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Escherichia Coli Causing Quick Start Neonatal Sepsis is A Growing Problem in Al-Furat Al-Awsat Region

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ABSTRACT

Subject: The neonatal sepsis is considered as a clinical syndrome manifested by systemic infection symptoms with or without associated bacteremia in newborns ≤1 month of age with clinical symptoms. The aims of this study were: Detection and characterization of *E. Coli* strains phenotypically and genotypically which are isolated from clinical samples and determination the distribution of *BlaCTXM*and *SHV* genes in addition to the study of MDR strains.

Methods: The recent study was done in Gynecology obstetrics at pediatrics hospitals of Al-Furat Al-Awsat region. During the period of November 2020 and February 2021. A number of 50 samples were collected for the bacteriological and molecular diagnosis of *E. Coli*. These samples were diagnosed using microbiological and biochemical tests and antibiogram was carried out using (antibiotics) and the frequency of blaSHV and blaCTXM were evaluated by using PCR.

Results: Our study revealed that of the 50 patients, 26 patients were positive for *E. coli* diagnosis. Additionally, the distribution of *BlaCTXM*gene was 7.6% and 26.9% in urine and blood respectively. While the distribution of *bla*SHV gene was 15.3% and 38.4% in urine and blood respectively. Highest of resistance were related to imipenem and meropenem and amikacin

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25(96.1%) and low resistance of related to ciprofloxacin 10(38.4%). Our study showed that the distribution of *bla*SHV gene was 4 (15.3%) and 10 (38.4%) in urine and blood respectively. In addition, the distribution of *BlaCTXM*gene was 2 (7.6%) and 7 (26.9%) in urine and blood respectively.

Conclusions: Our data showed *E. Coli*is distributed in a high frequency in Al-Furat Al-Awsat area/ Iraq, we have to managed antibiotic therapy and the antibiogram must be done before that any antibiotic used.

Key words: *E. Coli*, MDR, *blaCTXM*gene, *bla*SHV gene.

1. INTRODUCTION

The neonatal sepsis is a significant problem in the world. Actually, it is considered as a disease infecting newborn (with age ≤ 1 month). This infection is a main source of mortality and morbidity in the neonatal age, despite the high advances in intensive neonatal care units and the usage of extended antimicrobial spectrum agents(Schrag and Stoll, 2006).The neonatal sepsis is considered as a significant cause of both mortality and morbidity amongst infants in the developing countries in about (30-50) % of whole deaths yearly. In fact, it has been subdivided into early-onset neonatal sepsis (EOS) (from 0-7 day of age), and late-onset neonatal sepsis (LOS) (from 7-28 days of age)(Rasul, et al., 2007).A limited studies distinguish between the very early onset (during 24 h), EOS (during 24 h to 6 d), and LOS (during > 6-30 d) sepsis(Isaacs, 2003). EOS is belonging to the vertical transmission in birth or labor. It involves sepsis and/ or bacteremia, pneumonia and meningitis while LOS is belong to the vertical, horizontal or hospital aquired infection(Franz, et al., 2001).

World Health Organization (WHO) evaluates that there is approximately (5 million) neonatal mortality annually worldwide, 98% of them are arising in developed countries (Darmstadt, 2001).

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Some risk factors of the neonatal sepsis comprise; low birth weight or prematurity, premature or the prolonged membranes rupture, the traumatic delivery, preterm labor, maternalchorioamnionitis, male gender as well as low socioeconomic case and fetal hypoxia(Schrag and Stoll, 2006).The neonatal sepsis is a significant problem in the world (Karunasekera and Pathirana, 1999). It is considered as a clinical syndrome manifested by systemic contagion symptoms with or without associated bacteremia in newborns less than 1 month of age with detection of a bacteria in the bloodstream "positive blood cultures"(Marchant, et al., 2013).

Generally, Gram negative bacteria are more common mainly Klebsiella, E. coli, Pseudomonas, and Salmonella spp... while the most common Gram-positive organisms are S. aureus, coagulase negative staphylococci (CNs)(Baltimore, 2003), S. pneumoniae and S. pyogenes. E. Coli, Enterobacter, Enterococcus, and Listeria spp. are commonly linked with EOS disease(Movahedian, et al., 2006). While Klebsiella, Acinetobacter, and S. aureus are linked with EOS and LOS. Pseudomonas, Salmonella, and Serratia spp. are mostly associated with LOS. CNS are detected in both(Darmstadt, 2001). These pathogens have established an elevated drug resistance in last two decades (Movahedian, et al., 2006) therefor, the controlling of neonatal sepsis has become a main problem (Rasul, et al., 2007).

