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Molecular identification of extended-spectrum ß-lactamase-producing *E. coli* isolated from neonatal sepsis in Egypt: Expression of CTX-M-15 gene

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Abstract

Sepsis in newborns is a leading cause of neonatal mortality in poor nations. The study aimed to identify the bacteria that cause sepsis in neonates in Egyptian hospitals, to characterize their resistance profiles, and to facilitate the selection of the most effective medication combination for multidrug-resistant bacteria. Between March 2019 to March 2021, nine hundred eighty-nine blood samples were collected from newborns at Al Demerdash and Ain Shams University Specialized Hospital in accordance with established guidelines. The VITEK® 2 system was used to perform bacterial identification and antimicrobial susceptibility testing. The results revealed that 51.4% of patients were positive for blood cultures, of which 60.25% were males and 39.8% were females. Late-onset sepsis was detected in 95% of positive cases, while early-onset sepsis was observed in only 5% of all positive cases. AST-ST01 card (bioMérieux) by VITEK 2 revealed that 96% of *E. coli* isolates were resistant to ampicillin, 91% were resistant to ampicillin / sulbactam, and 83% were resistant to ceftazidime and cefotaxime. The bla_{CTXM15} gene expression was analyzed quantitatively using real-time RT-PCR and shown to be significantly higher in multidrug resistant (MDR) *E. coli* isolates (100 %) than in susceptible isolates, with an average change of 8.0458-fold versus 1.7581. In conclusion, *E. coli* was the most common cause of neonatal sepsis in Egyptian neonatal intensive care units (NICUs). Applying suitable antibiotic management systems in conjunction with infection prevention and control measures might aid in this attempt.

Keywords: neonatal sepsis, *E. coli* isolates, Quantitative real-time RT-PCR, multidrug resistant bacteria, antimicrobial susceptibility test AST, $bla_{CTX\text{-}M\text{-}15}$ gene expression, and treatment regimen.

1. Introduction:

Neonatal sepsis is one of the most prevalent infections in developing countries (**Vergnano, 2005; Waters et al. 2021**). The incidence of neonatal sepsis varies from 15/1000 births to

49170/1000 births (**Cortese et al. 2016; Johansson et al. 2019**). Mortality and morbidity are controlled by the newborn's age, the bacteria that cause the infection, and the availability of appropriate treatments. Neonatal sepsis is classified into two subgroups according to the newborn's age at the time of infection. Sepsis with early-onset (EOS) develops within the first 72 hours postpartum when the infection is transmitted by the mother and spreads through the mother's vaginal canal after birth. Sepsis, with a late onset (LOS), occurs after 72 hours but before 90 days. As a result, babies with neonatal sepsis cannot be ruled out unless the infant is older than 90 days (**Pathak et al. 2018; Ershad et al. 2019**).

In infants, LOS has a greater death rate than EOS, reaching 52%. LOS refers to infections that are spread by the environment, especially nosocomial infections in newborns (**Cortese et al. 2016; Tette et al. 2020**). Neonatal sepsis risk factors include low birth weight, infants with birth abnormalities as indicated by a low Apgar score, maternal perinatal risk factors, and infants with premature amniotic membrane rupture (**Cortese et al. 2016; Murthy et al. 2019; Goswami et al. 2020**).

Bacterial strains and antibiotic resistance are limiting concerns in LOS treatment. *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella* spp. are the most frequently encountered drug-resistant Gram-negative bacteria associated with LOS. Gram-positive bacteria, on the other hand, are mostly *Staphylococcus aureus*, Coagulase-negative *Staphylococcus aureus* (CONS), and *Streptococcus pneumoniae*. The challenge is not only in terms of bacterial infections and infection rates, but also in terms of developing safe and effective treatments that avoid bacterial resistance, and the incidence of bacterial resistance is rapidly increasing, particularly in developing countries, as a result of excessive antibiotic use (**Mohsen et al. 2017; Morris and Cerceo 2020)**.

E. coli infection rates have grown in recent years in hospitals. *E. coli* has been proven to develop resistance to a range of antibiotics and to negatively impact the hospital's microbial community. As a result, *E. coli* has established itself as the most frequently encountered bacteria in nosocomial infections. *E. coli* resistance to penicillin derivatives and third-generation cephalosporins as first-line therapy for neonatal sepsis (**CDC, 2019**).

