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Characterisation And Antimicrobial Resistance Of Sepsis Pathogens In Neonates

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ABSTRACT

The Blood infection that occurs in the infant younger is termed as a neonatal sepsis. The sepsis is correlated with the asset of microbial pathogens from the mother. The microbial infection occurred through hematology, transplacental spreading from an infected mother or from others or from the cervix infection. Bacterial pathogens that are accountable for children in the age group of 1-12 months with the presence of sepsis and 200 children were selected. From the positive blood culture, the age group 1-4 months neonates rate was 36%, the age group of 8 months was 20 % and 12 months was 25%. The organism most commonly isolated was Staphylococcus epidermidis from 41 cases of 56.70 % and Streptococcus canis was 4 cases (5%). The age-wise distribution of organisms was 56% of S. epidermidis in 1-4 months, 58% for 4-8months and 6% for 12 months. the S. epidermidis were sensitive to antibiotics Amikacin (55/80=70%) then Teicoplanin and Vancomycin, Ciprofloxacin is (53/80=67.5%) and 31% of S.canis sensitivity to the antibiotics such as Ampicillin, Cloxacillin and Oxacillin followed by 65% were sensitive to Cephalosporins and 100% to Piperacillin, Meropenam, Vancomycin and Teicoplanin respectively.

Key words: Bacteremia, Antibiotics, Amikacin, neonates.

INTRODUCTION

The Greek word denotes the sepsis is septikos 'the meaning is rotten or decaying'. It is defined as the extensive response of host to microorganism in earlier sterile tissue. The severe form of sepsis indicated by abundance dysfunction of organs and infection in the prime site. The sepsis is continuity and the infection caused in the both invasion of pathogen identification and beginning of tissue repair.

To minimize the risk of mistrust sepsis in under treatment to all patients by using the broad-spectrum antibiotics. The community-onset of sepsis to all patients or the association of the outcome of unnecessarily broad empiric treatment with the net prevalence of antibiotic-resistant pathogens (Barton,2000).

The neonatal septicemia is considered as a life threatening disease if correct therapy is not applied to the patients. The neonatal septicemia is very hard to diagnose because it has no specific signs and symptoms. There are many diagnostic methods for the identification of neonatal septicemia: C-reactive protein analysis, complete counting of blood, counting of platelets and sedimentation rate of erythrocyte and the blood culture is the standard method (Boman, 2000).

The risk factors those may be associated with neonatal septicemia are premature rupture of membrane, prolonged rupture, prematurity, urinary tract infection, poor maternal nutrition, low birth weight, birth asphyxia, and congenital anomalies (Engel et al., 1998). The spectrum of organisms causing neonatal septicemia shows variation in different countries and even varies in hospitals of the same region. Moreover, groups of organisms may be replaced by others over a period of time. In developed countries, gram-negative organisms are the most common organisms of neonatal septicemia (Wellington et al., 1992).

At the maternal, neonatal and child mortality, the sepsis is significantly caused. The sepsis is accorded with the attainment of Sustainable Development Goals (SDGs) by developing the rate of mortality in risk populations. It also causes the deaths of HIV, tuberculosis, malaria, and other infectious diseases. The sufficient vaccine coverage, quality universal health coverage, capacity to observe with the International Health Regulations, preparedness, and water and sanitation services are the prevention, diagnosis and management of sepsis. The challenge is the worldwide prevention, diagnosis and management of sepsis (Laegreid et al., 2009).

The early diagnosis of signs and symptoms of sepsis are used to treat the patients by antimicrobial therapy. The source control like abscess drainage in determining the infection source is dangerous (Khachatourians, 1998).The endangered clinical management of sepsis is required for the Antimicrobial resistance. In the starting stage of sepsis, the early fluid rescues to develop the volume status. And also the vasopressors are needed to develop and maintain perfusion of tissue. The monitoring essential signs and guide the suitable management of sepsis is the clinical management process (Mathew et al., 2005). The aim of this study is to observe the microbial pathogens involved in sepsis and their Antibiotic susceptibility in neonates.