Escherichia coli is one of the most common causative agentsof neonatal sepsis. These pathogens are considered as non-pathogenic when they found in the intestines, while when they reach the blood stream, they will become pathogenic. *E. Coli* harbor many virulence factors that are important to invade the host cells, allowing their adhesion, toxin and other factors that protect the bacteria from the immune system. Additionally, extended-spectrumβ-lactamase (ESBL) producing strains that regularly establish antibiotics resistance to other categories (i.e. quinolones,

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sulfonamides and aminoglycosides), making treatment strategies more complex (Fraser, et al., 2006).

It has been described that Enterobacteriaceae are the causative agent of several hospital acquired infections around the world(Elamreen, 2007). It is hard to control infections caused by rods Enterobacteriaceae causing the restriction of therapeutic choices that due to the rising antibiotics resistance. Actually, it has presented that ESBLs as one of the main famous resistance mechanisms in Gram negative bacilli (Gatchalian, et al., 1999).ESBLs are a set of enzymes that cause resistance increasing in Ceftazidime, Aztreonam, Cefotaxime, cephalosporins, penicillin and related Oxyimino-β-lactams, but Clavulanic acid inhibits them. SHV, TEM and CTX-M are the three ESBLs main types. CTX-M, has become more common than TEM and SHV, comprises a family which spread fast among an extensive range of clinically significant pathogens and among geographic regions (Meremikwu, et al., 2005).

Furthermore, the importance and diagnostic technique of ESBLs are not completely established by various laboratories; consequently, there might be a lack of sufficient resources in the laboratories to decrease the antibiotic resistance. A wide variation of ESBLs comprising TEM, SHV, CTX, OXA, AmpC; nevertheless, most of these ESBLs are derivatives of TEM, CTX-M and SHV enzymes which are mostly exist in E. coli and K. pneumonia (Adams-Chapman and Stoll, 2002). The aims of this study included as: 1- detection and characterization of *E. Coli* strains phenotypically and genotypically which are isolated from clinical samples and 2- determination the distribution of *BlaCTXM*and *SHV* genes in addition to the study of MDR strains.

2. Materials and Methods

2.1. Study population

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The recent study was done in Gynecology obstetrics and pediatrics hospitals of Al-Furat Al-Awsat region. During the period of November 2020 and February 2021.A total of 50 neonates' samples (0-29 days of age) admitted with suspected EOS (< 6 days of age).

2.1.1.Sample collection and handling

Using aseptic procedure by (70%) alcohol at the vein puncture site, (1 ml) blood was taken from the vein by a specialized nurse. (1 ml) of blood was inoculated into Brain Heart Infusion Broth (BHI) for culture. The samples were transported to the hospital laboratory. About 12 (46.1%) of the 50 admitted neonates, gain antibiotics on the admission day before collecting blood sample. whereas 14 (53.8%) of patients, who were positive for E. coli culture, did not take any antibiotics.About (10 ml) of urine samples were collected from patient in the morning in sterile containers, and made sure that patient did not take any drug for 3 days before collection. (1 ml) of urine was inoculated into BHI broth for culture.

2.1.2.Bacterial culture and identification

All BHI cultures were incubated aerobically at (37°C) for (18-24 hrs.). Then, they were subcultured by a sterile loop on blood agar, MacConkey, and EMB agar and incubated in bacteriological incubators under aerobic conditions at (37°C) for (18-24 hrs.). All positive blood cultures were studied by their hemolysis characteristic, Gram stain (Freedman, et al., 1981).

