SENSITIVITY PATTERN OF BACTERIAL ISOLATES IN NEONATAL SEPSIS: A HOSPITAL BASED STUDY

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ABSTRACT

OBJECTIVE: To determine the sensitivity pattern of bacterial isolates in neonatal sepsis at a tertiary care hospital of Peshawar.

METHODOLOGY: This cross sectional study was carried out at the Department of Paediatrics, Rehman Medical Institute, Peshawar, from 1st January 2006 to 30th June 2012. Out of 4900 neonates admitted to the department of neonatology during the study period, 2256 (46.04%) neonates had clinical signs and symptoms of sepsis. Only 440/2256 (19.5%) neonates with positive blood cultures were selected for the study. All the blood cultures were done following a standard protocol.

RESULTS: Out of 440 cases of neonatal sepsis, 309 (70.2%) were males and 131 (29.8%) were females. Mean age of patients was 8.93±8.70 days. Nineteen different micro-organism were isolated in which Staphylococcus Aureus (n=282; 64.1%), Enterococcus fecalis (n=61; 13.9%), Klebsiella pneumonia (n=30; 6.8%) and Escherichia coli (n=25; 5.7%) were most common organism. Gram positive organisms were mostly sensitive to vancomycin, imepenime, ceoftaxime, amikacin and amoxacillin, while gram negative organisms were mostly sensitive to amikacin and imepenime. Overall penicillin and cephalosporin has shown high resistance to gram negative organism, while ampicillin was found to be resistant to both gram positive and gram negative organism.

CONCLUSION: Staphylococcus Aureus, Enterococcus Fecalis, Klebsiella pneumonia and Escherichia coli are the commonest pathogens in neonatal sepsis in our set up. In era of highly resistant micro-organism, vancomycin, amikacin, cefotaxime, and imepinime may be considered as drug of choice for empirical treatment of neonatal sepsis on the basis of highest sensitivity amongst tested drugs.

KEY WORDS: Blood Cultures, Neonatal Sepsis, Sensitivity.

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INTRODUCTION

Sepsis neonatorum refers to systemic infection of the new born characterized by bacteraemia and clinical symptoms caused by micro-organism and their toxic products[1]. The neonatal sepsis can be either early onset (within first week of life) mainly acquired from pathogens of maternal genital tract, whereas late onset (after first week till 28 days of life) is mainly due to organisms acquired in community or in hospital. The neonatal sepsis has considerable contribution in the neonatal mortality and morbidity. Worldwide on average 1.6 millions of deaths are due to neonatal infection[2], of all the neonatal infections bacterial infection is considered to be the major cause for neonatal mortality[3]. The spectrum of bacteria most commonly implicated in neonatal sepsis are quite different in industrialized countries compared with middle and low income countries[2] and also the type of organism responsible for sepsis vary greatly depending on the region, its environmental condition, socioeconomic status, standard of living, mode of delivery and feeding.

Neonatal sepsis is the constellation of nonspecific symptomatology in association with bacteraemia. Early diagnosis, specific antimicrobial therapy and judicious supportive care are the key determinant of positive outcome in this serious paediatric emergency. It is estimated that almost 20% of all neonates develop infection and approximately 1% die of the serious systemic infection, surprisingly sepsis is the commonest admitting diagnosis among neonates at referral facilities[1].

The early diagnosis and prompt treatment of neonatal sepsis has prognostic significance and for that we need the detection of the microorganism that is mainly responsible for the infection. Beside clinical examination, history, specific or nonspecific symptoms the blood culture provide essential information for the evaluation of neonatal sepsis by isolating the causative organism. Though many infections in the neonatal age group can only be established on the basis of etiological agent recovered from blood, the positive blood culture alone cannot

confirm the infection as in some cases contamination of blood can occur, also some organisms like coagulase negative staphylococci (CONS), Corynebacterium or Candida spp are often difficult to interpret. Additional information beside the positive blood culture, like the density of bacterium, number of positive blood cultures, duration of incubation of the broth to obtain a positive culture, the technique used, presence of an underlying risk factor or disease is required to determine whether disease is present or not[4].

The use of antimicrobial no doubt has helped a lot in the reduction of neonatal mortality and morbidity but the overuse and misuse of antibiotic has become a serious concern around the world as antimicrobial resistance is alarmingly raised in these countries in the past five decades.