This resistance depends on the extended spectrum beta-lactamase activity of the newly acquired plasmid carrying the bla $_{CTXM15}$ gene throughout the *E. coli* species. It has been reported in various countries and results in significant resistance to penicillin derivatives and third generation cephalosporins, especially cefotaxime (**Goswami et al. 2020**).

Shibata et al. (2020) detected 1,456 strains resistant to either oxyimino-cephalosporin or cephalosporin. PCR identified 317 *bla* $_{CTX-M}$-positive bacteria in 57 of 132 clinical facilities. Fifty-seven, 161, and 99 strains had *bla* $_{CTX-M}$ genes from the *bla*$_{CTX-M-1}$, *bla*$_{CTX-M-2}$, and *bla*$_{CTX-M-9}$ clusters. Class A beta lactamase genes *bla* $_{CTX-M}$ and *bla* $_{SHV}$ may be identified in one-step reaction using the multiplex real time polymerase chain reaction (PCR) (**Roschanski et al. 2014; Nilsson, 2019)**. Extension of the spectrum of the beta-lactamase detection by real-time PCR has been reported in other studies (**Randegger and Hachler 2001; Chia et al. 2005; Correa-Martínez et al. 2019)**.

Third-generation cephalosporin susceptibility studies have shown that they are less effective in treating sepsis than the currently prescribed antibiotics benzylpenicillin and gentamicin (**Downey et al. 2013; Bielicki et al. 2020)**. Resistance to penicillin derivatives and third-generation cephalosporins limits the use of these antibiotics in neonatal sepsis, suggesting the use of a combination of antibiotics to overcome this resistance (**Abdul-Jabar et al. 2020**).

The combination of amikacin and cefotaxime, as well as piperacillin/tazobactam and cefotaxime, resulted in a significant increase in *E. coli* sensitivity, particularly with the amikacin combination.

The combination of amikacin and cephalosporins increases *E. coli* sensitivity to third generation cephalosporins via a chelating mechanism similar to that of EDTA for magnesium and calcium, which are components of cell wall integrity, thereby facilitating the action of third generation cephalosporins as a result, the availability of combinations will enable more clinical studies to circumvent bacterial resistance and reduce neonatal mortality **(Davis, 1982; Lin et al. 2019**).

The objective of the current study is to examine the correlation between the Cefotaxime minimum inhibitory concentration (MIC) and the fold change in *bla*$_{CTX-M-15}$ gene expression in Egyptian neonatal intensive care units (NICUs) where *E. coli* is the main cause of neonatal sepsis.

2. Materials and methods:

2.1. Study Design and Sample Collection

Blood samples from 989 newborns with sepsis, either EOS or LOS, were collected from the NICU at the Ain Shams Specialized Hospital and EL-Demerdash Hospital between March 2019 and March 2021. The study was authorized by the hospital's ethics committee. Infants were classified into two groups based on the timing of sepsis detection: EOS is identified within 72 hours of birth, whereas LOS is detected beyond 72 hours. A blood culture is the gold standard for diagnosing sepsis and should be performed on infants prior to commencing antibiotic therapy.

2.2. Bacterial culture and growth

Blood samples were cultured on a variety of media, including nutrient agar, MacConkey's agar, Mannitol salt agar, and Brain Heart infusion agar. For 24 to 48 hours, the plates were incubated at 37 ° C. Following incubation, the bacterial colonies were monitored and selected for further analysis.

2.3. Bacterial identification using ViteK2

A 5-ml positive bacterial culture was centrifuged for 5 minutes at 6,000 xg to precipitate the cells. Vitek Densichek bioMe'rieux was used to match 0.5 Macfarland standard in 0.45% sodium chloride solution in order to achieve the necessary turbidity of the bacterial suspension. Afterwards, the VITEK 2 ID-GNB card, the AST-NO09 card, and the bacterial solution was loaded manually. The VITEK 2 system automatically reports results with software update 2.01.

