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Neonatal sepsis: Bacteriological profile,molecular detection and antimicrobial susceptibility test among pre-term pediatrics in Erbil city, Iraq

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Abstract

Background and objective: Neonatal sepsis refers to infection occurring within the neonatal period, the first 28 days of life for a term baby, and up to 4 weeks beyond the expected date of delivery in a pre-term baby. This study was designed to determine the frequency of neonatal early onset sepsis and late onset sepsis in some hospitals in Erbil city.

Methods: Out of 170 neonate samples from Neonatal Intensive Care Unit in Rapareen Pediatric Teaching Hospital and Maternity Obstetric Hospital randomly were studied through isolation and identification of causative bacteria using the traditional approach, VITEK 2 compact system, molecular detection using PCR technique, moreover, C-reactive protein and white blood cell count detected as biomarker assay. The isolated bacteria tested against some widely used antibiotics.

Results: The sepsis was confirmed in 54 (31.76%) patients of total neonates with suspected cases of sepsis by clinical signs and symptoms. EOS was 42 (77.77%) were detected, and late onset sepsis was 12 (22.23%). Identification of the isolated bacteria by the traditional method, and VITEK 2 compact system were performed. In addition, molecular detection via the PCR system through the detection of the specific genes was performed. Antimicrobial Susceptibility test indicated that most of the isolated bacteria were developed resistant to the most widely used antimicrobials.

Conclusion: Our study showed a high incidence of neonatal early onset sepsis more than late onset sepsis, mostly associated with Gram-negative bacteria than Gram-positive bacteria.

Keywords: Neonatal Sepsis; Early onset sepsis; Late onset sepsis; CRP; VITEK 2 system.

Introduction

Neonatal sepsis is a clinical syndrome characterized by signs and symptoms of infection with or without accompanying bacteremia in the first month of life. It encompasses various systemic infections of the newborn such as septicemia, meningitis, pneumonia, arthritis, osteomyelitis, and urinary tract infections.[1] Neonatal sepsis is one of the most common causes of neonatal morbidity and mortality in newborn babies, it is estimated to cause 26% of all neonatal deaths worldwide. Neonatal sepsis accounts for 10% of all neonatal mortality. Neonatal infection is present in 8 of every 1000 live births and 71 of every1000 neonatal admissions.[2] It is the cause of 1.6 million deaths per annum in developing countries. Its incidence varies from country to country but is much higher in developing countries where it is responsible for about 30-50% of the total of neonatal deaths.[3] One of the most common reasons for admission to neonatal units in developing countries neonatal sepsis refers to generalized bacterial infection documented by positive blood culture in the first 28 days of life. The risk of sepsis in neonatal unit admissions is magnified by factors linked to prematurity and neonatal intensive care. Neonatal sepsis often develops rapidly with high

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mortality risk in the absence of specific therapy, demanding early empirical antibiotic therapy when sepsis is suspected.[2]Some of the risk factors for neonatal sepsis include; prematurity or low birth weight, pre-termlabor, premature or prolonged rupture of membranes, maternal chorioamnionitis, fetalhypoxia, traumatic delivery, male Gender and low socio-economic status.[3] Sepsis can be classified into two types according to the age of neonates. Early onset sepsis (EOS) presents within the first 72 hours of life, and in severe cases, the neonate may be symptomatic at birth. The source of infection for early onset sepsis is usually the maternal genital tract.[4] Late onset sepsis (LOS) presents after 72 hours of age. It is usually nosocomial as a complication of neonatal intensive care or community acquired. This classification is important as it helps in determining the most probable organism and mode of transmission.[4] Neonatal infections of viral or fungal etiology may also occur at seven days of life and must be distinguished from bacterial sepsis. The local epidemiology of neonatal sepsis should be constantly updated to detect changes in the pattern of causative organisms and their susceptibility to various antibiotics.[5] Early diagnosis and proper management of neonatal sepsis by rational antimicrobial therapy and supportive care can reduce mortality. Blood culture is the gold standard for diagnosis of sepsis, but blood culture reports are usually available after 24-72 hours. In addition, some biomarkers are diagnostic indicators for it, such as C-reactive protein and white blood cell count.[6] The most predominant microorganisms associated with neonatal sepsis are Coagulase Negative *Staphylococcus (CoNS) in developing countries. While* Group B *Streptococcus (GBS), E. coli and Listeria monocytogenes in developed countries.*[7] The antibiotic susceptibility of microorganisms also changes with time, with the emergence of multidrug resistant organisms since the discovery of antimicrobial agents, microorganisms have developed resistance to them through mechanisms such as mutations and increased enzyme production. Resistance to commonly used antibiotics is an important problem worldwide. A periodic survey of the causes of sepsis and their antibiotic sensitivity patterns is essential in the design of effective infection control programs and in guiding empiric antibiotic therapy.[8] Studying the neonatal sepsis according to the bacteriological profile among pre-term paediatrics is important. Therefore, this study aimed to determine the frequency of neonatal early onset sepsis EOS and LOS in Neonatal Intensive Care Unit (NICU) in some hospitals in Erbil city, and evaluate the socio-demographical characteristics of the neonate and the mothers in this city with particular focus on their molecular detection and antimicrobial susceptibility pattern.

Methods

A total of 170 neonate samples were obtained from Neonatal Intensive Care Unit (NICU) for EOS and LOS in Rapareen Pediatrics Teaching Hospital and Maternity Obstetric Hospital in Erbil city from 10th July 2018 to 10th March 2019. Fifty-four patients from a total neonate were studied. Patients were classified into two groups (proven and clinical sepsis) according to the clinical signs and blood cultures. They were recorded into some clinical data: gender, birth weight (gm), birth age (days), mother's age (years), residency area, and hospital's name**.** Neonate's sociodemographic characteristics were recorded including Gender (female and male), birth weight was categorized into early low birth weight (<1000 gm.), very low birth weight (1000-1500 gm.), and low birth weight (>1500 gm.), ages were designed into EOS ages (<7 days) and EOS ages (7–30 days), mother's age was ordered into early (<25 years) and late (>25 years), residency area of mother's and neonates were classified into urban,

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suburban and rural. Finally, the hospitals were named as Rapareen Pediatrics Teaching Hospital and Maternity and Obstetric Hospital, Erbil.