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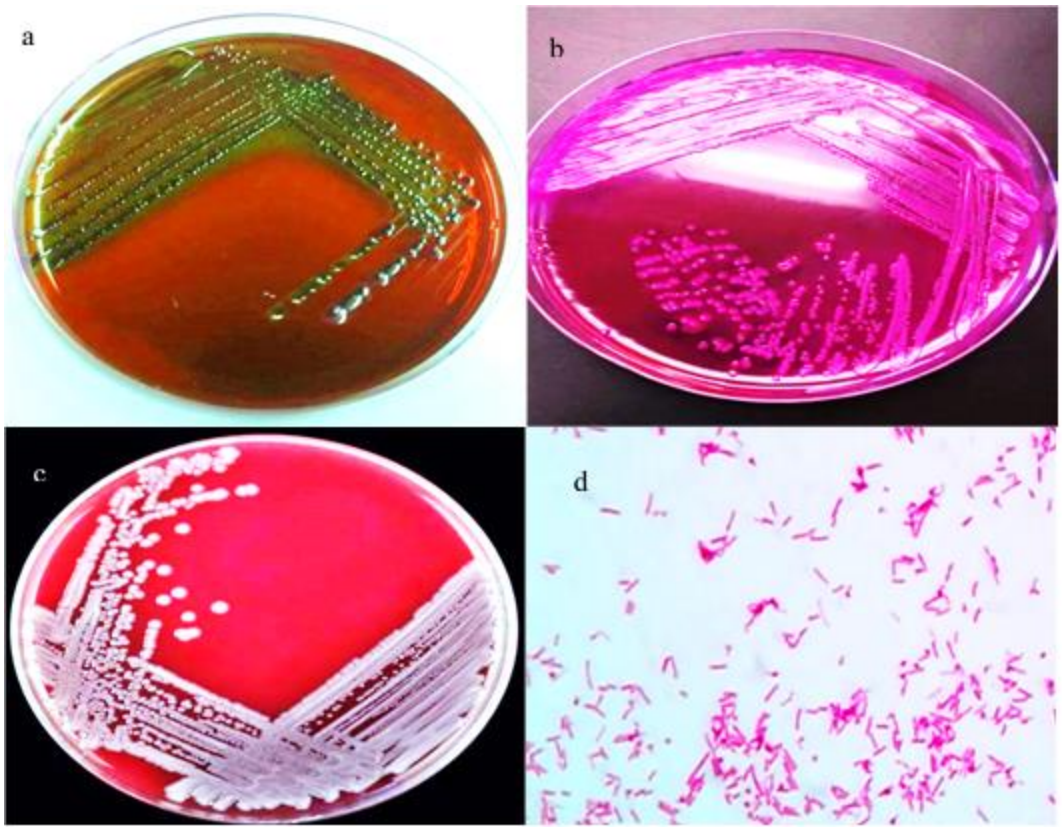

Figure1- A) E. Coli on EMB agar; B) E. Coli on MacConkey agar; C) E. Coli on blood agar and D) E. Coli on Gram stain

By microscopic examination of specimens excluding pus cells and other substances found in urine, culture and presumptive identification from each urine specimen, calibrated loops technique was used (WHO, 1996).EMB agar, bacterial colonies appear as green metallic sheen. On MacConkey agar it was lactose fermenter. In microscopic examination by Gram's stain bacterial cells appear as Gram negative bacilli. A subculture was done to get pure E. coli colonies(Figure 1) (Ghiorghis, 1997).

2.2. Blood hemolysis test

Blood agar media were streaked with bacterial culture in order to test hematolysis and incubated at 37°C for 24 to 48 h. The clear zone around the colonies reveals ß–hemolysis, while the green zone reveals α–hemolysis, and no hemolysis reveals γ- reveals(Ghiorghis, 1997).

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2.2.1. Antimicrobial susceptibility test

Antimicrobial susceptibility testing was accomplished for all E. Coli isolates. About (3-5) bacterial colonies were taken from the pure culture by sterile cotton swab to distribute the bacterial colonies on Mueller Hinton agar and streaked over the medium surface many times. Then, antibiotic sensitivity was examined by using seven antibiotic discs, according to the guidelines recommended by the CLSI (2016), corresponding to the routine medications testing on Enterobacteriaceae. Antibiotic discs (MASTDISCS) were placed on the Muller Hinton agar surface by sterile forceps. Afterward, the plates were incubated at (37°C) for 18-24 h. The Antibiotic were in the following concentrations: (GM) (10 µg), Amikacin (Ak) (30 µg), Ceftazidime (CAZ) (30 µg), Azitreonam (ATM) (30 µg), Ciprofloxacin (CIP) (5 µg), Imipenem (IMI) (10 µg), Meropenem (MEM) (10 µg) Figure (2).