2.4. Antimicrobial susceptibility testing

The VITEK 2 system was used to determine the antimicrobial susceptibility of *E. coli* isolates. Standard strain including *E. coli* ATCC 25922 was used as controls. Antibiotic susceptibility testing results for bacterial isolates identified using the VITEK 2 direct susceptibility technique were compared to those obtained using the broth microdilution MHB method (MIC-2000 System; Dynatech, McLean, VA) using pure cultures in accordance with NCCLS recommendations (**2001**). The 16 antibiotics tested were amikacin, cefepime, ceftazidime, ciprofloxacin, gentamicin, Ampicillin, Ampicillin/Sulbactam meropenem, Cefotaxime, Cefazolin, Cefoxitin, Trimethoprim/Sulfamethoxazole, piperacillin-tazobactam, and tobramycin, Levofloxacin, Nitrofurantoin. Susceptibility discrepancies were classified as very major discrepancies, which. occurred when the VITEK 2 system was sensitive but the reference method was resistant, major discrepancies, which occurred when the VITEK 2 system was resistant but the reference method was sensitive, or minor discrepancies, which occurred when the VITEK 2 system was susceptible or resistant but the reference test was intermediate, or vice versa. If inconsistencies arose, only pure cultures were retested using the reference procedures

2.5. Detection of *bla*$_{\text{CTX-M-15}}$ gene using Real time PCR (RT-PCR)

RNA isolation

The RNeasy Mini Kit Qiagen, Germany, GmbH, Cat. no. 74104 was used to extract RNA according to the manufacturer's instructions with minor modifications. Overnight inoculum was Sub cultured in 10 ml Mueller Hinton broth. Cultures were grown to OD600 = 0.5–0.6 in the mid-log phase. To preserve RNA from degradation, one ml of the RNAprotect Bacteria Reagent Qiagen, Germany, GmbH was added to one volume 0.5 ml of the broth and centrifuged for 10 minutes at 8000 rpm. The supernatant was decanted and the pellets were added to 200 µl of TE buffer with 1 mg/ml Lysozyme Biochemica, Applichem. Cleared lysates were transferred to RNeasy spin columns set in 2 ml collection tubes, spun for 1 minute at 14000 rpm, and the flow-through was discarded. RW1 Buffer (700 µl) was added to the spin column and centrifuged for 1 min. at 10000 rpm. The flow-through was discarded from the spin basket. RPE Buffer (500 µl) was added to the spin column and spun for 1 minute at 10000 rpm and the flow-through was discarded. The RNA was eluted by adding 50 µl RNase-free water to the spin column and centrifuged at 10000 rpm for 1 minute. To remove DNA contaminants from the RNA, TURBO DNase 4 U was added and incubated at 37°C for 30 minutes.

Primer Design

qRT-PCR was conducted with the 16S rRNA and *bla*$_{\text{CTX-M-15}}$ primers (**Table 3**), which amplifies a 253-bp and 281bp fragments. All primers were synthesized commercially Willowfort Birmingham Research and Development Park, Birmingham. **Table (3)** shows the primer sequences specific for the selected candidate gene for *E. coli* strains.

Quantitative real-time reverse transcriptase PCR (q-RT-PCR)

qRT-PCR (quantitative real-time reverse transcriptase polymerase chain reaction) was conducted on 1µg of total RNA using Thermo

Fisher Scientific RevertAid Reverse Transcriptase 200 U/L to synthesize the first strand cDNA. The primers that were utilized are listed in Table 1. Samples were made in triplicate using Thermo Fisher Scientific's SYBR Quantitect SYBR green PCR kit and expression was determined using the Stratagen MX3005P Real-time PCR system. The following were the cycling conditions: 50°C for 2 min, 95°C for 2 min followed by 40 cycles at 95°C for 15 sec, 55°C for 30 sec, and 72˚C for 30 sec. A melt curve was provided to assess the amplification's specificity, as well as a no template control to rule out contamination or primer dimers. Amplification curves were used to assess the results, and Ct values were calculated using the Stratagene MX3005P software. To determine the variance in gene expression on the RNA of the various samples, the Ct of each sample was compared with that of the control group according to the "ΔΔCt" ratio as described by **Yuan et al. (2006)** using the following ratio: $2^{-\Delta\Delta ct}$. Whereas ΔΔCt = ΔCt reference – ΔCt target and Fold increase = $2^{\Delta\Delta Ct}$

3. Results

3.1. Study Design and Sample collections

Nine hundred and eighty-nine blood samples have been obtained from sepsis newborns at EL-Demerdash and Ain Shams University Specialized Hospital; 39.8% were males and 60.25% were females. According to **Table (1)** there had been positive cases of sepsis in total, 26100 of which were early onset EOS and 483100 of which were late onset LOS. There was no significant difference between males and females in infection occurrence at $p \leq 0.05$.

