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Identification Common Cause of Neonatal Sepsis by Analytical Profile Index System (API)

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Abstract

Background Neonatal Sepsis is a bacterial infection of the blood in a neonate and an infant younger than 4 weeks of age. The analytical profile index or API is a classification of bacteria based on experiments, allowing fast identification. This system is developed for quick identification of clinically relevant bacteria.

Objective To identify the pattern of organisms in neonatal sepsis using API system in Baghdad City hospital, Al-Imamein Al-Kadhimein Medical City and Central Pediatrics Teaching Hospital.

Methods In this cross-sectional study, blood samples from 100 neonatal patients were inoculated into a blood culture bottle and incubated at 37 °C under aerobic conditions, subculture was done after 24 h of incubation, the growth was identified by phenotypic characteristics, gram's stain, and API system.

Results Positive blood cultures were detected in 82 (82%), according to API system the most prominent bacterial isolates from blood culture in neonates with early-onset sepsis were non-coagulase *Staphylococcus* (20.4%) *Staphylococcus aureus* (18.1 %), *Acinetobacter bumanii* (13.6%), *Pseudomonas aeruginosa* (11.36 %), *Streptococcus agalactiae* (11.3 %), while in late-onset sepsis the most common bacteria were *Staphylococcus aureus* (21.0%), non-coagulase Staphylococcus (13.1%), *Citrobacter freundii* and *Pseudomonas aeruginosa* (10.5 %) respectively.

Conclusion The API 20E may be useful for the identification of the bacterial species rarely described as pathogens in neonatal sepsis will help us to study the clinical burden resulting from the emergence of these species as causes for this neonatal infection.

Keywords Early-onset sepsis, late-onset sepsis, blood culture, API system

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List of abbreviations: EOS = Early-onset sepsis, LOS = Late-onset sepsis, API = Analytical profile index, PROM = Premature rupture of membranes, UTI = Urinary tract infection

Introduction

Neonatal sepsis is a bacterial infection of the blood in a neonate and an infant younger than 4 weeks of age. Babies with sepsis were listless, overly sleepy, floppy, weak, and pale [(1)]. Epidemiologists divided Neonatal Sepsis into two types of an early-onset sepsis (EOS) and late-onset sepsis (LOS). Of newborns with EOS sepsis, 85% present within 24 hours, 5% present at 24-48 hours, and a smaller percentage present within 48-72 hours [(2)]. LOS is sepsis occurring after 72 h in

NICU infants and 7 days of life in term infants, has been variably defined as occurring up to the age of <90 or 120 days, and may be caused by vertically or horizontally acquired pathogens [3]. It was found that neonatal deaths account for a third of global child mortality and that infection are a major cause of neonatal mortality [4]. The pathogens responsible of neonatal sepsis has also changed dramatically, Since the mid-20th century the infectious agents that cause neonatal sepsis have changed from Staphylococcus aureus and Escherichia coli which were the most common bacterial pathogens among neonates in the United States for decades to group B *Streptococcus* (GBS) as the most common gram-positive organism that caused early-onset sepsis [5]. At 1990s, *GBS* and *Escherichia coli* kept on being related with neonatal infections but coagulase-negative *Staphylococcus epidermidis* is presently more often watched. Additional organisms, for example, *Listeria monocytogenes*, *Chlamydia pneumoniae*, *Haemophilus influenza*, *Enterobacter aerogenes*, and types of *Bacteroides* and *Clostridium* spp. have likewise been recognized in neonatal sepsis [6]. The analytical profile index or API is a classification of bacteria based on experiments, allowing fast identification. This system is developed for quick identification of clinically relevant bacteria.

The goal of this investigation to identify the pattern of organisms in neonatal sepsis using API system in Baghdad City hospital, Al-Imamein Al-Kadhimein Medical City and Central Pediatrics Teaching Hospital.