MATERIAL AND METHODS

Study Design

All children in the age group of 1-12 months with the presence of sepsis (pediatric consensus on sepsis) and suspected bacteremia. This research work was done in the Institute of child health, Our Lady child care Hospital, Thanjavur.

Sample collection and processing

Totally 28 samples were collected with the help of sterile cotton swab sticks from the blood culture bottle and moisture with normal saline for smear preparation and cultured on to culture media plate.

BacT/Alert: an Automated Colorimetric Microbial Detection System

1. 16ml of complex media and 4ml of charcoal with the density of 1.0215 g/mL are present in the BacT/ALERT PF culture bottles

2. 0.1% w/v of brain heart infusion solids, 2.0% w/v of soybean-casein digest, 0.025% w/v of sodium polyethylene sulfonate (SPS), 0.0000625% w/v of menadione, 0.025% w/v of L-cysteine, 0.001% w/v of pyridoxine HCI, 0.000625% w/v of hemin and complex of carbohydrate and amino acid substrates present in the media.

3. The positive bottles are smeared and sub cultured and the false positive bottles are reloaded until they come positive.

4. The negative bottles are discarded as before smeared and sub cultured.

Preparation of media

The nutrient agar and citrate agar mediums were prepared, weighed, packed into the autoclave and sterilized for 15 min at 121˚C and cooled at 45˚C for further use**.**

Isolation and identification of bacterial isolates

After 24 hours incubation the growth in plates were noted then the isolates were sub-culture into the media plates for pure isolation. The pure culture isolation was kept in MacCartney bottles and they were applied for gram staining, biochemical identification and morphological appearance (CLSI - Clinical and Laboratory Standards Institute, 2012).

Gram staining techniques

A small part of microorganisms were emulsified by thin smearing from the old culture colony of 18–24 hours into normal saline on a slide. The smear was heat fixed, air dried, place it to light flame and flooded with crystal violet stain for 60 seconds and kept in the staining rack carefully. For 60 seconds the gram's iodine was added then smeared and washed gently with running water (Beach et al, 2002). The 70% ethanol was used as a decolouriser and stained with the safranin stain for 60 seconds, rinsed with water and dried well. Then the Gram positive organisms formed purple and the negative organisms showed the pink color under the examination of microscope.

Biochemical characterization of the isolates

The isolated organisms were identified and classified by using the following tests such as catalase test, oxidase test, Citrate utilization test, motility test, indole test and urease test(Jean F. Mac Faddin., 2000).

Antimicrobial susceptibility tests

The most commonly used method for the antimicrobial susceptibility is agar disc diffusion method (Kirby –Bauer method by Kirby and A. Bauer, 1950), with clinical significance except for predictable Antibacterial susceptibility patterns. In this method the test organism is inoculated into the agar plate and the antibiotic-impregnated filter paper discs were kept above the surface of the agar plate. After the incubation of 18-24 hours, the inhibition zone was measured against the bacterial growth and compared to the resistance of standard.

RESULTS AND DISCUSSION

From the overall positive cultures, there are 70 cases were obtained among the totally 200 cases and were selected for the further study to yield the overall positivity culture rate was 25%.

From the positivity culture, the age group 1-4 months neonates observed the positivity rate was 36%, the next increased rate of culture positivity was in the age group of 8 months was 20 %, it indicated the bacteremia prevalence was higher in this age group. The last isolated age group of positivity was observed in 12 months was 25%.

Our study observed the endemic findings of sepsis in neonates in low and middle income countries of the world. Next, this study work mainly depends on the high degree of methodological accuracy and its data are not cause a byproduct. From this work, the positive blood culture was observed in 25% (70 out of 200). Paulsen et al (1993) reported the similar percentage of isolated positive blood cultures in the rate of -25% (32/128). And also the Smith et al (2002) and You et al (2006) reported the 7.9% and 12.2% percentage of culture positivity in their studies.

