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ORIGINAL ARTICLE

Microbiological pattern of Neonatal Sepsis in Aswan University Hospital: hospital based study

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ABSTRACT

Keywords:

Neonatal sepsis, NICU, pathogen, antibiotic sensitivity

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Background: Neonatal sepsis is still considered as one of the major causes of morbidity and mortality in neonates. **Objective:** This study aimed to estimate the prevalence, microbiological pattern, and antibiotic susceptibility of neonatal sepsis in the Neonatal Intensive Care Unit (NICU) in Aswan University Hospital (AUH) in a specific time. **Methodology**: The study was a prospective cross-sectional study carried out on all neonates admitted to the NICU with suspected neonatal sepsis a from August 2017 to July 2018. All neonates were subjected to history, examination, and investigations which included complete blood count, C-reactive protein, and blood cultures at the time of presentation. **Results:** Our study included 100 neonates with suspected neonatal sepsis. Positive growth in blood culture was 23% of cases. Isolated organisms were 11% S. aureus, 7% K. Pneumonia and 5% Enterobacter cocci. Antibiotic sensitivity revealed that the three organism were sensitive to Ciprofloxacin. All cases were resistant to Erythromycin and Gentamycin. About 8.6% were leucopenic, 38% had leukocytosis and 95.7 % had high CRP. **Conclusion:** This study revealed increasing rate of neonatal sepsis with high degree of antibiotic resistance to commonly used antibiotics among both gram-positive and gram-negative isolates.

INTRODUCTION

Although the major advances in health care of neonatal management, sepsis is still considered as one of the major causes of morbidity and mortality in neonates. It is considered as the commonest cause of neonatal mortality reaching up to 30-50% of the total neonatal deaths each year in developing countries[(1)]. Timely recognition of sepsis, rational antimicrobial therapy, and aggressive supportive care. The difference in the causative organisms for neonatal sepsis varies between the developed and

developing countries. Also their antibiotic sensitivity to drugs differs in each hospital and region[(2)].

Neonatal sepsis classified into early/late-onset sepsis, depending on whether symptoms appear before/after 72 hours of age [(3)]**.** The signs of sepsis can be subtle including temperature instability, lethargy, poor feeding, hypotonia, development, or increased episodes of apnea and increased needs of respiratory support. Additional signs such as tachypnea, cyanosis, vomiting, diarrhea, and abdominal distention can be observed in sepsis [(4)]**.** Clinical diagnosis of sepsis is difficult due to nonspecific signs and symptoms. In addition, laboratory diagnosis is time-consuming. And so, it is vital to initiate empirical antibiotic therapy until the sepsis is ruled out. Moreover, increased multidrug-resistant organisms reduce the treatment options, delay the effective treatment and increase hospital stay and cost [(5)]**.**

This study aimed to estimate the prevalence of neonatal sepsis in Neonatal Intensive Care Unit (NICU) in Aswan University Hospital in a specific time, determine the microbiological pattern of neonatal sepsis and the antibiotic susceptibility of the isolates to evaluate the used empirical antibiotics.

METHODS

The study was a prospective cross-sectional study carried out on all neonates who admitted to NICU in Aswan University Hospital with suspected neonatal sepsis at the age of (1-28) days old from August 2017 to July 2018.

Definitions

Suggestive criteria of sepsis were the following: Fever (rectal temperature >38°C), hypothermia (rectal temperature <36°C), hypoactivity, skin mottling, delayed capillary refill time (>2 second), abnormal respiratory symptoms such as tachypnea (RR > 60 breath/min), or bradypnea (RR < 30 breath/min), abnormal white blood cell (WBC) count (leukocytosis ≥12,000/mm or leucopenia <5,000/ mm), CRP is positive if concentration > 6mg/dl [(6)].