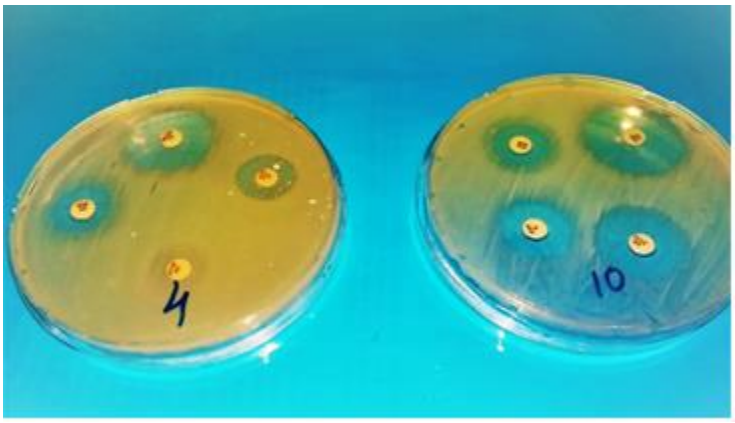

Figure 2- Antimicrobial Sensitivity of E. coli.

The inhibition zone diameters (including the disc diameter) were measured by a pair of electronic calipers; all the isolates were categorized as resistant, intermediate and sensitive, according to the standardized table provided by NCCLs (2000). Low, intermediate and High resistance level is determined if the resistance percentage is < 60%, 60-80% and >80% respectively (Kordek, et al., 2003).

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2.3. Molecular diagnosis of E. Coli by genomic DNA analysis using PCR technique

2.3.1. Extraction of deoxyribonucleic acid (DNA) from bacteria

DNA was extracted from all E. Coli samples using Geneaid Genomic DNA kit . The protocol was designed for extraction genomic DNA of gram-negative bacteria according to manufactures instructions as follows:Bacterial culture was transferred to a 1.5 ml microcentrifuge tube contains 100 µl D.W. then vortexed. After centrifuge for 30 sec at 14-16,000 the supernatant discarded. 180 µl of GT buffer+ 20 µl proteinase k was added to the bacteria pellets. The palateswere resuspendedby shaking vigorously or pipette, then incubated at 60oC for 10 min by inverting the tube every 3 minutes during incubation. 200 µl of GB buffer was added to the tube then vortexed. The tubes were incubated at 70ºC for 10 min and inverted every 3 min to ensure the sample lysate is clear/homogenous. Then 200 µl of ethanol 100% was addedto the sample and mix by vortex and placed in column. After that, 400 µl of W1 was added to the tubes, centrifuged at 13,000 for 30 sec and the tube discarded and new one was used. In to the new tube, 600 µl of wash buffer was added, centrifuge at 13,000 for 30 sec and the supernatant was discarded and again centrifuged at 13,000 for 3 min. Finally, it Placed intoEppendorf tube and 100 µl of elution buffer was added, then incubate at room temperature for 3 min. ultimately, the tubes were centrifuged at 13,000 for 30 sec and the supernatant was discarded and again centrifuge at 13,000 for 3 min.

2.3.2. Polymerase chain reaction (PCR) detection

To detect the target genes, PCR was used as a diagnostic method. The extracted DNA was amplified as follows:

2-3-2-1. Primer's solution preparation

All the primers that used in the recent study were produced by alpha company (Alpha DNA) as a lyophilized of different pmolconcentrations, and dissolved in nuclease-free water DDH2O to

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provide a final concentration of 100 pmol/µl of suspension in the master tube, then 10 pmol/µl was prepared as a working solution by taking 10µl from master tube and completed the volume to 100 µl by adding DDH2O.

2-3-2-2. The PCR amplification

All PCR reactions were amplified in a thermal cycler (labenet-USA). The reaction mixtures were set up as follows: 13µl 0f Master Mix was provided by (wizbio), which contained deoxynucleosides (dNTP), MgCl2, reaction buffer, Taq DNA polymerase, and blue dye that allow progress monitoring during electrophoresis, 2 µl of each primer (10pmol), 4 µl of DNA template, nuclease-free water DDH2O was added to achieve a total volume of 25 µl (Table 1)All amplification reactions were performed going on ice under aseptic condition in a laminar air flow cabinet.

Table 1- The mixtures adopted in PCR analysis for all primers

PCR reaction components	Final concentration	Volume for (1) tube
Deionized water	--------	4 µl
Master mix	1X	13 µl
F- primer	10 picomol	2 µl
R- primer	10 picomol	2 µl
Template DNA	6 ng/µl	4 µl
Total reaction volume	----	25 µl

2.3.2.3. Molecular detection of E. Coli by blaCTXM and blaSHV genes: Specific primers

Two oligonucleotide specific primers were used for identifying E. Coli isolates. The primers and their sequences were listed in table 2.