The organism most commonly isolated from the culture was Staphylococcus epidermidis and it was isolated from 41 cases of 56.70 % culture positivity and Streptococcus canis was recorded in 4 cases (5%). The Gram staining result for the isolated organism Staphylococcus epidermidis is a discretionary anaerobic and a gram positive bacteria. This organism was a hardy consists of non motile, Gram positive cocci and arranged as grape like clusters. After the overnight incubation the organism formed the white cohesive colonies of 1–2 mm in diameter. The biochemical test for these organisms showed the presence of oxidase, catalase, citrate and urease tests (Table-1). Streptococcus canis was observed as a Gram positive organism and beta-hemolytic species of Streptococcus group G. The G group denotes the human – specific species and has a various chemical composition of phenotype. Oxidase and citrate tests noted as positive.

Table-1: Biochemical tests for the isolated organisms

S.no	Isolated organism	Gram staining	Oxidase test	Catalase test	Indole Test	Citrate utilization test	Urease test
1	Staphylococcus epidermidis	Gram positive	-	+	-	-	+
2	Streptococcus canis	Gram Positive	+	-	-	+	-

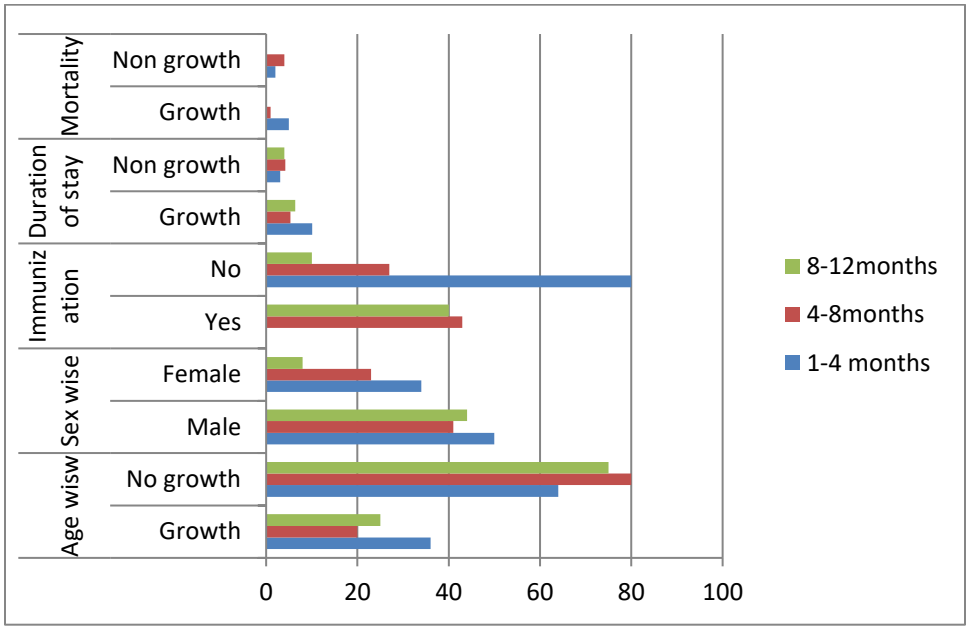
(-) negative

(+) positive

The blood culture automated system was used for higher result and with antibiotics with inactivating agents. From the study, sepsis earlier occurrence at or before 72hr of life identified the classic onset pathogens. Staphylococcus epidermidis spp was the highly emerged organism and has the high antimicrobial resistance to even reserve antibiotics. The most common organism in all groups was Staphylococcus epidermidis had 56% of organism grown in 1-4 months, in the age of 4 to 8 months was noted as 58% and the 8-12 months of neonates observed in 6% only and Streptococcus canis in the age groups of 1-12 months was 52%, 4 to 8 months was 48% and 8 to 12 months has 2% distribution.

From this study, the children who had completed their vaccination schedule against H.Influenza were observed in the age group of 3 months and all the three doses were received at the rate of 74.74%. Clearly noted that below 3 months infants have finished their three doses of vaccine H.Influenza (Fig-1). Another main collision of introducing the vaccine of H.Influenza has no isolates among the culture positive cases.