Methodology

All neonates were subjected to full history taking, complete physical examination, and investigations. Full history taking including: 1- maternal data such as gestational age at delivery based on last menstrual period, and mode of delivery. 2- ~~N~~eonatal data such as sex, birth weight, and neonatal morbidity including underlying disease and clinical presentation were recorded. Investigations included CBC, CRP, and blood cultures at the time of suspicions of sepsis, and antimicrobial sensitivity

Sample collection

In our study, we performed blood culture to all neonates admitted to NICU and had any symptoms or signs of neonatal sepsis before any interference or antibiotic therapy. We followed the strategy of the BACTEC system in performing blood cultures to the neonates in our NICU, before any interference or antibiotic therapy while in case of negative results of early signs of sepsis, another blood cultures were withdrawn. Neonates were diagnosed as being septic if they had positive blood cultures. The blood cultures were processed in a conventional 2-bottle broth blood culture system (BACTEC; Becton Dickinson, MD, United States of America). All of the isolates were identified using standard procedures as described by the National Committee for Clinical Laboratory Standards (NCCLS) guidelines. Routine susceptibility testing was performed by the disk diffusion method. Susceptibility to common antibiotics were determined by the Kirby-Bauer disc diffusion method. The susceptibility of the bacterial isolates were tested against Ciprofloxacin (5mg), Penicillin (10mg), Clindamycin (5mg), Imipenem (10mg), Chloramphenicol (30mg), Rifampicin (5mg), Erythromycin (15mg), Gentamycin (10mg), Tetracycline (30mg) and Cefaxine (30mg). The zones

of inhibition were measured and compared with NCCLS guidelines.

Statistics

Summary of measures was reported as mean ± standard deviation (SD) for quantitative variables such as age, weight, hospital stay, and percentages for categorical variables such as sex and type of organism. The variations in distribution were evaluated using: T-test was used to compare the difference in means between the two groups, Chi-square analysis was used to compare the difference in proportions and significance level is considered when $p \leq 0.05$

RESULTS

Our study included 100 neonates (full-term and preterm ≥28wks) with suspected neonatal sepsis. Socio-demographic characteristics of the studied cases were as followings mean age was (4.65 ± 0.5) days, mean gestational age was (35.92 ± 3.2) weeks, 56% of cases were male, 81% were delivered by C.S, and mean hospital stay was (3.03 ± 0.2) days. Pre-eclampsia was the most frequently encountered maternal obstetric disorder while RDS was the major cause of admission followed by neonatal sepsis.

Hypothermia, bradycardia, delayed capillary refill time and mottled skin were the most common clinical presentation of our patients as in shown in Table (3)**. While laboratory findings** showed that hemoglobin level ranges from (6.5 – 21) g/dl, WBCs range from (2.5 - 64) ×103, while platelets range from (21-516) ×103 and CRP was positive in 76% of cases.

Our results revealed that the percentage of positive growth in blood culture was 23% of cases. Isolated Organisms were S. aureus K. Pneumonia and Enterobacter cocci with antibiotic sensitivity revealed that S. aureus and K Pneumoniae were sensitive to Ciprofloxacin, Clindamycin, Rifampicin, Penicillin, Erythromycin, Gentamycin, Cefotaxime, and Tetracycline while Enterobacter was sensitive only to Ciprofloxacin.

Comparative analysis of the studied groups (positive vs. negative blood culture), revealed that gestational age and weight were the same in both groups, with no significant difference regarding sex, place, and mode of delivery between 2 groups. Maternal risk factors were presented in 30.4% of positive blood culture cases with preeclampsia and DM were the predominant risk factor as shown in Fig (1).

RDS was the most encountered cause for admission in our cases and also presented in 57% of positive blood culture cases as shown in Fig (2)

The relationship between the results of blood culture and lab findings showed that there was no significant difference regarding hemoglobin level and platelets count while there was a significant difference regarding the WBCs Count between both groups. CRP was positive in 95.7% of positive versus 70 % of negative blood culture cases with highly significant P-value (**0.012**). Relationship between in/outpatient and isolated organism in blood culture as shown in Fig (3) revealed that organisms in inpatients were S. aureus followed by Enterobacter then K. Pneumoniae, while organisms in Outpatients was S. aureus and K. Pneumoniae.

DISCUSSION

In our study, out of 100 clinically suspected septicemic patients, 23% had bacteriologically positive blood cultures. Similar results were found by **Mudzikati et al [(7)], Ansari et al [(8)]**, and **Thapa & Sapkota [(9)]** as they reported (9.8%), (12.6%) and (10.8%) blood culture positivity rate in their unit. Lower incidence rate (8.9%) was reported by **Raha et al [(10)]**. Much higher incidence rate was reported by **El Badawy et al [(11)], Sarasam et al [(12)]** and **Siddaiah et al [(13)]** as they reported 69.3%, 36.4% and 42% of their patients had bacteriologically positive blood cultures respectively.