Sample Collection

Sample collection was conducted under the supervision of medical staff, four milliliters of blood samples were collected in the primary hours after birth but before taken antibiotics for EOS, whereas the blood samples were taken after the first week of birth for LOS, applying either povidone iodine or 70% ethyl alcohol to the skin over the area,[9] by clinicians using sterile syringe and needle by venipuncture, then carefully the needle was changed and immediately transferred into a *BacT/ALERT*®PF Plus pediatric blood culture bottles. The remaining blood was divided into two tubes, one tube for C-reactive protein (CRP) determination, and the other was used for whole blood count by an automated hematology analyzer. These bottles were specifically designed to provide rapid and sensitive detection of microorganisms (bacteria and yeast) when only a small blood volume was available. They contained a smaller volume of broth, thereby still providing optimal blood to broth ratio when a limited volume of blood was collected. The *BacT/ALERT*®PF Plus blood culture bottle could accommodate up to four mL of blood.[10] As such, it was appropriate for blood cultures from pediatric patients weighing and labeled with the identification number, date, gender, birth weight (kg), birth age (kg), mother's age, Residency area, and hospital's name. Then it was taken into *BacT/ALERT*®3D 120 Combo surprisingly compact, remarkably complete by recording barcodes, all blood cultures have been observed for at least one week to appear either infectious or noninfectious samples of blood by their screen.[10]

CRP Determination

The CRP latex test kit is for the qualitative and semi-quantitative estimation of CRP in neonate serum through specific reagents (CRP Latex kit, India). Fresh clear serum was obtained by centrifugation of blood. The sample was stored at 2-8°C for 48 hours before performing the test. For a longer period of time the serum was frozen. Contaminated serum sample was discarded.

White Blood Cells Count

The WBC count was performed for all suspicious neonates, one ml of blood samples received to testing according to Horbia apparatus.

Identification of Bacteria

At first, the infectious sample was taken into four different plates media and conditions: Blood agar and Chocolate agar used for the anaerobic condition using candle Jar, while other Blood agar and MacConky agar were used for the aerobic condition, all cultures were incubated at 37˚C for 18-24 hours. Sub-culture onto the differential selective medium was achieved by inoculating a single colony into the plate by quadrant method. The inoculated plates were incubated for 24 hours. Identification of bacteria by using conventional methods, including gram staining, depend on cultural characteristics: colonies of bacteria, morphological characteristics: bacterial shape colonial morphology, and changed the exhibition on the media like hemolysis, lactose fermentation, or non-lactose fermentation and motility. The biochemical tests which comprise: indole production, methyl red, voges-proskauer, citrate utilization (IMViC), catalase, oxidase, urease, and coagulase. In addition, the identification was supported through both VITEK 2 compact system[7] and the molecular approach.

VITEK 2 System

The first step to identify isolates by VITEK 2(BioMerieux, Inc, France) was performing gram stain to determine which card will be depended. Pure colony of each isolate was taken re-cultured on nutrient agar, then incubated at 37˚C for 24 hours. Three to five colonies were suspended in 2.5 ml of a 0.45 % saline. The suspended bacteria were used adjusted to a McFarland turbidity standard of (0.50 - 0.63)

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depending on the instructions of the system. Inoculated cards were passed by a mechanism, which cuts off the transfer tube and seals the card prior to loading into the carousel incubator. The carousel incubator can accommodate up to 30 or up to 60 cards. All card types were incubated on line at 35.5 + 1.0 ºC. Each card was removed from the carousel incubator once every 15 minutes, then transported to the optical system for reading, then returned to the incubator until the next read time. Data were collected at 15-minute intervals during the entire incubation period.[11]

Molecular Detection

For supporting the identification of isolated bacteria, the molecular basis method was performed by using the PCR system through the detection of one target gene that related to some commons isolated bacteria. Genomic DNA was extracted from pure cultures, for Gram-negative Bacterial Genomevia *GeNet*Biokit (Canada), and for Gram-positive via AmpliSens® (Russia). Both kits were used according to the fitting protocols in the manufacturer's instructions; 100 µl as a final elution amount. Extracted DNA was stored at -20°C until used. All primers and PCR programs were demonstrated in (Table 1), were provided by (Bio Lab Company), South Korea in lyophilized forms, dissolved in sterilized deionized distilled water to obtain 10 µM as a final concentration. For detection of genes that related with isolate bacteria, 1.0 µl from extracted bacterial DNA and controls were amplified with 10µl of Master Mix (2x RED AMPLICON). PCR products were separated on 1.2% agarose gel with 1 TAE buffer, and DNA bands were visualized with safe dye.

Antibiotic Susceptibility Test

Antimicrobial Susceptibility testing was carried out using Kirby Bauer disc diffusion method according to the (CLSI, 2018) formerly known as (National Committee for Clinical Laboratory Standards).[17] The following antimicrobials were used for Gram-negative bacteria as follows: Ampicillin-Sulbactam (SUM) 20/10 µg, Ceftriaxone (CRO) 30µg, Gentamicin (GN) 10µg, Amikacin (AN) 30µg, Ciprofloxacin (CIP) 5µg, Meropenem (MEM) 10µg, Streptomycin (S)1µg, Tetracycline (TE) 30µg, Piperacillin (PIP) 100µg, Aztreonam (AM) 30µg, Imipenem (IPM) 10µg, Chloramphenicol (C) 30µg, Ceftazidime (CAZ) 30µg, Trimethoprim/ Sulfamethoxazole (STX) 1.25/23.75µg and Cefepime (FEP) 30µg). While antibiotics