In Pakistan, problem of antimicrobial resistance has been reported in neonatal sepsis and different studies have been conducted to address this issue.[5-9] We planned this study to determine the sensitivity pattern of bacterial isolates in neonatal sepsis at a tertiary care hospital of Peshawar.

METHODOLOGY

This was a cross sectional study carried out at the Department of Neonatology, Rehman Medical Institute, Peshawar from 1st January 2006 to 30th June 2012. Rehman Medical Institute, Peshawar is a tertiary care hospital with a grade IIIA care NICU.

Out of 4900 neonates admitted to the department of neonatology, 2256 neonates having clinical signs and symptoms of sepsis like fever, lethargy, poor feeding, jaundice, hypothermia, poor perfusion, diarrhoea, vomiting, abdominal distension, prolong capillary refill, weak or excessive cry, grunting, apnoea, bulging anterior fontanel or any maternal risk factor like maternal pyrexia (within first week of prenatal and 48 hours of postnatal, foul smelling/purulent vaginal discharge, PROM> 18 hours, maternal UTI in last 4 weeks of gestation and instrumental delivery were included. All the neonates were examined in detail by the paediatric trainees in NICU. Neonates with birth asphyxia, Meconium stained liquor, low birth weight (< 1500 grams), preterm babies (<32 weeks), blood cultures with mixed growth or contaminated growth or neonates who were already on antibiotics therapy were not included in the study.

Out of 2256, 440 neonates with confirmed blood culture were included in the study. The data was recorded on a data sheet. Each neonate was carefully examined according to the criteria of sepsis. Parents' consent was taken regarding data collection and its use in research purpose.

Blood was taken for blood cultures under strict aseptic condition. Samples were sent to the microbiology lab where it was processed following a standard protocol.

Blood Culture:

The blood was collected under strictly hygienic condition to avoid contamination. The blood was then drawn from a vein following a standard procedure; 1-3ml of blood was drawn and aseptically introduced into the blood culture bottle (BD BACTEC Peds plus/F culture vials) which contains 40ml of culture media. The culture media bottle was kept at 37 C in BACTEC machine, which automatically detected growth of organisms. Upon detection the machine give bleep. The culture media is then taken out of the machine and put on a culture media plates (Blood, Chocolate and Meccon-key media are used). Blood cultures were considered negative if there was no growth after 7 days continuous incubation. Enquiry about any growth in culture made every day. Sensitivity was determined by using Kirby-Baur-Disc diffusion method.

RESULTS

Among all isolates, gram positive organisms were 296 (67.3%) and gram negative organisms were 144 (32.7%). Common gram positive organisms were Staphylococcus aureus (n=282; 64.1%), while common gram negative organisms were Enterococcus fecalis (n=61; 13.9%), Klebsiella pneumonia (n=30; 6.8%) and E coli (n=25; 5.7%).

Gram positive organisms were mostly sensitive to Vancomycin, Imepenime, Ceoftaxime, Amikacin and Amoxacillin, while gram negative organisms were mostly sensitive to Amikacin and Imepenime. Overall Penicillin and Cephalosporin has shown high resistance to gram negative organism, while ampicillin was found to be resistant to both gram positive and gram negative organism.

A total of 440 neonatal cases were studied during the study period. These included 309 (70.2%) males and 131 (29.8%) females. Their ages ranged from 1 to 28 days with a mean age of 8.93 ± 8.70 days with no significant difference between genders.

A number of microorganisms were isolated from the neonates as given in Table 1; there were no significant differences in the types or numbers of organisms between the genders.

Table II provides the results of sensitivity testing against 18 antibiotics for the microorganisms. The major classes and types of antibiotics have been tested for resistant or sensitivity of the microorganisms. Only the major patterns of sensitivity or resistance are presented here. Frequencies of sensitive and resistance microorganisms are provided for each antibiotic tested; thus a Sensitivity / Resistance 'score' (S/R score) is obtained for each microorganism as well as for each

TABLE I: DISTRIBUTION OF MICROORGANISM ISOLATED FROM NEONATES (N=440)