62% of our patients with suspected neonatal sepsis were early onset sepsis (≤

3 days) where 56.5% were proven by blood culture. In agreement with **El Badawy et al [11], Hafsa et al [14], Assudani et al [15]** and **Adatara et al [16].** In contrast to us, **Mohammad et al [17] and El-Din et al [18]**, a study done in Egypt, as they found late onset sepsis in 55.8% of proven sepsis cases.

58% and 52.2% of clinically suspected and culturally positive septicaemic neonates were full-term respectively. The predominance of full-term babies was evident. As in the study of **Ellahony et al [19], Siddaiah et al [13] and Adatara et al [16],** they reported 90% and 71.8% of their patients were full-term in clinically positive blood culture sepsis respectively. In contrast to **Dhumal et al [20], they** concluded 58% and 52% of their patients were preterm in proved and probable septicemia respectively. **Rugolo et al [21] found** mean gestational age was 29 ± 2.2 weeks and **El Badawy et al [11]** found 90% of their patients were preterm. In a study done by **Mehar et al [22]**, Preterm were having 1.49 [CI (0.95, 2.35)] times risk of developing septicemia as compared to term neonates.

51% and 52.1% of our patients in clinically suspected and culturally positive septicaemic neonates were low birth weight (<2500 g). Similar to **El Badawy et al [11], El-Din et al [18], and Dhanalakshmi & Sivakumar [23]** as they found 69.7%, 75.5% and 65% of their patients < 2.5 kg . In contrast to **Adatara et al [16]**, they found that the majority (77.7%) of their neonates had normal birth weight above 2.5 kg.

Male predominance was observed in clinically suspected (56%) as well as positive blood culture (60.9%) infants. Similar to **El Badawy et al [11], Ellahony et al [19] and Siddaiah, et al [13]** as they reported that 73%, 62.9%, and 64% had positive blood culture sepsis respectively. In contrast to **Abou Farrag et al [24]**, they found that there was no statistically significant difference in the incidence of sepsis regarding sex. An increased incidence of septicemia in males than female infants may be due to the presence of immunoregulatory genes located on the "X" chromosome or probably because of more attention paid by the parents to male neonates in our locality **[25].**

CS delivery was 81% in clinically suspected neonates, of them 91.3% had positive blood culture. CS was 11 times that of VD in a positive blood culture group. Similar to **El-Din et al [18] and Adatara et al [16]** as they found that 69.7% and 66% of their patients were delivered by C.S. In contrast to our study, *Mehar* **et al [22]** *and* **Siddaiah et al [13]** as they reported that 83.5% and 88% of their patients were delivered vaginally. An explanation for this result is that newborns delivered through CS are not exposed to vaginal and fecal bacteria, but they often experience prolonged hospital stay and late initiation of breastfeeding. Late initiation of breastfeeding after CS may deprive the neonate of the protective effect of colostrum against different pathogenic microbes which have harmful effects on the survival of the newborn baby and its ability to provide immunity for the neonate **[26].**

Although the percentage of neonates delivered inside the hospital was about 4 times of those admitted from outside, there was no statistically significant difference in the rate of infection between the 2 groups. The relationship between the site of delivery and isolated organism in blood culture in our study revealed that Staphylococcus and Klebsiella Pneumoniae were presented in both groups with an increased percentage in neonates delivered inside our hospital. Enterobacter cocci revealed only from neonates delivered inside our hospital. In contrast to our study, **Black et al., [27]** reported increase Infections by E. coli in the outpatient setting (44%), whereas S. aureus and Gram-negative organisms other than E. coli were more frequently isolated from inpatients (21%, and 24%, respectively).

Maternal risk factors were presented in about one-third of positive blood culture cases with preeclampsia and DM were the predominant risk factor. **Adatara et al., [16]** concluded that bleeding disorder during pregnancy was significantly related to the increased risk of neonatal sepsis, and **Siakwa et al [28]** reported that maternal age was the main maternal obstetric complication associated with neonatal sepsis.