Table 1: The PCR program used for amplifying genes *ntrA*, *uspA, blaOXA-51, 16S* rRNA, and *eda-1.*

Target gene	PCR Program							References
	Initial Denaturation (°C/min)	Denaturation (°C/sec)	Annealing (°C/sec)	Extension (°C/sec)	Final Extension (°C/min)	Final Store (°C/min)	Cycles	
ntrA	95/10	95/45	58/45	72/60	72/10	5/10	35	[12]
uspA	95/5	95/15	56-63/45	72/60	72/10	5/10	40	[13]
blaOXA-51	94/3	94/45	57/45	72/60	72/5	5/10	35	[14]
16S rRNA	94/5	95/30	59/45	72/40	72/5	5/10	35	[15]
eda-1	94/5	94/30	51/60	72/30	72/5	5/10	40	[16]

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were used for Gram-positive bacteria as follows: (Penicillin (P)10µg, Gentamicin (GN) 10µg, Cefoxitin (CN) 30µg, Ciprofloxacin (CIP) 5µg, Oxacillin (OX) 1µg, Linezolid (LZD) 30µg, Erythromycin (E) 15µg, Tetracycline (TE) 30µg, Vancomycin (VAN) 30µg, Ofloxacin (OFX) 5µg, Trimethoprim/Sulfamethoxazole (STX) 1.25/23.75 µg, Clindamycin (CL) 2µg, Rifampin (R) 5µg, Amikacin (AN) 30µg, Tobramycin (TOB)10µg and Netilmicin (NET) 30µg). Mueller-Hinton agar (MHA), was used as growth media, then the results were compared with standard Clinical and Laboratory Standards Institute (CLSI),[17] In addition, antimicrobial susceptibility testing was performed by VITEK 2 system for all isolates bacteria through specific cards to Gram-positive and Gram-negative bacteria.

Results

Out of 54 neonate patients during eight months were collected from two Government Hospitals (Rapareen Pediatric Teaching Hospital and Maternity Obstetric Hospital) in NICU/Erbil. The result of this study indicated that proven 42 (77.77%) were EOS, while 12 (22.23%) were LOS, see in (Table 2). In this study, the Gram-negatives bacteria were 31 (57.40%), divided in EOS 25 (80.64%) and was 6 (19.36%) that associated with LOS. while Gram-positive bacteria were 23 (42.60%), was 17 (73.91%) that linked with EOS and 6 (26.09%) that connected with LOS, and the names of isolated Bacteria, as shown in (Tables 4 and 5).

Table 2: Neonatal Sepsis detection according to the neonatal ages, frequency, and percentage.

Neonatal ages (days)	No.	%
Early onset sepsis (< 7 days)	42	77.77
Late onset sepsis (7 – 30 days)	12	22.23
Total	54	100

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Table 3: Neonatal Sepsis detection according to neonates' sociodemographic characteristics, CRP ratio (I.U/ml) and total WBC count (cell/mm^3).

Characteristics	No.	%
Gender		
Female	34	62.97
Male	20	37.03
Birth Weight		
Early low birth weight (< 1000 gm.)	3	05.56
Very low birth weight (1000-1500 gm.)	23	42.59
Low birth weight (>1500 gm.)	28	51.85
Mothers Age (years)		
Early age (<25 years)	28	51.85
Late age (>25 years)	26	48.15
Residency Area		
Urban	17	31.49
Suburban	11	20.37
Rural	26	48.14
Hospitals Name		
Rapareen Pediatrics Teaching Hospital	14	25.93
Maternity & Obstetric Hospital	40	74.07
CRP Ratio		
1/2 Dilution (12 I.U/ml)	8	14.81
1/4 Dilution (24 I.U/ml)	12	22.22
1/8 Dilution (48 I.U/ml)	19	35.19
1/16 Dilution (96 I.U/ml)	15	27.78
Total WBC Count		
< 10000/mm^3	5	09.26
>10000/mm^3	49	90.74

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Table 4: Distribution of isolates bacteria according to early onset sepsis and late onset sepsis.

Microorganisms	EOS	%	LOS	%	Total (%)
Gram-negative bacteria	25	80.64	6	19.36	31(57.40)
Gram-positive bacteria	17	73.91	6	26.09	23(42.60)
Total	42 (77.77%)		12 (22.23%)		54(54.76)

Table 5: The isolated bacteria from blood cultures by VITEK 2 system.

Names of Bacteria	No.	%
Gram-negative Bacteria		
Klebsiella pneumonia	14	25.92
Escherichia coli (E. coli)	12	22.22
Acinetobacter baumannii	5	09.26
Total	31	57.40
Gram-positive Bacteria		
Staphylococcus aureus	12	22.22
Enterococcus faecalis (GDS)	4	07.41
Staphylococcus epidermidis (CoNS)	3	05.57
Staphylococcus haemolyticus (CoNS)	2	03.70
Streptococcus agalactiae (GBS)	1	01.85
Koccuria kristina	1	01.85
Total	23	42.60

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Table 6 shows the identification of common isolates bacteria that detection by amplicon size according to base pair genes through the molecular approach. The results of antimicrobial sensitivity tests for all isolated Gram-negative bacteria against some antibiotics showed varying sensitivity patterns in (Tables 7 and 8).

Table 6: The five primers used to isolated Gram-negative and Gram-positive bacteria in this study.

Bacterial strains	Target gene	Primer	Sequence (5`- 3`)	Amplicon size bp.
K. pneumoniae	*ntrA*	Forward Reverse	5′-CATCTCGATCTGCTGGCCAA-3′ 5′-GCGCGGATCCAGCGATTGGA-3′	90
E. coli	*uspA*	Forward Reverse	5′-CCGATACGCTGCCAATCAGT-3′ 5′ACGCAGACCGTAGGCCAGAT-3′	884
A. baumannii	*blaOXA*-51	Forward Reverse	5′-TAATGCTTTGATCGGCCTTG-3′ 5′-TGGATTGCACTTCA CTTGG-3′	353
S. aureus	*16S* rRNA	Forward Reverse	5′-GTTGACTGCCGGTGACAAAC-3′ 5′-GCTGTTACGACTTCACCCCA-3′	372
E. faecalis	*eda-1*	Forward Reverse	5′- GGGGACAGTTTTGGATGCTA -3′ 5′-TCCATATAGGCTTGGGCAAC -3′	648

Table 7: The percentage of susceptibility pattern of Gram-negatives bacteria to 15 various antibiotics used the Standard Kirby-Bauer disk diffusion method.