Table 1: Description of the new-born positive cases with sepsis in collected blood samples.

Sex	Total Cases (%)	Positive Cases (%)			
		Early Onset	Late Onset	Total Cases (%)	***P*-value**
Male	394 (39.8)	11(42.3)	191(39.5)	202(39.7)	**0.0422**
Female	595(60.2)	15(57.7)	292(60.5)	307(60.3)	
Total	**989(100)**	**26(100)**	**483(100)**	509(100)	

3.2. Total microorganisms isolated from neonatal positive cases with sepsis

There were 184 Gram-negative bacteria found in total, including 5 from EOS cases and 179 from LOS cases. In all, 311 Gram-positive bacteria were found, including 21 from EOS patients and 290 from LOS cases. From the LOS cases, only fourteen *Candida albicans* were detected. There were 23 *E. coli* isolates from LOS patients in all (**Table 2**). Sepsis with a LOS was detected in 95% of positive cases, whereas sepsis with an EOS was detected in 5% of positive cases. *Klebsiella pneumoniae* being the most prevalent among the gram-negative bacteria, followed by *E. coli* and *Acinetobacter baumanii*. There was a significant difference between gram reaction values and infection incidence (p>0.05).

3.3. Antibiotic susceptibility test (AST)

Antibiotic susceptibility testing by AST-ST01 card BioMérieux VITEK 2 with sixteen different common antibiotics was performed on the total number of drug resistant bacteria that were isolated from positive cases including Ampicillin, Ampicillin /Sulbactam, Piperacillin/Tazobactam Cefazolin, Cefoxitin, Ceftazidime, Cefotaxime, Cefepime, Meropenem, Amikacin, Gentamicin, Tobramycin, Ciprofloxacin, Levofloxacin, Nitrofurantoin, and Trimethoprim + Sulfamethoxazole. The results showed that 96 % of *E. coli* isolates were resistant to Ampicillin, 91% to Ampicillin/sulbactam, and 83% to Ceftazidime and Cefotaxime (**Fig. 1).** *E. coli* isolates were reported to be multidrug resistant in 7/23 (30%).

3.4. RT-PCR amplification

The target gene $bla_{CTX\text{-}M\text{-}15}$ and the reference gene 16S- rRNA was chosen for RT-Q-PCR analysis in *E. coli*. Primers for the $bla_{CTX\text{-}M\text{-}15}$ and 16S rRNA genes were validated using conventional PCR followed by agarose gel electrophoresis prior to the Real-time PCR assays. The existence of a single PCR product of predicted size on agarose gel electrophoresis validated the amplification specificity (**Fig. 2**). **Table 3** summarizes the PCR primer sequences and amplicon sizes for selected candidate genes, amplified from *E. coli* isolates.

Table 2: The Frequency of microorganisms identified from newborn sepsis positive cases between 2019 and 2021 using ViteK 2 system.

Microbial isolates	Frequency			
	Early Onset EOS	Late Onset LOS	Total	%
Gram-negative organisms	**4**	**139**	**143**	**30.6%**
Klebsiella pneumoniae	0	101	101	21.6%
E. Coli	0	23	23	4.9%
Pseudomonas aeruginosa	0	13	13	2.8%
Burkholderia cepacia	1	1	2	0.4%
Citrobacter freundii	1	1	2	0.4%
Enterobacter cloacae	1	0	1	0.2%
Neisseria gonorrhoeae	1	0	1	0.2%
Gram-positive organisms	**20**	**290**	**310**	**66.4%**
Coagulase negative Staphylococcus	2	249	251	53.7%
Staphylococcus aureus	3	29	32	6.9%
Streptococcus viridans	4	9	13	2.8%
Enterococci	9	2	11	2.4%
Bacillus spp.	1	1	2	0.4%
Diphtheroid	1	0	1	0.2%
Fungi	**0**	**14**	**14**	**3.0%**
Candida albicans	0	14	14	3.0%
Total	**26**	**483**	**467**	