Table 2- primers sequences and PCR products for detection of *E. Coli*

primers	Primer sequence (5´-3´)	Product size (bp)
BlaCTXM -F	ACCGCCGATAATTCGCAGAT	
BlaCTXM- R	GATATCGTTGGTGGTGCCATA	585
BlaSHV - F	AGCCGCTTGAGCAAATTAAAC	

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BlaSHV - R	ATCCCGCAGATAAATCACCAC	713

The program conditions of mixtures were adopted in PCR analysis of E. Coli as follows:PCR amplifications consisted of 36 cycles of denaturation at 94°C for 5 min, annealing at 52°C for 40 sec. and extension at 72°C for 5 min in a thermal cycler.

2.3.2.4.Quantitation of mRNA of two types of E. Coli Using Real-Time PCR

The total RNA was extracted from two types of *E. Coli* with the TRIzol reagent (Invitrogen, USA) following the manufacturer's instructions. The RNA quality (intact rRNA 28S/18S) was evaluated by agarose gel electrophoresis. One microgram of the total RNA was then reverse transcribed to cDNA using superscript III two-step reverse transcript kit (Invitrogen, USA).

Escherichia Coli BlaCTXM-F BlaCTXM-R, and BlaSHV-R and BlaSHV-F and β-actin genes were amplified using TaKaRa SYBR Green PCR Master Mix (TaKaRa, Japan). Primers' sequences were shown in Table 2. The quantitative real-time PCR analysis was performed on a Bio-Rad Real-Time PCR detection system (Bio-radicycler version 3.0a, Bio-Rad, USA). Amplification was conducted with denaturation for 15 min at 95°C, followed by 40 cycles of denaturation for 5s at 95°C, and annealing/elongation for 30s at 60°C, and a final melting curve analysis. All target genes were normalized to the endogenous reference gene β-actin based on the previous analysis (Bai, et al., 2008). Relative gene expression data were analyzed using 2^[-delta delta C (T)] method (Livak and Schmittgen, 2001).

2.3.2.5. Detection of two types of E. Coli by automated sequencing

According to the result of PCR product, five DNA samples from both genes of E. Coli isolates were subjected to sequencing by NCBI using applied biosystem machine, which gave the identity of the genes comprised with the original genes in Gene bank in NCBI.

3. RESULTS

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3-1. Study populationand clinical characteristics

The sex distribution of neonates with suspected sepsis investigated for bacterial infection is presented in Table 3. Among 50 patients,EOSof males andfemale patients were 48 and52 percent respectively.Maternal data showed that 58 percent of thew were preterm (gestational age < 37 weeks). In the current study, the mean birth weight was 2300 ± 650 g and about 68 % of neonates with sepsis had low birth weight (<2500 g). The most predominant clinicalfeatures like lethargy, feed failure, respiratory suffering and hypothermia (Temp <36oC)were 30, 66, 70 and 82 percent among the 50 EOS patients, respectively (Table 3).

Table 3- Sex distribution, neonatal and maternal data of the neonates' sepsis

Sex distribution (EOS <6 days of age)		Neonatal and maternal data			Symptoms of neonate's sepsis	
Sex	n (%)	Criteria	Type	n (%)	Clinical Signs	n (%)
Male	24 (48)	Gestation age	<37 wk (Pre term)	29 (58)	Lethargy	15 (30%)
			37-42 wk (Term)	21 (42)	Feed failure	33 (66%)
Female	26 (52)	Delivery mode	Vaginal	27 (54)	Respiratory suffering	35 (70%)
			Caesarian	23 (46)	Hypothermia	41 (82%)
Total	50 (100)	Birth weight	<2.5 kg (LBW)	34 (68)		
			2.5-4 kg(Normal)	16 (32)		

LBW= Low Birth Weight

3.2.Bacterial culture

On EMB agar, bacterial colonies appear as green metallic sheen. On MacConky agar it was lactose fermentor. On blood agar it was non heamolytic (γ- heamolytic). In microscopic examination by Gram's stain bacterial cells appear as Gram negative bacilli. A subculture was done to get pure *E. coli* colonies.