Laboratory findings in positive blood culture cases revealed 35% of them were anemic and 38% had thrombocytopenia with no statistically significant difference between 2 groups while 8.6% had leucopenia and 35% had leukocytosis in positive blood culture, while WBC was significantly higher in positive versus negative blood culture group. Similar to a study that was done by **Mel-S et al [29]**. Total leukocyte counts are particularly unreliable indicators of infection during the first several hours of early-onset sepsis because they are normal at the time of initial evaluation in more than one-third of infants with proven bacteremia **[30]. Mehar et al [22]** signifies that in early-onset sepsis, the total count shown a more significant increase in early-onset neonatal sepsis as compared to late-onset sepsis.

CRP was high in 95.7% of neonates with positive blood culture. Similar to **Dhumal et al [20]** and **El-Din et al [18],** in which CRP was high in 61.2% and 85.3% of their cases. In contrast to **Siddaiah et al [13],** CRP was high only in 22% of their septic patients. CRP is an acute-phase protein rise-up to thousand fold and then fall rapidly due to its short half-life (19 hours) as soon as the source of infection has been eliminated **[31]**.

The percentage of positive growth in blood culture in our cases was 23% of cases. Isolated Organisms were S. aureus K. Pneumonia and Enterobacter cocci. Similar to studies done by **Dhumal et al [20], El-Din et al [18]** where they found that the main isolated organisms from their positive blood culture were staphylococcus followed by Klebsiella pneumonia. In contrast **Siddaiah et al [13] as they** reported that the commonest isolated organisms were K. pneumonia, enterobacter then E coli.

Antibiotic sensitivity shown 15% of cases (including the three isolated organism) were sensitive to Ciprofloxacin. Enterobacter was sensitive to Ciprofloxacin only. Similar to finding reported by **Fahmey et al [32],** the isolated organisms from their blood cultures were sensitive to ciprofloxacin and imipenem. In contrast to **Thapa &Sapkota [9]**, as they reported that amikacin was the most effective drug for both gram-positive and gram-negative isolates. **Rajesh, et al., [33]** reported that staphylococcus were sensitive to linezolid and vancomycin (85%). and K. pneumonia sensitive to cefoperazone, sulbactam, piperacillin, and Tazobactam (98%).

In the present study, antibiotic resistance among the gram-positive and gram-negative bacteria was quite high to recommended drugs like Penicillin, Cephalosporin, Erythromycin, and Gentamycin. A higher trend of the resistance in the last 5 years may be primarily due to the emergence of resistant strains as a result of the overuse of antibiotics at private clinics and primary health care facilities from which neonates are referred to our unit.

This study is limited by lack of randomization and follow-up of patients.

CONCLUSION:

The current study revealed that the prevalence of neonatal sepsis in the NICU in Aswan University hospital was 23%. The most common pathogens implicated were S. *aureus*, K. Pneumoniae, and Enterobacter cocci. A high degree of antibiotic resistance was observed to commonly used antibiotics among both gram-positive and gram-negative isolates. Ciprofloxacin was the most sensitive antibiotic for isolated organisms. Erythromycin and Gentamycin were the most resistant antibiotics for

isolated organisms, more than half of neonates with positive blood culture neonates were full-term, more than 2.5 kg, male patients while CS delivery were predominant in positive blood culture neonates. About one-third of positive blood culture cases were anemic and thrombocytopenic while 8.6% were leucopenic and 38% had leukocytosis. CRP was high in 95.7% of them.

We recommend that it is mandatory to perform routine antimicrobial susceptibility surveillance and periodic review of hospital and national antibiotic policy to reduce the burden of antibiotic resistance and further epidemiological and clinical studies are vital to predict the changes in microorganism pattern causing neonatal septicemia

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Table (1): Socio-demographic characteristics of the studied cases

Variable	N = 100
Age in days (Mean ± SD)	4.65 ± 0.5
Sex:	
• Female	44%
• Male	56%
Gestational age in weeks (Mean weeks ± SD)	35.92 ± 3.2
Gestational age category	
<37 week	58%
>37 week	42%
Mode of Delivery	
CS	81%
VD	19%
Weight in Kg (Mean ± SD)	2.41 ± 0.8
Hospital Stay/days (Mean ± SD)	3.03 ± 0.2
In/Out-patient	
Inpatient	79%
Outpatient	21%