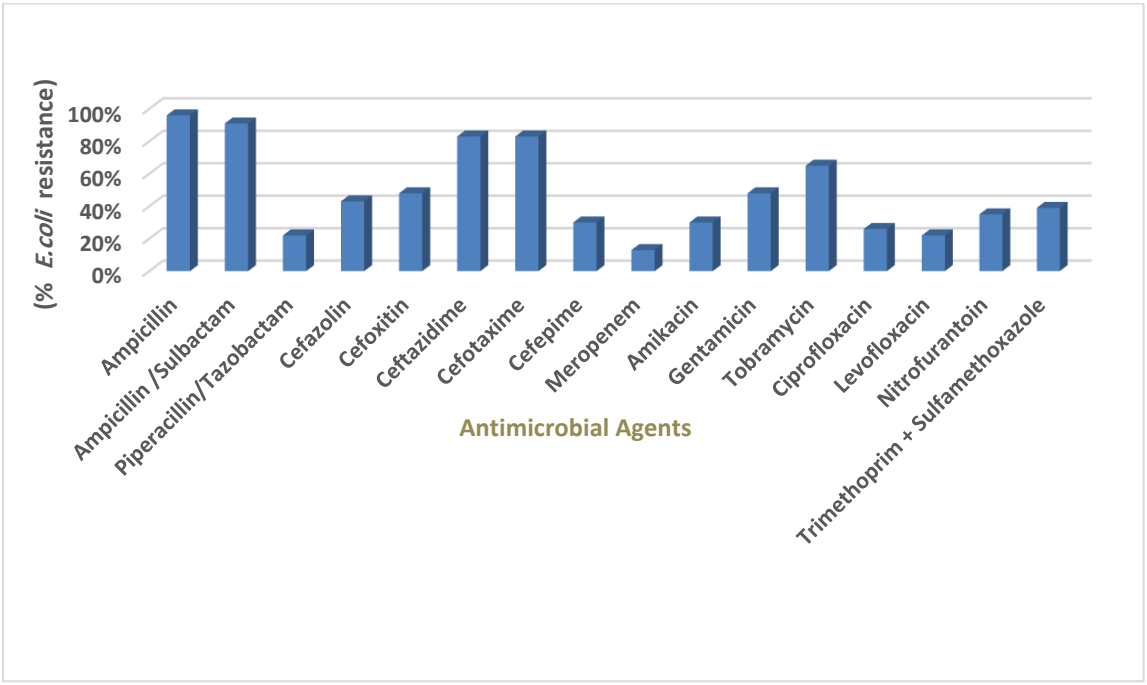

Fig. 1. Antibiotic susceptibilities of *Escherichia coli* isolates isolated from neonatal sepsis cases as determined by the VITEK 2 system.

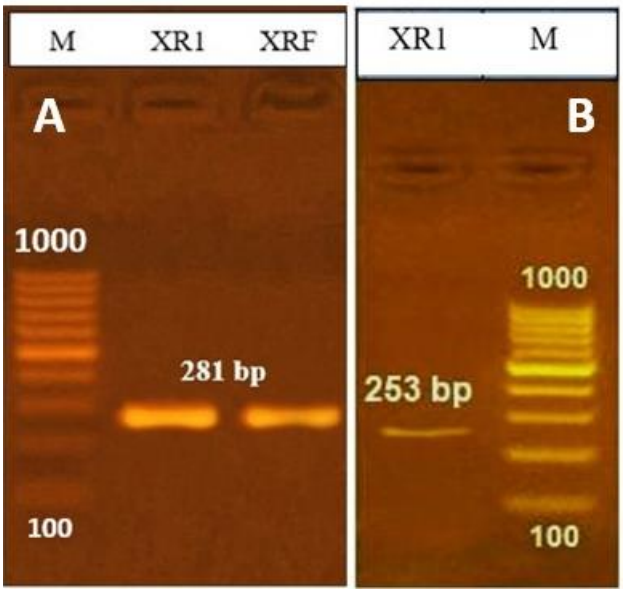

Fig. 2. Agarose gel electrophoresis showing the RT-QPCR products amplified from *E. coli.* A: PCR products 281 bp of *bla*$_{CTX-M-15}$ gene amplified from *E. coli* (XR1) and reference strain *E. coli* ATCC 25922 (XRF). B: PCR product 253 bp of 16S rRNA gene. M: 100 bp DNA Ladder.