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3.3. Antimicrobial sensitivity

The results of the antibiotics susceptibility testagainst *E. Coli*isolated from neonatal sepsis against 7 antibiotics showed that *E. Coli* have resistance in ratios as follows: 96.1%against Imipenem, 100% against Meropenem, 96.1% against Amikacin and 96.1% against Gentamicin.In addition, the current study revealed that, *E. Coli* showed resistance level of 84.6% against ceftazidime, 65.3% against Aztreonam whereas it had low resistance level 38.4% against Ciprofloxacin. Generally, Ciprofloxacin was the most effective antibiotic against *E. Coli*.

3.4. Molecular analysis of blaSHV and blaCTX-M genes by PCR

In the current study, we used both phenotypic and genotypic (PCR for *bla*SHV and *bla*CTX-Mgenes) approaches for *E. Coli* detection.Distribution of *bla*SHV gene was 15.3% and 38.4% in urine and blood respectively (Figure 3a). In addition, distribution of *BlaCTXM*gene was 7.6% and 26.9% in urine and blood respectively (Figure 3b). We concentrated our study on the genes which are the most occurrences in *E. coli* strains.

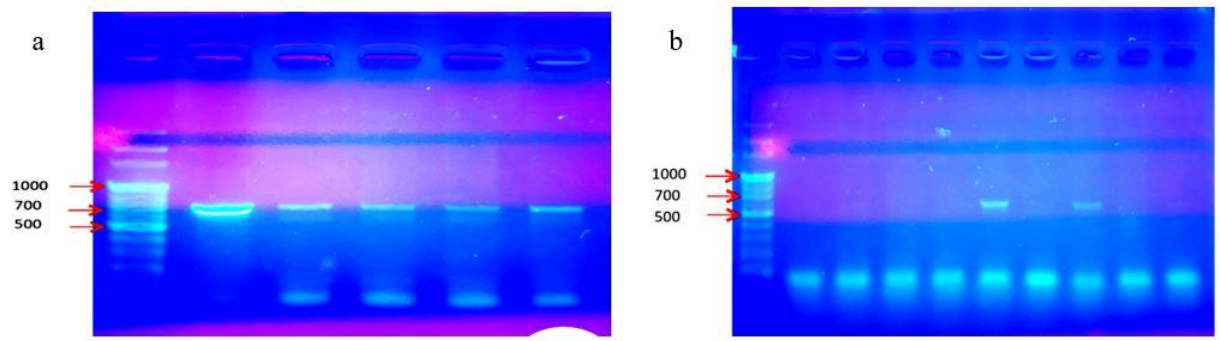

Figure 3-(A) Agarose gel electrophoresis for PCR reaction of *bla*SHVgene products (713) bp for DNA samples. Bands were fractionated by electrophoresis on a 2% agarose gel (1.5hr, 5V/cm, 1XTris-borate buffer) and observed under U.V. light. Lad:100-1000bp DNA marker. (b): Agarose gel electrophoresis for PCR reaction of *bla*CTX-Mgene products (585) bp for DNA samples. Bands were fractionated by electrophoresis on a 2% agarose gel (1.5hr, 5V/cm, 1XTris-borate buffer) and observed under U.V. light. Lad: 100-1000bp DNA marker.

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The investigation of *E. Coli* strains with the detection for *bla*SHV and *bla*CTX-M genes, which are established by phenotypic approaches as they produce extended-spectrum β-lactamases. Accomplished study using of PCR and specific primers for *bla*CTX-M and *bla*SHV genes showed differences in prevalence of these genes in tested *E. coli* strains (Table 4).

Table 4- Distribution of *bla*SHV and *bla*CTX-M gene among samples (%)

Gene	Blood	Urine
*bla*SHV	38.4	15.3
*bla*CTX-M	7.6	26.9

3.5. Gene sequencing

According to the positive results of PCR products, several DNA samples from *E. Coli* were subjected to sequencing, which gave the changes in amino acid sequences of SHV gene (figure 3a), results of Table 5 revealed that there was a change in the domain 33 from the amino acid Arginine (R) to the amino acid glycine (G), as well as changes in the domain 7 from the amino acid proline (p) to the amino acid phenylalanine (F).