Isolated bac.	*K. pneumonia*			*E. coli*			*A. baumannii*		
Antibiotic's Symbols	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**
SUM	0	7.14	92.85	11..2	5.2	83.6	5	0	95
CRO	85.60	0	14.40	86.20	0	13.80	33.4	19.2	47.4
GM	83.30	2.20	14.40	90.2	0	9.80	8.9	4.1	87
AN	06.6	0	93.30	0	3	97	2.9	0	97.1
CIP	28.57	64.28	0.92	81.9	12.4	5.7	0	0	100
MEM	3.30	0	96.60	97	0	3	69	0	31
S	28.57	0	71.42	50	50	0	30	2	68
NET	80	0	20	87.3	0	12.7	79.4	0	20.6
PIP	90.20	0	9..80	100	0	0	95	5	0
AM	100	0	0	100	0	0	100	0	0
IPM	7.14	0	92.85	22.1	12.8	65.1	0	0.9	99.1
C	10.71	10.71	78.58	0	2.1	97.9	92.1	7.9	0
CAZ	100	0	0	100	0	0	89.1	10.9	0
STX	75.9	0	24..1	100	0	0	58	8	39
FEP	97.85	0	2.15	88.9	28.4	11.1	87.8	12.2	0

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Table 8: The percentage of susceptibility pattern of Gram-negatives bacteria to 16 various antibiotics used the VITEK 2 compact system.

Isolated bacteria	***K. pneumonia***			***E. coli***			***A. baumannii***		
Antibiotic's Symbols	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**
AMP	100	0	0	100	0	0	100	0	0
SUM	100	0	0	60.9	10	39.1	?	?	?
TZP	50.5	49.5	0	10.8	20.2	69	100	0	0
CZ	100	0	0	90	0	10	100	0	0
CXM	100	0	0	75.4	10.1	14.5	×	×	×
CRO	50.9	49.1	0	70	10	20	100	0	0
CEP	50	0	50	70.7	0	29.3	100	0	0
EM	54.2	0	45.8	0	0	100	×	×	×
IMP	55.9	0	44.1	0	0	50	100	0	0
AN	49.1	0	50.9	0	0	100	×	×	×
GM	51.2	0	48.8	0	0	100	100	0	0
MN	39.5	2.4	58.1	10.4	0	89.6	100	0	0
CIP	0	0	100	22.9	0	77.1	100	0	0
L	0	0	100	15.9	1.9	82.2	0	0	100
N	0	48.8	51.2	12.2	11.9	75.9	×	×	×
STX	48.2	0	51.8	22.9	0	77.1	100	0	0

×: not performed.

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The results of antimicrobial sensitivity tests for all isolated Gram-positive bacteria against 15 antibiotics showed Varying sensitivity patterns see in (Tables 9 and 10).

Table 9: The percentage of Antibiotics Susceptibility of Gram-positives Bacteria to 16 various antibiotics that used the Standard Kirby-Bauer disk diffusion method.

Isolated bac.	*S. aureus*			*S. epidermidis*			*S. haemolyticus*			*St. agalactiae*			*E. faecalis*			*K. kristina*		
Antibiotic's Symbols	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**
P	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
GM	0	25	75	80.2	19.8	0	100	0	0	0	0	100	100	0	0	100	0	0
CN	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
CIP	79.2	0	20.8	34.1	0	65.9	90	10	0	0	2.2	97.8	55	0	49	0	0	100
OX	100	0	0	100	0	0	90.9	9.1	0	100	0	0	0	0	100	100	0	0
LZD	65.5	0	34.5	0	0	100	15	0	85	100	0	0	0	0	100	0	0	100
E	100	0	0	49	0	51	50	0	50	100	0	0	100	0	0	100	0	0
TE	90	10	0	15.9	0	84.1	15	10	75	0	0	100	0	0	100	0	0	100
VAN	0	14.9	85.1	10	55	35	80	20	0	100	0	0	0	100	0	0	0	100
OFX	75.6	0	24.4	25.2	0	74.8	90	10	0	10	0	90	0	0	100	0	0	100
STX	20.4	10.1	69.5	0	8	92	0	0	100	100	0	0	100	0	0	0	0	100
CL	0	0	100	10	10	80	0	0	100	100	0	0	100	0	0	100	0	0
R	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
AN	0	0	100	0	0	100	0	0	100	70	22.2	7.8	51	49	0	0	0	100
TOB	74.5	0	25.5	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
NET	0	0	100	0	31.2	68.8	0	0	100	20.1	39.1	40.8	0	50	50	0	0	100

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Table 10: The percentage of Antibiotics Susceptibility pattern of Gram-positives Bacteria to 15 various antibiotics that used the VITEK 2 system.