Methods

About (1.5-3 ml) of venous blood was obtained from 100 neonates who admitted to neonatal care unites of Baghdad City hospital, Al-Imamein Al-Kadhimein Medical City and Central Pediatrics Teaching Hospital during the period from January 2017 to March 2017. Clinical manifestations including poor feeding, lethargy, temperature instability, respiratory distress, abdominal distention and seizures were determined by consultation of a pediatric specialist and verification of the information in the medical record. Inclusion criteria for selecting children were the age group (from zero time to 30 days), and diagnosed clinically with sepsis while exclusion criteria neonates with obvious congenital anomalies and neonates with neonatal respiratory distress syndrome (NRDS).

Pre-term including live born infant delivered before 37 weeks from the last menstrual period. Term infants are an infant who delivered after 37 weeks of gestation. Post-term infants are those born after 24 weeks of gestation [7].

A consent letter was signed by each neonate parents and the study were approved by the Research Ethical Committee College Medicine of Al-Nahrain University. This cross-sectional study was conducted in the Microbiology Department at College Medicine of Al-Nahrain University. The following data were collected: gestational age, birthweight gender and whether the baby was born inside the hospital or outside the hospital and then transferred to the nursery.

Samples were immediately transferred to Brain heart infusion medium vial which prepared specially for bacterial cultivation, and incubated in 37 °C, then samples subcultured in MacConkey and blood aerobically while chocolate agar incubated under CO_2 conditions, the initial reading was recorded after 24 hours and the results continued to be recorded for 72 hours. The result is considered negative after this time if there is no growth. API 20E system for *Enterobacteriaceae*, API Staph System for identifying 23 species of *Staphylococci*, API 20 Strep System according to the procedure suggested by the manufacturing company (bio-Merieux) were used to recognize the species level.

Statistical analysis

Statistical analysis was performed with GraphPad Prism version 6 software, percentages were used for the comparison between samples of the study. Data analysis was done using Chi-square for the comparison of categorical data.

Results

The participants neonates were grouped in to two categories according to type of onset, the first was early onset group included children in age groups from (0-7 days), while other late onset group included children from (8-30 days). The characteristics of the study population are shown in table (1).

Table 1. Demographic character of the study neonates

Variable		Mean±SD (Range)
Age /days		1 30 9.86 ±8.76
		%
Gender	Female	43
	Male	57
Gestational age	Pre-term	47
	Full-term	53
Mode of delivery	cesarean section	57
	Vaginal	43
Place of delivery	Hospital in born	74
	Out born	26
Birth weight	<2.5 kg	54
	>2.5 kg	46
Presentation (days)	≤7 (EOS)	51
	>7 (LOS)	49

EOS: Early-onset sepsis, LOS: Late-onset spesis

According to the mother's clinical presentation for neonatal sepsis patients, this result demonstrated that the higher percentage of neonatal sepsis found in those mothers who had history of premature rupture of membranes (PROM) 72 (72%), UTI 79 (79%) and previous abortion (37%), while less percentage found in women who had fever 22 (22%) and Hemorrhage 10 (10%) (Table 2).

Table 2. Maternal risk factors for neonatal sepsis

Clinical presentation	% of mother
Premature rupture of membranes (PROM)	72 (72%)
Mother fever	22 (22%)
UTI	79 (79%)
Hemorrhage	10 (10%)
Previous abortion	34 (37%)

Conventional methods for diagnosis of neonatal sepsis

Blood culture

One hundred samples of the patient's blood were implanted in the culture media that attended to this purpose, positive blood cultures were detected in 82 (82%), gram

negative was the major causative pathogen 43 (43%) followed by gram positive 38 (38%) and one sample showed growth of candida species, the patients with negative blood culture were 18 (18%). Table (3) explains the result of blood culture.