Table 5- Results of gene sequencing of SHV gene

SHV gene variant	Sample type	AA change	Other seq. change	Accession #/ID.
SHV-98	Urine	33 R→G	57 C>T	AB731686.1 /BAM25051.1
SHV-98	Urine	33 R G	57 C>T	AB731686.1 /BAM25051.1
SHV-98	Blood	33 R G	57 C>T	AB731686.1 /BAM25051.1
SHV-102	Blood	7 P F	79 G>T	AB731686.1 /WP_063864606.1
SHV-102	Blood	7 P F	79 G>T	AB731686.1 / WP_063864606.1

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In addition, the change in amino acid sequences of *bla*CTX-Mgene (Figure 3b), Table 6 datademonstrated that there was a change in the domain 117 from the amino acid Aspartate (D) to the amino acid Asparagine (N), as well as change in the domain 143 from the amino acid Serine (S) to the amino acid Alanine (A).

Table 6- Results of gene sequencing of *bla*CTX-M gene

*bla*CTX-M type	Sample type	AA change/ location			Accession #/ ID
CTX-M-9	Urine	117 D	N, 143 S	A	AF252621.2 /AAF72529.1
CTX-M-9	Urine	117 D	N, 143 S	A	AF252621.2 /AAF72529.1
CTX-M-9	Blood	117 D	N, 143 S	A	AF252621.2 /AAF72529.1
CTX-M-9	Blood	117 D	N, 143 S	A	AF252621.2 /AAF72529.1
CTX-M-9	Blood	117 D	N, 143 S	A	AF252621.2 /AAF72529.1

4. DISCUSSION

Approximately 5 million of neonatal deaths happened worldwide yearly, about (98%) in developing countries. Sepsis account for (30-50) % of neonatal deaths in developing countries(Murty and Gyaneshwari, 2007). In general, neonatal sepsis mentions to systemic symptomatic bacterial, viral and fungal infections (Bindayna, et al., 2006). Neonatal sepsis is a life-threatening disease and any delay in treatment can cause death. The organisms' spectrum causing the neonatal sepsis varies over time and changes from area to another(Agnihotri, et al., 2004). Additionally, these pathogens have established multi-drug resistance during the last decade's (Lim, et al., 1997).

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The current study focused on the *E. Coli* pattern in neonates with clinical signs of sepsis admitted at Al-Furat Al-Awsat region Hospitals, from November 2020 and February 2021. A total of 50 admitted neonates (0 to 28 days of age) with suspected cases of sepsis were investigated for bacterial infection between November 2020 and February 2021. Among the 50 patients who have EOS, 24 (48%) were males and 26 (52%) were females.A previous study from developing countries have established EOS ratio, for instance in Bangladesh (70.7%) (Mokuolu, et al., 2002), Iran (77.5%) (Movahedian, et al., 2006), Pakistan (42%) (Bhutta and Yusuf, 1997), Saudi Arabia (39%) (Dawodu, et al., 1997)and Libya (31%) (Al Magri, et al., 2006). Frequently the early symptoms of neonatal sepsis are not-definite, for example breathing difficulty, instability of temperature, poor feeding and lethargy (Bindayna, et al., 2006).

4.1. Antimicrobial sensitivity

Overall, 26 *E. coli* isolates obtained from blood and urine samples were studied, and their antibiotic resistance pattern against 7 different antibiotics were tested. The present results showed that *E. coli* resistance differs extensively to various antibiotics. In the current study, out of 50 cases of neonate's sepsis, 26 (52%) were positive for *E. Coli*, which is comparable to the results reported in India (52.6%) (Khaneja, et al., 1999). Lower isolation ratio was described in Iran (6.6%) (Bindayna, et al., 2006; Bromiker, et al., 2001).

In the case of resistance to Imipenem, Meropenem, Amikacin and Gentamicin our results were comparable to another study done in Iraq which revealed that the antibiotic resistance pattern was as follows: 100% Imipenem, 100 % Meropenem, 95.8 % Amikacin, 95.8% Gentamicin (Janjindamai and Phetpisal, 2006). In addition, in the case of resistance toCeftazidime, Aztreonam andCiprofloxacin, the results of current study was similar to previous studies which revealed that generally *E. Coli* isolate showed low resistance levels to ciprofloxacin (Leibovitz, et al., 1997;

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Schrag and Stoll, 2006) while another study from Sydney has revealed that *E. Coli* were sensitive to gentamicin and cephalosporin (Woldehanna and Idejene, 2005).