CS: caesarean section, VD: vaginal delivery

Table (2): Maternal obstetric disorder and underlying disease of the studied cases

Maternal obstetric disorder	N = 100	**Underlying disease of the studied cases**	N = 100
History of obstetric complication	33%	RDS	57%
		Neonatal Sepsis	21%
Preeclampsia	17%	TTN	5%
PROM	10%	MAS	5%
Diabetes millets	6%	HIE	2%
Anti-partum Hemorrhage	4%	Gastroenteritis	2%
		Ohers	8%
Others	5%		

HIE: Hypoxic ischemic encephalopathy, MAS: Meconium aspiration syndrome, RDS: respiratory distress syndrome, TTN: Transient tachypnea of newborn

Table (3): Clinical presentation and laboratory Findings of the studied Patients

Variable	**N= 100**	**Variable**	**N = 100**
Body Temperature		**HGB (g/dl)**	
Hypothermia	80%	Mean ± SD	14.69 ± 2.5
Hyperthermia	1%	Median (Range)	14 (6 - 21)
Delayed capillary refill time	77%	**WBCs *10^3**	
		Mean ± SD	12.56 ± 1.1
Skin Mottling	72%	Median (Range)	10 (2.5 - 64)
RR: Tachypnea	10%	**PLT *10^3**	
Bradypnea	1%	Mean ± SD	189.67 ± 10.1
HR: Tachycardia	5%	Median (Range)	173 (21 - 516)
Bradycardia	79%	**CRP**	
Hypoactivity	34%	**Positive**	76%
Lower Limb Oedema	1%		
Pallor	1%		
NEC	1%		
Jaundice	1%		

CRP: C-reactive protein, HGB: hemoglobin, HR: heart rate, NEC: necrotizing enterocolitis. RR: respiratory rate, WBC: white blood cells.

Table (4): Isolated organisms and antibiotic sensitivity of blood culture

Variable	N = 100	%
Growth		
Yes	23	23%
No	77	77%
Organism		
S. aureus	11	11%
K, Pneumoniae	7	7%
Enterobacter cocci	5	5%
Antibiotic Sensitivity		
Ciprofloxacin	15	15%
Clindamycin	7	7%
Rifampicin	6	6%
Penicillin	4	4%
Erythromycin	3	3%
Gentamycin	3	3%
Imipenem	3	3%
Cefotaxime	2	2%
Chloramphenicol	1	1%
Tetracycline	1	1%

Table (5): Comparative Analysis of the studied groups (Positive vs. negative Blood Culture)

	Blood Culture Positive (No=23)	**Blood Culture Negative (No=77)**	**P-value**
Gestational Age/weeks	35.96 ± 3.3	35.91 ± 3.2	0.951
Weight/kg	2.40 ± 0.7	2.41 ± 0.8	0.955
Inpatients Outpatients	18 (78.3%) 5 (21.7%)	61 (79.2%) 16 (20.8%)	0.564
Sex: Male Female	14 (60.9%) 9 (39.1%)	42 (54.5%) 35 (45.5%)	0.385
Delivery Mode: CS VD	21 (91.3%) 2 (8.7%)	60 (77.9%) 17 (22.1%)	0.126
Maternal Risk: No Yes	16 (69.6%) 7 (30.4%)	51 (66.2%) 26 (33.8%)	0.488
HGB (g/dl)	14.90 ± 2.3	14.01 ± 2.8	0.126
WBCs $*10^3$	11.58 ± 0.7	15.84 ± 3.4	**0.016***
PLT $*10^3$	189.49 ± 11.3	190.26 ± 22.9	0.975

CRP: No	23 (29.9%)	1 (4.3%)	**0.012**
Yes	54 (70.1%)	22 (95.7%)	

CRP: C-reactive protein, CS: caesarean section, HGB: hemoglobin, VD: vaginal delivery, WBC: white blood cells. Independent-samples T Test and Chi-square test were used. *P-value <0.05: significant, **p-value <0.001: highly significant.