Isolated bacteria	*S. aureas*			*S. epidermidis*			*S. haemolyticus*			*St. agalactiae*			*E. faecalis*			*K.kristina*		
Antibiotic's Symbols	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
GM	33.3	0	66.7	100	0	0	50	0	50	73	27	0	100	0	0	0	0	100
CIP	88.9	0	11.1	100	12	88	50	0	50	0	11	89	0	14.1	85.9	0	0	100
L	17.9	82.1	0	×	×	×	0	59.1	40.9	0	0	100	0	0	100	×	×	×
MO	9.9	0	90.1	0	0	100	0	0	100	×	×	×	×	×	×	0	0	100
E	0	19.8	80.2	100	0	0	100	0	0	91.2	8.8	0	0	7.4	92.6	100	0	0
CL	13.1	1.2	88.1	50	0	50	50	0	50	100	0	0	100	0	0	100	0	0
T	×	×	×	0	0	100	×	×	×	100	0	0	×	×	×	0	0	100
SYD	17.8	0	82.2	×	×	×	67.1	0	32.9	100	0	0	100	0	0	×	×	×
LI	28.1	2.2	69.7	0	28.7	71.3	0	2.2	97.8	90	0	10	5.5	19.4	75.1	0	0	100
VAN	0	0	100	0	0	100	32	0	68	100	0	0	0	0	100	0	0	100
TE	0	0	100	97.9	0	2.1	60	0	40	0	0	100	91.8	0	8.2	0	0	100
TI	0	19.2	80.8	×	×	×	0	0	100	0	18	82	0	0	100	0	0	100
N	0	15.8	84.2	×	×	×	0	0	100	×	×	×	97.8	2.2	0	×	×	×
R	0	9.8	90.2	0	0	100	55.5		44.5	100	0	0	×	×	×	0	0	100
STX	71.1	0	29.9	100	0	0	100	0	0	0	0	100	100	0	0	100	0	0

×: not performed.

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Discussion

Neonates' Sociodemographic Characteristics

The sources of infection for EOS come from maternal obstetric factors, Bacterial pathogens may reach the fetus across the placenta, or as the fetus passes through the colonized birth canal. Bacteria usually reach the blood stream through the fetal swallowing of contaminated amniotic fluid, resulting in bacteremia, while LOS from hospital environmental delivery.[18] Thirty four (62.9%) of the samples were female, and male were twenty (37.1%). The reason of difference gender to sepsis was unknown, or it may be attributed to sex-linked immunoregulatory genes.[19] According to our results, the maternal and neonatal factors which had a significant point such as the Low birth weight (>1500 gm.) was 28 (51.8%), Very Low birth weight (1000-1500 gm.) was 23 (42.60%), and Early low birth weight (<1000 gm.) was only 3 (5.6%), were recorded. These differences may be attributed to the variations in geographical location, population characteristics, and in predisposing factors such as rate of prematurity, low birth weight, and also could vary from hospital to hospital. Our results revealed that the very low birth weight was associated with LOS.[20] reported that premature infants have an increased incidence of sepsis; the incidence of sepsis was significantly higher in infants with a birth weight of less than 1000 gm (26 /1000 live births) than in infants with a birth weight of 1000-2000 gm (8 /1000 live births). Another important point early mother's age to married (< 25 years) was 28 (51.9%), while late mothers to married (> 25 years) was 26 (48.1%). About to residency area urban was 17 (31.5%), suburban was 11 (20.3%), and rural was 26 (48.2%). It means that the early mothers age to married and rural of residency area were more prone to sepsis than other neonates' sociodemographic characteristics. In all races, the incidence of bacterial sepsis, especially with gram-negative enteric bacilli was higher in males than in females. Black infants have an increased incidence of Group B Streptococci disease and LOS. This was observed even after the risk factors of low birth weight and decreased maternal age have been controlled,[21,22] have studies in some different Arabic countries these differences may be attributed to the variations in Neonates' sociodemographic location, population characteristics and in predisposing factors such as Gender, birth weight, mothers age, residency area and also could vary from hospital to other hospital.

Biomarker Assays

In this study, all neonate patients were positive for CRP, and WBC were increased degrees than the normal ranges, most of the positive samples were not positive during the *BacT/ALERT*® PF Plus blood culture bottle, and these samples were ignored. The normal value of neutrophil depends on age, with a peak during the first 12-14 hours of age (7800 – 14500 cells/mm^3). While full-term infant, the value ranged (2700 – 13000 cells/mm^3). The ideal biomarkers can aid in both the early diagnosis and risk stratification and prognosis. Serological marker as C-reactive protein (CRP) and hematological marker as blood cell counts were two importance of biomarkers to early diagnosis of neonatal sepsis, as part of the inflammatory variables.[23] Our results indicated that no significant correlation was found between CRP and WBC tests. CRP has been used as better than the total WBC test for bacterial sepsis. It means that CRP is not definitely a specific marker for neonatal sepsis; this difference can be attributed to that the CRP is a non-specific response to that disease. CRP is not considered to be a useful marker. This indicates that no significant correlation was found between total WBC count with proven cases of neonatal sepsis.[24]

Isolates of Pathogenic Bacteria

The predominant pathogenic bacteria were *K. pneumonia* 14 (25.9%), and the second

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common pathogens were followed by *E. coli* 12 (22.2%) and *A. baumannii* 5(9.3%). It means that *K. pneumonia* was identified as the most common bacteria responsible for neonatal sepsis. In contrast, the total predominant Gram-positive bacteria were 23 (42.6%), that isolated were 17 (31.5%) associated with EOS and was 6(11.1%) LOS, as *S. aureus* was 12 (22.2%) the most common bacterial isolates such as *E. faecalis* was 4 (7.4%), *S. epidermidis* was 3 (5.5%), *S. haemolyticus* was 2 (3.7%), only one isolate (1.9%) for *St. agalactiae* and *Koccuria kristina* found in EOS, it may be attributed to their conditions. The predominant cause of LOS followed by *S. aureus and E. Faecalis*,[25,26] revealed in Kirkuk and Duhok pediatrics hospital that Gram-negatives bacteria were most common and represented by *K. pneumonia, E. coli* and *Pseudomonas sp.* While Gram-positive bacteria; CoNS, *S. aureus, St. pneumoniae* were most commonly isolated.