S. No.	Microorganism isolated	Males	Females	Total
1.	Acinetobacterbaumanii	03	—	03
2.	Aerobic species	05	01	06
3.	Chryseobacteriumindologenes	—	01	01
4.	Citrobacterfreundii	02	—	02
5.	Citrobacterkoseri	01	—	01
6.	E. coli	15	10	25
7.	E. vulnaris	01	01	02
8.	Enterobacteraerogenes	02	—	02
9.	Enterobacter cloacae	03	02	05
10.	Enterococcus fecalis	43	18	61
11.	Enterococcus spp.	05	02	07
12.	Klebsiellapneumoniae	21	09	30
13.	Morganellamorgani	01	—	01
14.	Neisseria spp.	01	—	01
15.	Pseudomonas aeruginosa	01	—	01
16.	Pseudomonas spp.	03	—	03
17.	Staphylococcus aureus	194	88	282
18.	Streptococcus spp.	11	02	13
19.	Streptococcus viridians	01	—	01
	Total	309	131	440

Staphylococcus aureus (n=282; 64.1%), while common gram negative organisms were Enterococcus fecalis (n=61; 13.9%), Klebsiella pneumonia (n=30; 6.8%) and E coli (n=25; 5.7%).

antibiotic which helps to identify useful antibiotics as well as the troublesome (resistant) microorganism.

Table III provides the results of the common isolated organism's age and sex wise. It can be seen that among all isolated organisms Staph aureus is the common organism and has highest incidence in the first week of life, affecting a total of 174 (61.7%) neonates. The second in the list is the Enterococcus affecting a total of 46 (75.5%) neonates in the first week of life. On average the incidence of sepsis is highest in the first week of life, affecting a total of 295 (67%) neonates. We can also note significant difference between males and females in the first week of life, a total of 204 (69%) male are recorded to have culture proven sepsis while only 91 (31%) females are isolated to have septicaemia.

DISCUSSION

The emergence and dissemination of antimicrobial resistance has been well documented as a serious problem worldwide.[10] Septicaemia is one of the major causes of morbidity and mortality in the neonatal period, and it often has a rapid and fulminant course. The incidence and major pathogens of the infection vary with geographical regions and among nurseries, and within the same nursery at different time. The identification of causative organism is important because it will not only help in the proper treatment but will also help in reduction of cost of treatment and hospital stay.[11]

Neonatal sepsis and resistance to antimicrobial is the major cause of morbidity and mortality in the developing countries of the world.[12] For the better outcome and proper treatment it's mandatory to select an empirical therapy to reduce neonatal mortality and any disability that can be caused by inappropriate therapy.[5]

In our study gram positive organism Staph aureuswas isolated in 282 (64.1%) of the total 440 culture proven sepsis which is similar to other study of Shah MN and Desai PB.[13] This is in contrast to reports from other parts of the World, In western countries group B Streptococci and E coli are the most common gram positive and gram negative microorganism respectively.[12,14] Our study is also comparable with the studies done by Anwar et al[126], Abdul-Karem JM Al-Bahadle et al,[15] and other reports[16,17] in which the Gram Positive Staph aureus was the leading cause of sepsis in neonates.

The results of our study revealed that among the Gram negative organism, K pneumonia (6.8%) and E coli (5.7%) are predominant isolates, our study in this regard is comparable with the study done in Fatmawati Hospital Jakarta,[18] comparing our study to other studies done in which the E coli is also the predominant organism for sepsis but their percentage was far more high than ours.[7] Date from other studies done in Pakistan reveal that Staph aureus, K pneumonia and E coli are the commonest organism isolated in neonatal units at Karachi and Peshawar, and most of these strains are multidrug resistant.[6,8] Similarly, prevalence of gram negative organism (E coli/ K pneumonia) as a cause of sepsis in neonates has been recorded by several Nigerian authors,[19] reports from eastern and central Nigeria. In contrast to our study, in India[20-22] report shows that E coli and K pneumonia are the most common isolates followed by Staph aureus.

Contrary to our study the Streptococci spp was the main causative organism

TABLE II: DISTRIBUTION OF MAJOR SENSITIVITY AND RESISTANCE PATTERNS OF TESTED MICROORGANISMS ISOLATED FROM NEONATES (N=440)