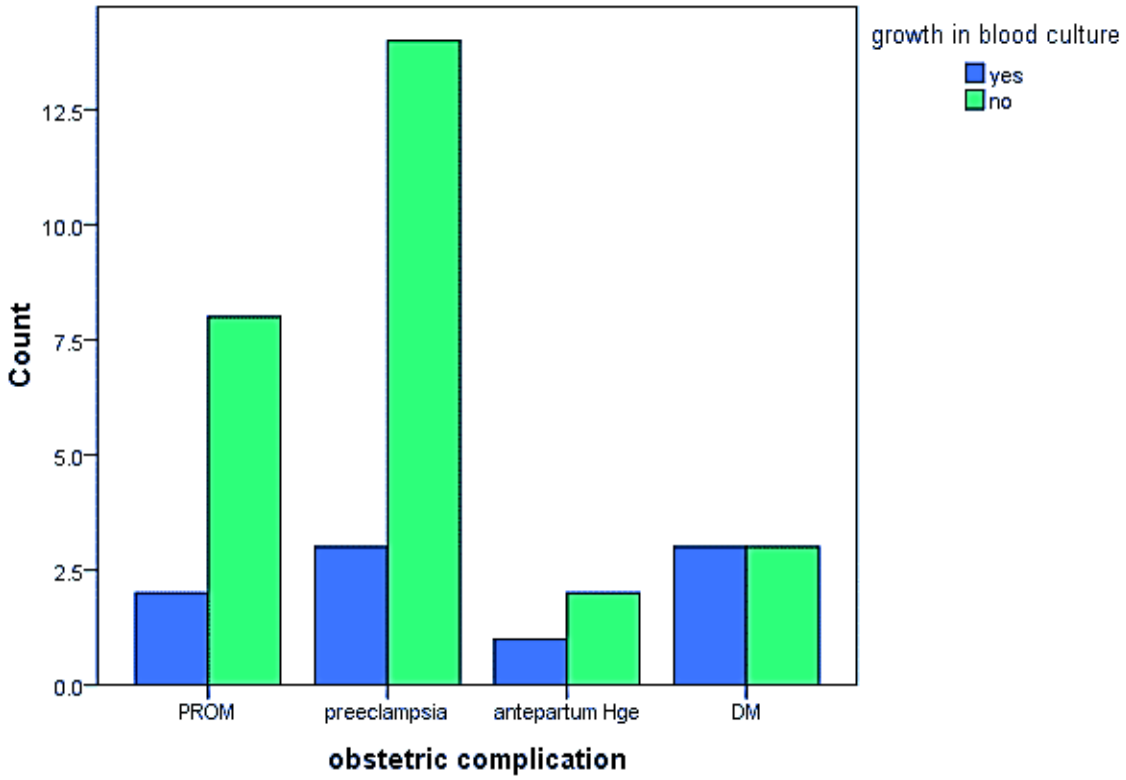

Figure 1: Comparative Analysis of the studied groups (Positive vs. negative Blood Culture) regarding maternal complications