Table 3. Pathogens isolated from neonatal sepsis by blood culture

Blood culture	Percentage
Gram negative	43%
Gram positive	38%
Candida species	1%
Total positive	82%
No growth	18%

Bacterial distributions by API system strips

A total of 81 bacterial positive culture were examined by the different API strips system which were tailored to certain groups of microbes to identify the bacterial isolate. The distribution of bacterial isolates in EOS and LOS were represented in table (4).

Table 4. Distribution of bacterial species according to API system

Bacteria	EOS	%	LOS	%
Acinetobacter bumanii	6	13.6%	1	2.6 %
Pseudomonas aeruginosa	5	11.36 %	4	10.5 %
E. coli	4	9 %	0	0.0%
Serratia marcescens	3	6.81 %	2	5.26 %
Citrobacter freundii	2	4.45%	4	10.5 %
Pantonia	2	4.45 %	0	0.0%
Klebsiella pneumonia	1	2.27 %	3	7.89 %
Aeromonas hydrophilia	1	2.27%	0	0.0%
Pseudomonas cepacian (Burkholderia cepacian)	1	2.27%	1	2.6 %
Enterobacter	0	0.0%	1	2.6 %
Serratia phymuthica	0	0.0%	2	5.26 %
Staphylococcus aureus	8	18.1 %	8	21.0 %
Staphylococcus xylosus	3	6.81%	2	5.26 %
Staphylococcus lentus	3	6.81 %	1	2.6 %
Staphylococcus auricularis	1	2.27 %	0	0.0%
Staphylococcus cohnti	1	2.27 %	0	0.0%
Staphylococcus heamolyticus	1	2.27 %	2	5.26 %
Streptococcus agalactiae	5	11.3 %	1	2.6 %
Streptococcus pneumonia	1	2.27 %	0	0.0%
Veridanis streptococcus	0	0.0%	1	2.6 %
Total	48	100%	33	100%

Discussion

Based on the current data, the percentage of neonatal sepsis was 82% out of 100 patients enrolled in this study, this observation disagreement with a study done in Iraq by Albahadle and Abdul Abass [8] where sepsis was constituted 89.76% of the studied neonate, another study conducted by Ibrahim and Rahma [9] Stated that neonatal sepsis was 12.4% However, a study by Al-Hamadani found that the sepsis was 58 % [10].

This discrepancy in such results may be due to different reasons such as blood culture technique, administration of antibiotic in mother, difficulty in sample collection, development of much more antibiotics resistant bacterial strains.

In current study, (86.0%) of neonates were male and (75.4%) of them were female male to female ratio was (1:1.4) This result comes incompatible with those obtained in others studies of Albahadle, and Abdul Abass [8] who found that male was (40.16%) and the female was 59.84%, Ibrahim found (68.7%) neonates were males and (31.2%) were females [11], while another study by Ibrahim and Rahma [9] found the (60.3%) were males and (39.7%) were the females, which may be attributed to neonatal admission in our societies, medical attention is predominantly male to female children, and it is noteworthy to mention that many studies in our country found that regarding gender admission, boys more than girls [12] furthermore genetic, socioeconomic factors also play a role in the development of infections and may partially explain the observed differences [13].

For the successful administration of neonatal sepsis, information about bacteriological profiles, which play an essential part in the management and antibiotic administration , so for the purpose and for rapid identification we conducted in this study analytical profile index (API) system , were included API-20E, , API-Staph, and API- Strep , The most prominent bacterial isolates from blood culture in neonates with EOS were *Non-coagulase Staphylococcus* (20.4%) *Staphylococcus aureus* (18.1 %), *Acinetobacter bumanii* (13.6%), *Pseudomonas aeruginosa* (11.36%), *Streptococcus agalactiae* (11.3 %), while in LOS the most common bacteria were *Staphylococcus aureus* (21.0%), *Non- coagulase Staphylococcus* (13.1%), *Citrobacter freundii* and *Pseudomonas aeruginosa* (10.5%) respectively.