Molecular Based Detection of Bacteria

One target genes were detected for most common isolated Gram-negative bacteria. To the identification of *K. Pneumonia isolates* by detection of amplicon size 90 base pair *ntrA* gene (Lane:7 and lane13 Figure 1). *E. Coli isolates* by detection of 884 bp.*usp A* gene (Lane 6 and line 12 Figure 2), *A. baumannii* isolates by detection of 353 bp.*blaOXA-51* gene (Lane 4 Figure 3). While Gram-positive bacterial isolates, to the detection of *S. Aureus* isolates by recognition of 372 bp.*16S* rRNA (Lane:2 and 12 Figure 4), and *E. Faecalis* isolates by detection of 648 bp.*eda-1* gene (Lane:4 Figure 5), all detection isolated bacteria were showed in. The molecular methods have an advantage of being rapid as compared to conventional blood culture method and also useful in detecting non-cultivable microorganisms.[27] PCR was used to measure highly conserved extraction of genomic DNA in a variety of isolated Gram-positive and Gram-negative bacteria by agarose gel electrophoresis techniques. This method has the potential to provide a more rapid diagnosis of bacteria and virus infectious, while studies are demonstrating increasing benefit, such tools are not available in all microbiology departments.[27] In addition,[28] reported that various studies had shown a sensitivity of 41% to 100% and specificity of 86% to 100% in comparison to blood culture. There are many reasons why molecular methods cannot replace blood culture as the gold standard. The most important reason is that molecular methods do not differentiate between live and dead bacteria as they are nucleic acid based.[1]

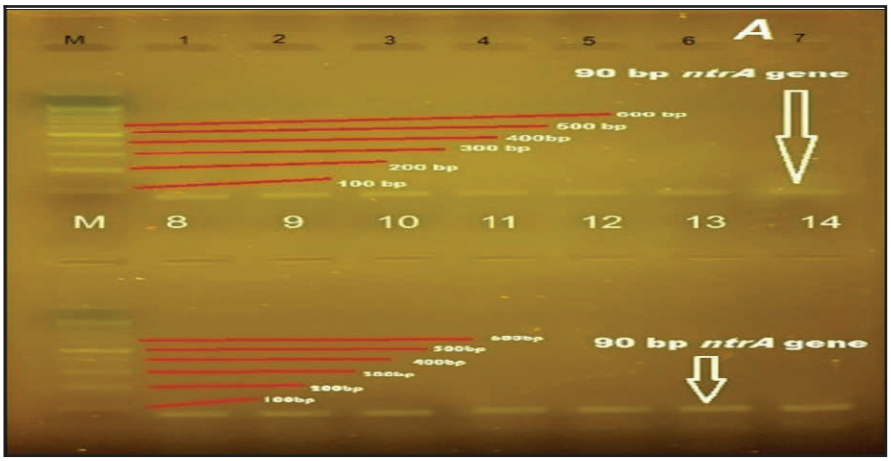

Figure 1: Agarose gel showing amplification of the PCR products. **M**: DNA ladder. **No.**: 1, 2, 3, 4, 5, 6, 7, 8, 9,10,11,12, 13, 14: Bacterial isolates. Lane:7 and lane13 identification of *K. pneumonia* isolates by detection of amplicon size 90 base pair *ntrA*gene.

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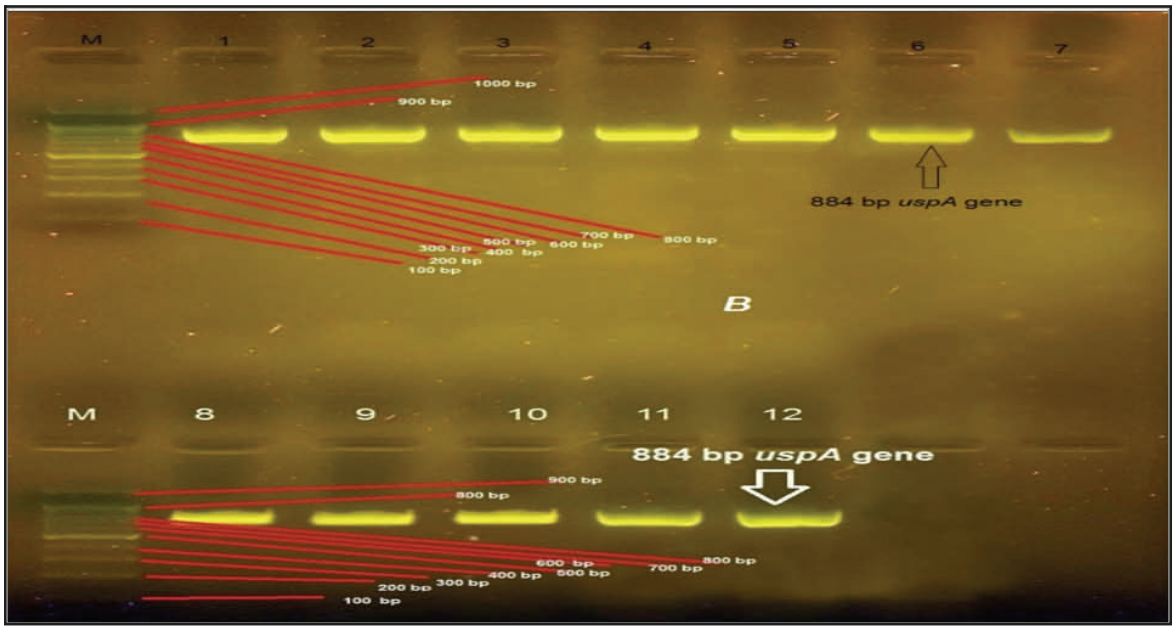

Figure 2: Agarose gel showing amplification of the PCR products. **M**: DNA ladder. **No.**: 1, 2, 3, 4, 5, 6, 7, 8, 9,10,11,12: Bacterial isolates. Lane:6 and lane12 identification of *E. coli* isolates by detection of amplicon size 884 base pair *uspA*gene.