DM: diabetes mellitus, PROM: premature repute of membrane

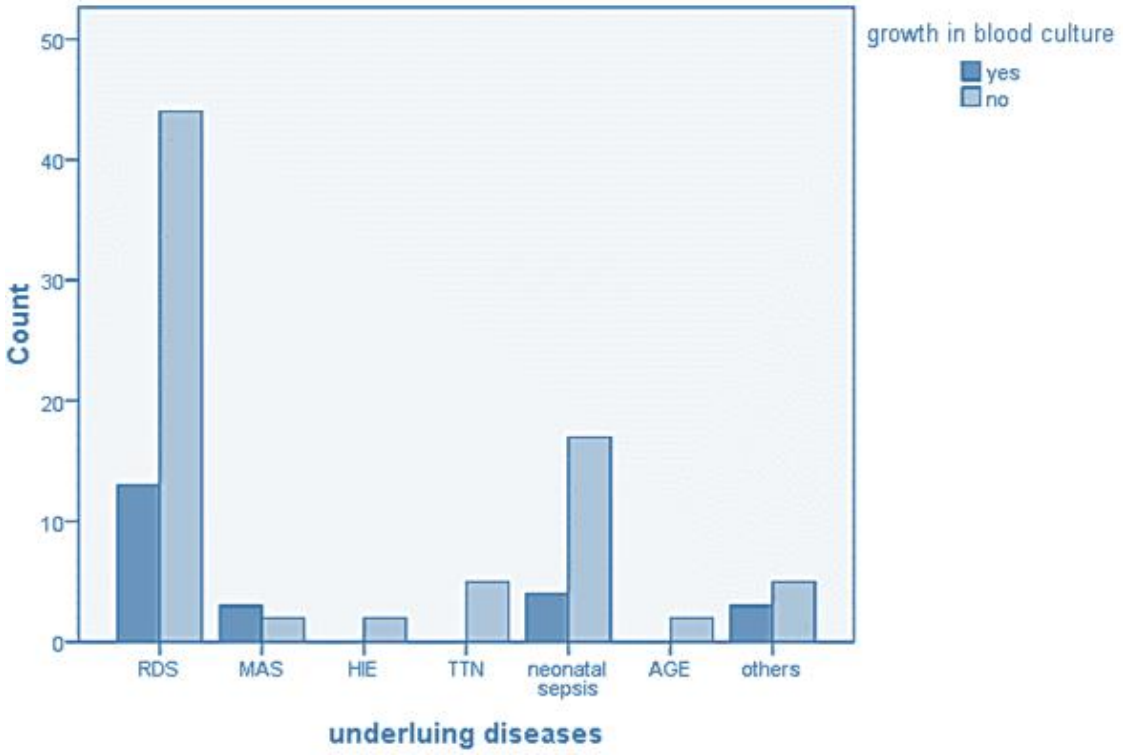

Figure 2: Comparative Analysis of the studied groups (Positive vs. negative Blood Culture) regarding underlying diseases

AGE: acute gastroenteritis, HIE: Hypoxic ischemic encephalopathy, MAS: Meconium aspiration syndrome, RDS: respiratory distress syndrome, TTN: Transient tachypnea of newborn

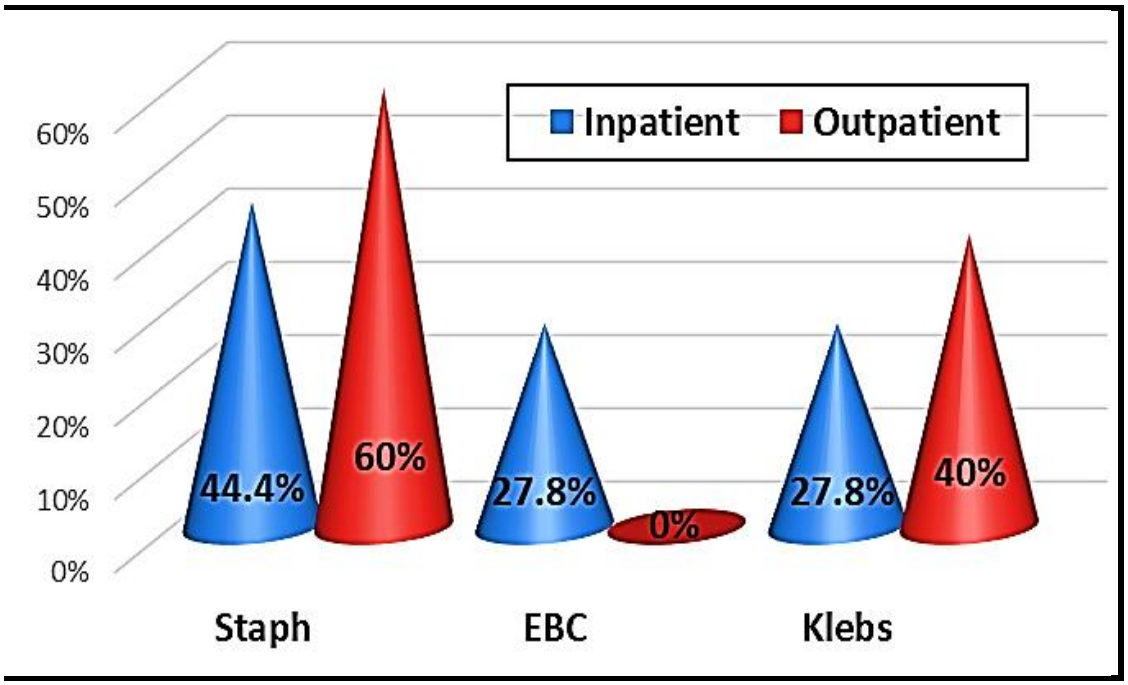

Fig. 3: Relationship between In/Outpatient and resulted organism of Blood culture