S. No	Microorganisms	Sensitivity / Resistance (S/R) to Antibiotics																		Total
		Ami	Amo	Amp	Aztr	Cefo	Ceft	Ceph	Chlo	Cipr	Imp	Cotr	Doxy	Gent	Moxi	Oxac	Sulb	Tazo	Vanc	S/R
	Acinetobacterbaumanii	3/0	3/0	0/3	3/0	3/0	NT	0/3	0/3	3/0	40/2	3/0	3/0	3/0	3/0	NT	3/0	3/0	NT	73/11
	Aerobic species	4/0	4/1	0/6	NT	NT	NT	3/0	6/0	3/1	4/0	1/3	5/1	4/1	5/1	4/1	NT	NT	6/0	49/15
	Chryseobacteriumindologenes	0/1	NT	NT	0/1	NT	1/0	NT	1/1	1/0	NT	1/0	NT	NT	1/0	NT	1/0	1/0	NT	7/3
	Citrobacterfreundii	1/1	0/2	0/2	0/2	0/2	0/2	0/1	1/1	1/1	NT	0/2	1/1	0/2	1/0	NT	2/0	1/1	NT	8/20
	Citrobacterkoseri	1/0	1/0	NT	1/0	1/0	1/0	NT	1/0	1/0	NT	1/0	0/1	1/0	1/0	NT	1/0	1/0	NT	12/1
	E. coli	23/1	17/8	2/22	23/2	21/4	20/3	1/4	18/3	22/3	70/5	1/23	1/6	5/19	7/1	NT	23/2	21/4	NT	275/110
	E. vulnaris	1/1	0/2	NT	0/2	0/2	0/2	NT	NT	0/2	NT	0/1	NT	1/1	NT	NT	0/2	1/1	NT	38/24
	Enterobacteraerogenes	1/1	0/2	0/2	0/2	0/2	0/2	0/2	0/2	1/1	33/1	0/2	0/2	0/2	1/1	NT	1/0	1/0	NT	5/23
	Enterobacter cloacae	1/1	0/5	0/5	5/0	0/5	0/5	0/5	0/4	3/2	0/1	0/4	1/4	1/4	4/0	NT	4/0	5/0	0/1	19/46
	Enterococcus fecalis	30/0	NT	43/10	NT	NT	1/0	30/1	51/3	NT	NT	NT	10/41	45/8	NT	NT	0/54	0/54	47/3	257/174
	Enterococcus spp.	NT	NT	5/1	NT	NT	NT	NT	6/1	NT	NT	NT	3/4	4/3	NT	NT	0/7	0/7	5/0	18/23
	Klebsiellapneumoniae	24/6	3/27	0/30	9/21	1/29	1/29	0/10	23/5	4/24	29/1	2/28	20/8	0/27	23/1	NT	12/18	11/18	NT	162/282
	Morganellamorgani	1/0	0/1	NT	0/1	0/1	0/1	NT	NT	1/0	NT	0/1	NT	1/0	NT	NT	0/1	0/1	NT	3/7
	Neisseria spp.	NT	NT	1/0	1/0	1/0	NT	NT	1/0	0/1	0/1	NT	1/0	NT	NT	NT	0/1	0/1	NT	5/4
	Pseudomonas aeruginosa	1/0	NT	NT	1/0	NT	NT	NT	NT	1/0	NT	NT	NT	NT	NT	NT	1/0	1/0	NT	5/0
	Pseudomonas spp.	0/3	0/3	0/3	0/3	0/3	NT	NT	0/3	0/3	NT	3/0	0/3	0/3	3/0	NT	3/0	3/0	NT	12/27
	Staphylococcus aureus	159/3	257/16	1/280	NT	116/4	NT	19/127	142/5	145/130	94/20	282/14	264/9	143/127	282/267	26/18	NT	NT	171/0	2101/2020
	Streptococcus spp.	NT	NT	11/2	NT	0/1	NT	NT	2/0	11/1	NT	NT	1/11	NT	NT	NT	NT	NT	13/0	38/15
	Streptococcus viridians	NT	NT	1/0	NT	1/0	NT	NT	NT	NT	1/0	NT	NT	NT	1/0	NT	NT	NT	1/0	4/0
Total		250/18	285/67	64/366	43/34	144/53	24/44	53/153	252/31	197/40	271/31	293/78	310/91	208/197	329/271	30/19	51/85	49/87	243/4	3089/2797

Amik = Amikacin; Amox = Amoxacillin; Ampi = Ampiclox; Aztr = Aztreonam; Cefo = Cefotaxime; Ceft = Ceftazidime; Ceph = Cephradin; Chlo = Chloramphenicol; Cipr = Ciprofloxacin; imp = Impenem; Cotr = Cotrimoxazole; Doxy = Doxycyclin; Gent = Gentamicin; Moxi = Moxifloxacin; Oxac = Oxacillin; Sulb = Sulbactum; Tazo = Tazobactum; Vanc = Vancomycin; NT = Not done