Pattern of organisms in this study do not agree with most studies at the level of our country [14,15] but it is interesting this study was recorded to our knowledge and to the first of some types of bacteria that caused the neonatal sepsis in Iraq from neonatal intensive care unit (NICU), which includes *Aeromonas hydrophilia* and *Pantoea agglomerans*. Hochedez et al. (2010) reported Bacteremia Caused by *Aeromonas hydrophila* Complex in the Caribbean Islands [16].

Others case report by Padmaja et al. in 2011, Okumura et al. in 2013 [17,18] reported a case report study sepsis caused by *Aeromonas hydrophila*, during literature search in this study 15 reports of Aeromonas Blood stream infection (BSI) in 14 articles published in English language found [19-31], seven were pediatric patients (including two neonates) [20-22,29-31].

In our study, one cases were diagnosed with sepsis caused by *Aeromonas hydrophila,* the baby had episodes of hypotension, seizures, apnea, bradycardia, diarrhea, and temperature instability and the patient was died.

Sepsis caused by *Aeromonas hydrophila* remains uncommon life-threatening conditions among the spectrum of infections occurring in neonates, with high mortality [31].

In this study, other two unique neonatal sepsis cases were reported caused by *Pantoea agglomerans*. It is an opportunistic pathogen and very rarely causes disease in healthy individuals [32] disease with *Pantoea* species related to exogenous source, *Pantoea agglomerans* the most widely recognized human pathogen mainly septicemia due to contaminated blood products, parenteral

nutrition, intravenous fluid and the anesthetic agent [33].

These two cases represented by neonates were referred to neonatal intensive care unit 48 (early onset sepsis) an hour after delivery presented with fever, tachypnea and Respiratory distress. The weight of the children was less than 2.5 kg, the ages of their mothers range from 19-20 years came from a rural area, she gave a history of PROM approximately 8 to 10 hours before delivery. One of them noticed a foul-smelling discharge after rupture of membrane. One of the children died two days after being admitted to the hospital, second one was responded well to antibiotic.

Results in current study similar to many cases reported through the world [34,35] and its quietly similar to results obtained by Senanayake et al in-Sri Lanka who reported that out of 55 blood cultures, 14 were positive for *P. agglomerans* [36].

Other interesting results reached in this study 7 (8.6%) bacterial isolates were identified as Serratia species, 5 isolates belong to *Serratia marcescens* and *Serratia phymuthica*.

Noteworthy five patients out of seven infected with Serratia species were die, many researchers reported that Serratia species and mainly *Serratia marcescens* is a well-recognized pathogen of severe nosocomial infections with highly mortality rate as found by Mahdi in isolation and molecular identification of a *Serratia spp*. from suspected neonatal sepsis in intensive care unit (ICU) of Basra Province, Iraq) [37].

The possible explanation for increase mortality in neonatal sepsis infected with *Serratia spp.* ability to produce a beta-lactamase that confers resistance to broad-spectrum beta-lactam antibiotics, which often complicates therapy [38,39].

In conclusion, the most common cause of neonatal sepsis in current study was *Staphylococcus aureus* and *coagulase negative Staphylococcus* of gram-positive bacteria while *Pseudomonas aeruginosa* and *Acinetobacter bumanii* were the most common gram-negative bacteria. *Achromobacter xylosoxidans*, *Pantonia agglomerans* and *Aeromonas hydrophilia* should be included as one of the most important causes of neonatal sepsis. These findings suggest that the API 20E may be useful for the identification of the bacterial species rarely described as pathogens in neonatal sepsis will help us to study the clinical burden resulting from the emergence of these species as causes for this neonatal infection.

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Authors' contribution

Al-Mossawi: Samples collection, molecular diagnosis, design and acquisition of data. Dr. Hassan: Drafting the article and revising it critically for important intellectual content and interpretation of results. Dr. Al-bashier: DNA extractions and conventional methods diagnosis. Dr. Al-Omrani: Patients selections and statistical analysis.

Conflict of interest

There is no conflict of interest.

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