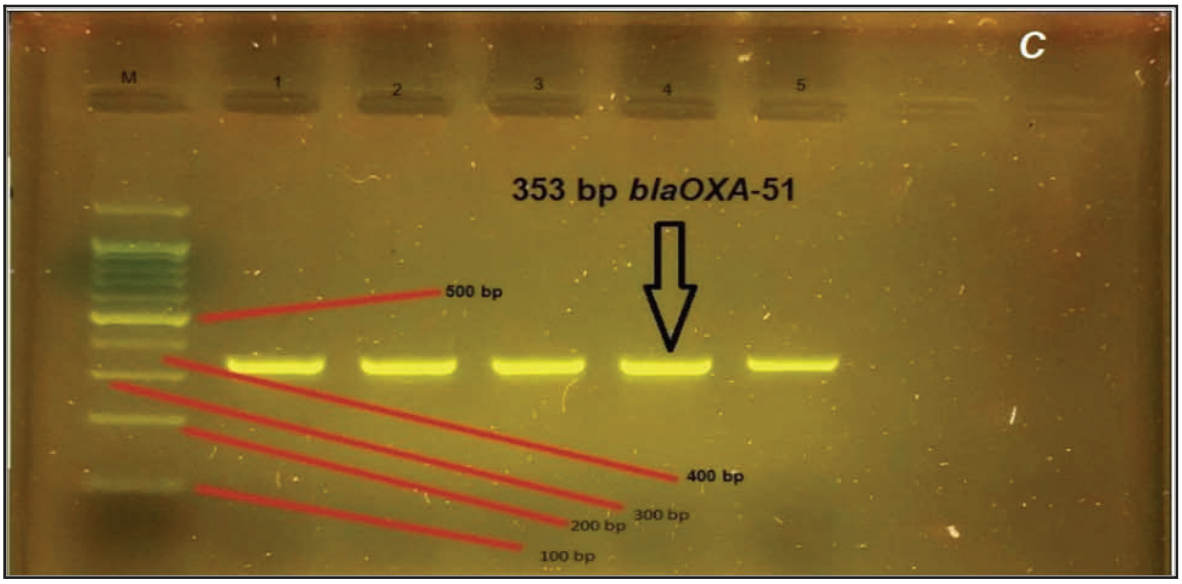

Figure 3: Agarose gel showing amplification of the PCR products. **M**: DNA ladder. **No.**: 1, 2, 3, 4, 5: Bacterial isolates. Lane:4 identification of *A. baumannii* isolates by detection of 353 bp.*blaOXA-51* gene.

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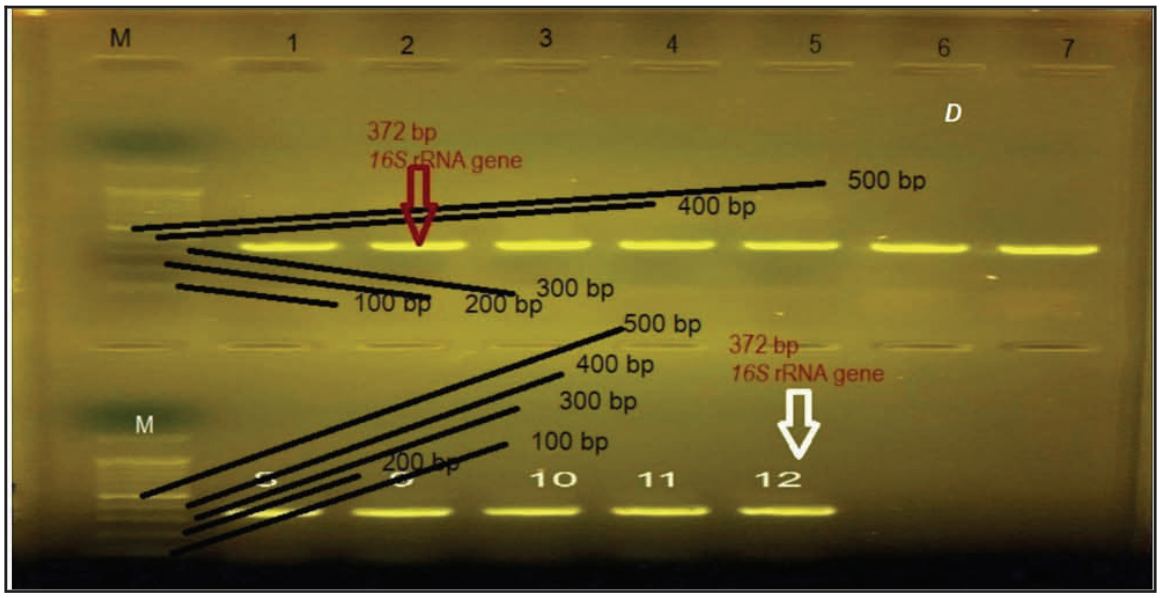

Figure 4: Agarose gel showing amplification of the PCR products. **M**: DNA ladder. **No.**: 1, 2, 3, 4, 5, 6, 7, 8, 9,10,11,12: Bacterial isolates. Lane:2 and Lane 12: *S. aureus* isolates by recognition of 372 bp.*16S* rRNA gene.