TABLE III: COMMON ISOLATED ORGANISM AGAINST AGE AND SEX

Organism	Age 1-7 days		Age 8-14 days		Age 15-21 days		Age 22-28 days		Total
	Male	Female	Male	Female	Male	Female	Male	Female	
Staph aureus	115	59	24	11	13	4	42	14	282 (64.1%)
Enterococcus	34	12	6	0	0	3	4	2	61 (13.9%)
K pneumonia	14	5	3	0	1	1	3	3	30 (6.8%)
E. coli	12	9	0	0	1	0	2	1	25 (5.7%)
Enterobacter	12	2	0	0	0	0	0	0	14 (3.2%)
Streptococci	11	2	0	0	0	0	0	0	13 (2.9%)
Pseudomonas	2	0	0	0	1	0	0	0	3 (0.7%)
Aerobic spp	2	0	0	0	0	0	0	0	2 (0.45%)
Others	2	2	3	1	0	0	2	0	10 (2.3%)
Total	204	91	36	12	16	8	53	20	440

of neonatal sepsis in most of the developed countries of the world,[12] while in our study only a total of 13 (2.9%) cases were isolated with the causative organism Streptococci. The results of our study are also similar to the study done in India and other tropical countries, in which also the infection is infrequent with Streptococci.[23]

The bacteria responsible for sepsis showed variable pattern of resistance to commonly used antibiotic, bacterial resistance to ampicillin was quite high in our study a total of 366 (83.1%) isolates were resistant to ampicillin. This antibiotic showed high resistance to Staph aureus (99.2%), E coli (88%), and Klebsiella pneumonia (100%). The studies done by Waseem R, et al[24] and Muhammad Z, et al[7] found almost similar resistance pattern. The resistance pattern to Gentamycin was also high and a total of 197 (45%) isolates were resistant to Gentamycin. The antibiotic showed high resistance to Staph aureus (45%), E coli (76%) and Klebsiella pneumonia (100%) the study done by Waseem R, et al[24] found almost the similar pattern of resistance.

Vancomycin is still the drug of choice for S aureus; a similar trend is expected in the developing countries due to its low cost and easy availability. A combination of Vancomycin and Amikacin is the best choice for infection due to Staph aureus and Amikacin alone is the drug of choice for infection due to Klebsiella pneumonia. The Ciprofloxacin has also shown significant sensitivity to Staph aureus but its use in neonates is still experimental due to lack of safety data.[25,26]

In our study Amoxicillin showed highest sensitivity pattern against gram positive organism and gram negative organism, our finding is in contrast to the study done by A. Sobhani, H. Shodjai and S. Javanbakht.[27] Our study showed that Cefotaxime is still sensitive against the Gram positive Staph aureus and Gram negative organism E coli, our this finding is in contrary to the study done by Zardad Muhammad, Ashfaq Ahmad in which the organisms isolated showed high level of resistance against the Cefotaxime.[7]

The finding of our study also showed that imipenem has highest sensitivity to the isolated organisms like Staph aureus 82.5%, E coli 93% and Klebsiella pneumonia 96.5%, due to its highest sensitivity pattern imipenem can also be used as a drug of choice in neonates, and this finding of our study is similar to other studies.[9,24]

CONCLUSION

Staphylococcus Aureus, Enterococcus Fecalis, Klebsiella pneumonia and Escherichia coli are the commonest pathogens in neonatal sepsis in our set up. In era of highly resistant micro-organism, vancomycin, amikacin, cefotaxime, and imepinime may be considered as drug of choice for empirical treatment of neonatal sepsis on the basis of highest sensitivity amongst tested drugs. From our study we suggest that cefotaxime and amikacin should be used as first line, while vancomycin and imepenime should be used in severe cases. Multiple antimicrobial resistances are one of the greatest challenges for prompt treatment of neonatal sepsis. Slow progress in the development of newer antimicrobials and rapidly rise in resistance, wise policies should be carried out in using antimicrobial therapy.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

AZJ: Conception and design, acquisition and analysis of data, drafting the manuscript, final approval of the version to be published

SBZ, SA: Acquisition of data, critical revision & drafting the manuscript, final approval of the version to be published

CONFLICT OF INTEREST

Author declares no conflict of interest

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