There was no significant difference in *E. Coli* culture positivity in both neonates receiving antibiotic/s 12 (46.1%) and those who did not receive any antibiotics 14 (53.8%), however, the culture positivity ratio in patients receiving antibiotics is high indicating the significance of culture and antibiotics sensitivity in the neonatal sepsis management. Multiple drug resistance (MDR) was showed in tested *E. Coli*. The high prevalence of *E. Coli* sepsis is associated with MDR resistance to the commonly used antibiotics in the recent study establishes that the septicemia was actually nosocomial infection(Robillard, et al., 1993). Generally, it is difficult to compare antibiotic sensitivity pattern among countries because the infection epidemiology is extremely variable. Many studies revealed the increasing resistance to common antibiotics (Chandra and Milind, 2001; Yoder, et al., 1983).

The spectrum of *E. Coli* causing neonatal sepsis is comparable to that in other reports which indicated that *E. Coli* plays an important role in most neonatal sepsis cases (Agnihotri, et al., 2004).These results were dissimilar to other studies revealed that gram^{+ve} bacteria were the most common pathogen of neonatal sepsis (Levine, et al., 1999; Stoll, et al., 2003).In general, the pattern of *E. coli* causing neonatal sepsis in the recent study is comparable to that reported in developing countries, with gram^{-ve} bacteria (especially *E. coli*)are the main causative agent (Akova, 2016; Dannstadt, 2002). However, this pattern is slightly differing from the results reported in India(Kuruvilla, et al., 1999) and Iran (Movahedian, et al., 2006), where showed that *Pseudomonas aeruginosa* was the major cause of neonatal sepsis followed by *E. Coli.*

The infections of neonates are intensely associated to their suitable detection and administration. Establishing neonatal sepsis is a challenge, as clinical symptoms are frequently nonspecific.

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Consequently, assessment optimum patients care for example antibiotic resistance or probable adverse events, may be problematic. The studying of the risk factors for neonatal sepsis is enormously significant in the clinical management, as it is important in the diagnosis. As well as, this understanding helps to regulate strategies that can reduce the mortality and morbidity, accordingly, the great costs related with nosocomial infections (Ghiorghis, 1997; Robillard, et al., 1993; Shitaye, 2008). Though *E. coli* is considered as one of the most-studied bacteria internationally, its features are changing. Else ways, one significant universal problem is the increasing of the antibiotic resistance by bacteria, which is as "threatens the accomplishments of present medicine" (Lim, et al., 1997).

4.2. Molecular analysis of blaSHV and blaCTX-M genes by PCR

In the current study, we used both phenotypic and genotypic (PCR for blaSHV and blaCTX-M genes) approaches for *E. coli* detection. Our study showed that the distribution of blaSHV gene was 4 (15.3%) and 10 (38.4%) in urine and blood respectively (Figure 3a). In addition, the distribution of *BlaCTXM*gene was 2 (7.6%) and 7 (26.9%) in urine and blood respectively (Figure 3b). We concentrated our study on the genes which are the most occurrences in *E. coli* strains.Many researches revealed that the genes that produce CTX-M β-lactamases were more common in tested *E. coli* strains than the genes encoding SHV-type β-lactamases. The blaCTX-M gene was found in all tested in tested *E. coli* strains. These results are comparable with studies describing CTX-M-family enzymes are predominant (Brook, 2002; Manzoni, et al., 2007).

The investigation of *E. coli* strains was with the detection for blaSHV and blaCTX-M genes, which are established by phenotypic approaches as they produce extended-spectrum β-lactamases. Accomplished study using of PCR and specific primers for blaCTX-M and blaSHV genes showed differences in prevalence of these genes in tested *E. coli* strains (Tables 5 and 6). Many researches

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5. CONCLUSIONS

At the condition similar to the correct study, it is concluded thatall *E. Coli* isolates were developing resistance to the most common antibiotics, indicate that the using of these antibiotics only can be ineffective and biomarker is an important tool for a high diagnostic reliability and accuracy as a guiding tool for assessment of the infection risk and therapy antibiotic.Low birth weight and prematurity were highly related with bacterial culture established neonatal sepsis.Ciprofloxacin was the major effective drug in comparing with other antibiotics tested against *E. Coli* isolates.The discovery of multi-drug resistant (MDR) isolates can limit therapeutic choices. It is recommended that antibiotic sensitivity patterns reveal that ciprofloxacin is the most appropriate drug for the neonatal sepsis treatment.

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