Fig-1: Distribution of age, sex, immunization, duration of stay and mortality of sepsis neonates



Among all the isolates the mean period of stay was observed as 10.65 days. The duration of average stay between positive culture cases was 13.2 days and for the negative cases 9.5 days. Compared to all age groups the high duration of stay was observed at the months of infants' 1-4 category. From the gross mortality of 200 cases, the 12 cases have 4.35% only 6 cases were observed in the positive culture cases. These are all brought out by the organism Staphylococcus epidermidis. Among all the isolated culture cases the positivity was noted in 7.65% ($P<0.05$) these were statistically significant.

Traditionally, the most common organisms found in the early stage of sepsis in neonates includes E coli, Streptococci, Listeria monocytogenes, and Enterococcus spp. and the isolates in high income countries were Acinetobacter spp, coagulase negative Staphylococci, and Klebsiella spp were dominant (Levesque et al., 1995).From the above results the most commonly isolated organism was Staphylococcus epidermidis. Among the 45 isolated growths the isolates of S. epidermidis were sensitive to antibiotics Amikacin then Teicoplanin and Vancomycin. The highest sensitivity observed against all organisms was Amikacin in the range of 55/80=70% coverage. Next the maximum activity antibiotic sensitivity was found in the antibiotic Ciprofloxacin in the range of (53/80=67.5%) against all isolates. The culture positivity among the 70 patients, the S.epidermidis was observed in 46(57.5%). For antibiotic sensitivity the highest activity found in 89% (41/46) of S.epidermidis and Ciprofloxacin 75.2 % (36/46) was followed.

The national and global estimation loads the antimicrobial resistance and the net prevalence of antibiotic resistance among the culture sites in positive cultures community onset sepsis. The two-thirds of sepsis patients received the therapy of broad spectrum directed to the organism of resistance (Agnihotri et al., 2004).The percentage of S.epidermidis was 36.1% (18 of 46). The antibiotic sensitivity was 88.9% to vancomycin next to 81.3% of amikacin. Overall resistance was observed to vancomycin in 16.3% (8/36) and to 14.2 % (7/46) of Teicoplanin. The low antibiotic sensitivity was noted to the Linezolid antibiotic.

The S.canis showed the 31% of all the isolates were sensitivity to the antibiotics such as Ampicillin, Cloxacillin and Oxacillin followed by 65% were sensitive to Cephalosporins. 100% of isolates were sensitive to Piperacillin, Meropenam, Vancomycin and Teicoplanin (Table-2).

Table-2: Antimicrobial susceptibility tests

		ANTIBIOTICS												
Organism		**Amikacin(AMI)**	**Teicoplanin(TEI)**	**Vancomycin(VAN)**	**Ciprofloxacin(CIP)**	**Meropenem(MER)**	**Piperacillin(PIP)**	**Flurooquinolones(FLO)**	**Penicillin(PEN)**	**Cephalosporins(CEP)**	**Linezolid(LIN)**	**Ampicillin(AMP)**	**Cloxacillin(CLO)**	**Oxacillin (OXA)**
S.epidermis	R	21	30	31	16	18	5	19	21	9	20	32	0	12
	S	45	17	14	35	25	20	14	32	40	13	0	0	7
S. canis	R	3	4	4	2	2	5	3	1	1	8	11	6	0
	S	0	2	2	3	7	7	6	2	3	4	3	1	1

S-Sensitive

R-Resistant

Our antimicrobial resistance observations showed the threatening alarm for the policy maker implicating in the risk of mortality to sepsis culture positive cases. The positive culture sepsis attributable population risk by multi drug resistant was higher than the sepsis by non multi drug resistant isolates (15·7% vs 12·0%). From the recent studies by the "The Review Antimicrobial Resistance' seek to generate the economic burden of laciness in the field. Because of the unsuitability for cost of effectiveness or allocated models. This lack of scientific survey and clarity, questioning the methodology.

CONCLUSION

This study concludes neonatal sepsis is the common infection found and most widely isolated pathogens were isolated from the septic patients. The more utilization of antibiotics such as Ampicillin, Cloxacillin and Oxacillin etc., need a plan to control the antibiotic uses. Pathogenic culture, resistance and sensitivity observed from the local patients of sepsis could be used as a data representation to select a particular applied therapy of antibiotic to reduce the mortality and morbidity in the patients of sepsis.

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