINANT BACTERIA	%
Karthikeyan et al.	1997-2001	Staphylococcus aureus	61.5
Ni Chung lee et al.	1999-2001	Coagulase negative staphylococcus	29
Shashi kala et al.	2000	Klebsiella spp.	64.87
Kapoor et al.	2005	Klebsiella pneumonia	34
Upadhyay et al.	2006	Klebsiella pneumonia	17
Vinod kumar CS et al.	2008	Klebsiella pneumonia	26.9
Sahila Latif et al	2009	Klebsiella spp. S.aureus	24.10% 24.10%
Kairavi et al .	2010	Klebsiella spp.	47.14
Yasser A.Elbayouni et al.	2011	CONS	39%
Arpita Jigar et al.	2012	E.coli	20%
Present study	2013	Klebsiella spp.	32%(J.L.N) 37.5%(R.M.C)

The present study of J.L.N & R.M.C Nursery where the Gram Negative sepsis Klebsiella spp. presented with some degree of resistance with some important antibiotics likewise in gram positive organism isolated. .(TABLE-6)

TABLE-6
Comparative Analysis: % prevalence of antibiotic drug resistance of predominant flora gram negative isolates

AUTHOR	Cephalosporins	Carbepenams	Aminoglycosides
Kavita Nimboor et al .2006	26.67%	◎	53.34%
Komal tak et al .2008	100%	4%	100%
Sahila Latif et al.2009	93.7%	6%	◎
Kairavi et al .2010	24.18%	–	76.30%
Yasser A.Elbayouni et al .2011	37.50%	0%	37.50%

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Arpita Jigar et al .2012		35.34%	19%	90%
PRESENT STUDY	J.L.N	88.86%	0%	80%
	R.M.C	94.33%	0%	72.20%

In the present study Gram Negative sepsis Klebsiella spp. showed 88.86%(J.L.N) & 94.33%(R.M.C) resistance to all cepahalosporins and aminoglycosides 80%(J.L.N) &72.20%(R.M.C) resistant isolates were found.100% sensitivity was seen to Imipenem at both the nurseries ,Sensitivity to Tetracycline were found to be 33.4%(J.L.N) 61.2%(R.M.C),Chloramphenicol 73.34%(J.L.N) 83.34%(R.M.C) , Arpita Jigar et al.reported resistance 35.34% to cephalosporins, 90% to aminoglycosides & 19% to imipenem .So 52.9% ESBL producers were found at J.L.N & 25% at R.M.C. Komal tak et al. reported 100% resistance to cephalosporins ,68% to aminoglycosides & 96% sensitive to imipenem and 60% to chloramphenicol .This data can be co-related with present study .

In present study of Gram positive sepsis, Coagulase negative Staphylococcus(CONS) were found 100% sensitive to Vancomycin, Linezolid, Tetracyclins at both the nurseries. Enterococcus spp. were found to sensitive to Vancomycin ,Linezolid at both the nurseries Vancomycin resistant strains were not reported during the period of study.

Staphylococcus aureus were found to sensitive to Vancomycin , Linezolid, where as 15.1% (J.L.N)12.5%(R.M.C) Methicillin resistant (MRSA)strains were seen . Arpita Jigar at al. reported methicillin resistance in S.aureus was seen in as high as 50% isolates.& methicillin resistance noted in CONS was 68%.Inducible Clindamycin resistance was noted in 9%.

Present study showed increased resistance to penicillins(100%) which is a primary drug against gram positive organisms. Resistance to macrolides is also increasing. Linezolid and Vancomycin is 100% sensitive in all isolates. It was also noticed against aminoglycosides which are commonly used for empirical therapy. According to our antibiogram of both the nurseries, carbepenems were effective against gram negative organisms. The drug treatment given to the protocol showed marked improvement in the outcome of patient.

CONCLUSION

It is evident from this study that Gram-negative organisms (Klebsiella), CONS, and Enterococci are the leading cause of neonatal sepsis in this study, and most of them are resistant to multiple antibiotics. Surveillance of antimicrobial resistance and antibiotic policy is necessary to formulate. Depending on the antibiotic sensitivity pattern of the isolates, antibiotics should be used and stop the indiscriminate use of antibiotics, which is currently considered to be a menace in our society.

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