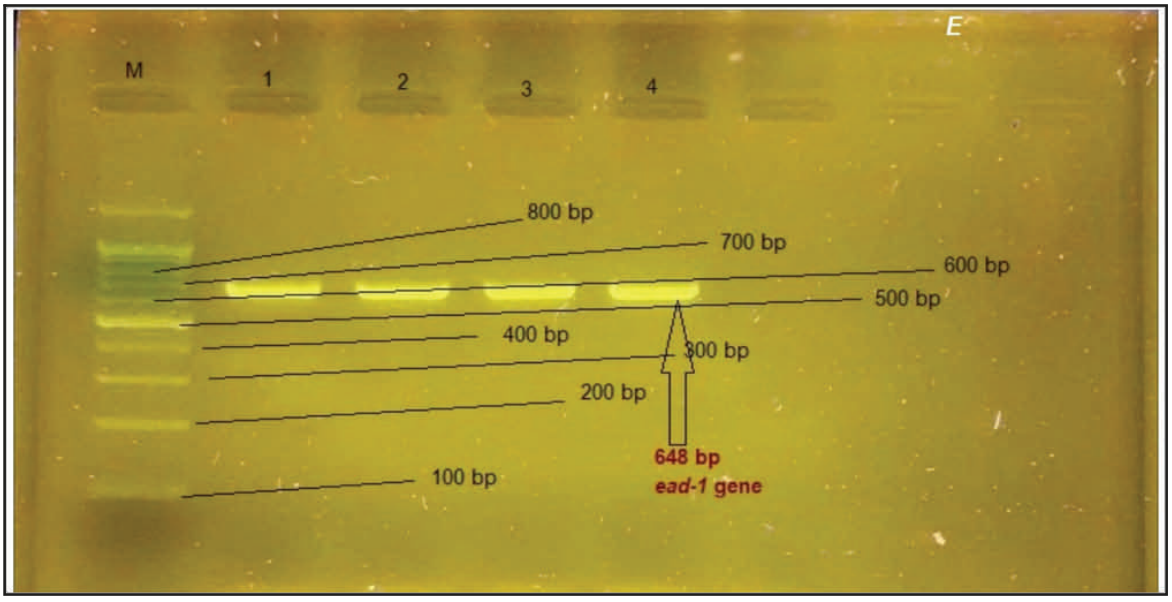

Figure 5: Agarose gel showing amplification of the PCR products. **M**: DNA ladder. **No.**: 1, 2, 3, 4: Bacterial isolates. Lane:4: Identification of *Enterococcus faecalis* isolates by detection of *eda-1* gene.

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Antibiotic Sensitivity Test

The highly resistance percentage was against Ampicillin, Aztreonam, Ceftazidime, Piperacillin, Cefazolin, and many were becoming resistant to Cefepime, Ampicillin/ Sulbactam, Ceftriaxone, and Gentamicin. Whereas, all isolated Gram-negative bacteria were highly sensitive to Imipenem, Ceftriaxone, Levofloxacin that used disk diffusion method, and VITEK 2 system. Antibiotic resistance is now a global problem. Studies of multi drug resistant bacteria causing neonatal sepsis in developing countries are increasing, particularly in NICU.[21] Gram positives bacteria in this study were high resistance 100% against some antibiotics such as *S. aureus*to Penicillin, Cefoxitin, Oxacillin, Erythromycin, and Rifampin when used disk diffusion method, but in VITEK 2 system it becomes decreases degree. Antibiotics were 100% resistant to *St. agalactiae*, as Clindamycin, Teicoplanin, Quinupristin/Dalfopristin, Vancomycin, and Rifampin when used VITEK 2 system. Whereas in Kirby-Bauer disk diffusion method noticed high resistant 100% to Penicillin, Cefoxitin, Oxacillin, Linezolid, Erythromycin, Vancomycin, Trimethoprim/ Sulfamethoxazole, Clindamycin, Rifampin, and Tobramycin. Some variation was noted between both methods to the same antibiotics, such as *K. pneumonia* was resistant to Ampicillin/Sulbactam 100%, Ceftriaxone 50.9%, Amikacin 49.10%, Gentamicin 51.2%, Ciprofloxacin 0%, and Trimethoprim/Sulfamethoxazole 48.2% through VITEK 2 system. While using disk diffusion method were 0%, 85.60%, 06.6%, 83.30%, 28.57%, 75.9%, respectively. While *E. coli* was resist 60.9%, 70%, 0%, 0%, 22.9%, 22.9% during VITEK 2 system, and 11.2%, 86.20%, 0%, 90.20%, 81.9%, 100%, when disc method used respectively. The last bacteria of Gram-negative bacteria were *A. baumannii,* had a partial different degree for both methods when compared with *K. Pneumonia* and *E. coli*. Gram-negative bacteria and Gram-positive bacteria were multidrug resistant (MDR), the antibiotic sensitivity pattern differs in different studies as well as at different times in the same hospitals, because of the emergence of resistant strains as a result of indiscriminate use of antibiotics in from South African.[8] Köksal, Hacimustafaoğlu[29] from India reported that Gram-negative bacteria were resistant to Ampicillin, Amoxicillin, Ticarcillin, Cefazoline, Cefotaxime, Ceftazidime, Ceftriaxone and Aminoglycosides. Only 57.14% of the *E. coli* were sensitive to Gentamicin. *A. baumannii* was mostly sensitive to Amikacin (65%) and Ceftazidime (75%), with high resistance to Gentamicin (85%) and Meropenem (80%), all isolated *A. baumannii* strains were resistant to Tazobactam/Piperacillin. Also, in the current study all isolated *E. coli* were sensitive to Meropenem and Cefotaxime, with high sensitivity to Amikacin (65%) and Tazobactam/Piperacillin (92.8%)[8] from South African. In contrast, most isolated Gram positives bacteria were resistant 100% to Penicillin, Cefoxitin, Rifampin, but 100% were sensitive to Moxifloxacin. But dissimilar with the results reported by El-Din et al.[30] from Egypt. Moreover, Vergnano, et al.[31] reported that It is difficult to compare antibiotic resistance between countries because the epidemiology of neonatal sepsis is extremely variable, that showed in some countries, *S. aureus* is the most common cause of neonatal sepsis, and methicillin resistant strains (methicillin resistant *S. aureus* (MRSA)) are widespread).

Conclusion

Our results showed a high incidence of late onset sepsis in NICU/Erbil, mostly associated with Gram-negative bacteria than Gram-positive bacteria. Early mothers age married (<25 years), and rural area as a residency factor were more prone to neonatal sepsis. In addition, the most pathogens leading to neonatal sepsis are acquired from the mother's genital tract, suggests routine screening of pregnant women to determining appropriate

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treatment for positive cases before delivery. Finally, all isolated Gram-negative and Gram-positive bacteria were developing resistance to commonly used antibiotics, signify that the use of these antibiotics alone may be inefficient.

Competing interests

The authors declare no competing interests.

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