3.5. Q-RT-PCR

A high degree of confidence in real-time PCR quantification was achieved with PCR efficiencies ranging from 95.0% for *bla*$_{CTX-M-15}$ to 98.00% for the 16S rRNA sequence (**Table 4** & Supplementary Data S1).

Moreover, the standard curves demonstrated an acceptable correlation coefficient R2, validating the primer pairs' accuracy in the RT-qPCR assay.

The fold change was determined by using the ΔΔCt method (**Table 4**), which provided relative estimate of the expression of *bla*$_{CTX-M-15}$ copies belonging to each sample relative to the house keeping gene 16S rRNA as described under materials and methods. Gene expression levels of the *bla*$_{CTX-M-15}$ gene are significantly higher in resistant *E. coli* isolates (XR1-XR17) than in susceptible ones (XS2-XS6) with a mean fold change of 8.0458 vs 1.7581.

4. Discussion

Neonatal sepsis has mild and variable clinical signs and symptoms, making early identification difficult, and it can affect the treatment of other life-threatening disorders, such as necrotizing enterocolitis and perinatal asphyxia (**English et al. 2004**). Sepsis frequently results in a systemic inflammatory response characterized by the production of proinflammatory cytokines such as tumour necrosis factor alpha, interleukin 1 beta (IL-1), IL-6, and IL-8 (**Mikkelsen et al. 2013; Li et al. 2020**). Sepsis in newborns continues to be a major public health problem, with high rates of morbidity and fatality. While initiating adequate empiric antimicrobial treatment is critical, its effective application needs ongoing monitoring of the local epidemiology and antimicrobial sensitivity profiles of causal bacteria. Severe sepsis treatment is complicated by a lack of accurate data on the optimal use of antibiotics and the development of antibiotic-resistant bacteria.

Table 3. Primers used for the specific amplification of *E. coli.*

Name	Gene	Primer sequence 5'-3'	Size of PCR amplicon	Reference
Pf	16S rRNA	GCTGACGAGTGGCGGACGGG	253 bp	Tivendale *et al.*, 2004
Pr1		TAGGAGTCTGGACCGTGTCT		
CTX-M15	*bla*$_{CTX-M-15}$	CGGAAAAGCACGTCAATGGG	281 bp	GenBank: DQ302097.1
CTX-M15'		GCTGTCGCCCAATGCTTTAC		Liu *et al.* 2009

Table 4: Results of real-time PCR, Ct, and fold change of *bla*$_{CTX-M-15}$ in sensitive and resistant *E. coli* strains.

Gp ID.	Sample ID.	*E. coli 16S rRNA*	*bla*$_{CTX-M-15}$	
		CT*	CT	Fold change
Control	XS1	17.82	22.31	-
Sensitive	XS2	19.31	23.20	1.5157
	XS3	19.24	23.15	1.4948
	XS4	18.52	22.47	1.4540
	XS5	19.61	22.96	2.2038
	XS6	20.33	23.61	2.3134
	Mean	**19.40**	**23.08**	**1.7581**
Resistant	XR1	18.25	18.80	15.3482
	XR2	20.07	20.92	12.4666
	XR3	20.10	21.16	10.7779
	XR4	21.11	22.04	11.7942
	XR5	19.72	20.79	10.7034
	XR6	19.81	21.27	8.1681
	XR7	18.05	19.42	8.6939
	XR8	17.92	19.18	9.3827
	XR9	19.24	20.66	8.3977
	XR10	18.33	19.76	8.3397
	XR11	20.12	21.78	7.1107
	XR12	20.53	22.20	7.0616
	XR13	19.51	21.60	5.2780
	XR14	19.81	21.84	5.5022
	XR15	20.24	22.43	4.9246
	XR16	21.44	23.44	5.6178
	XR17	20.20	22.35	5.0630
	Mean	**19.67**	**21.16**	**8.0458**

CT= Cycle Threshold

Blood culture continues to be the gold standard for diagnosing septicemia (**Gohel et al. 2014**). Pathogen identification and antibiotic susceptibility tests are critical for initiating appropriate therapy and managing long term problems (**Philippe et al. 2012**).

The current study found two distinct classes of microbial isolates: bacterial and fungal isolates. 453 of 467 isolates were identified as bacterial, while only 14 were identified as fungal. *Candida albicans* was isolated as a fungal isolate, whilst bacteria (453 isolates) included a variety of Gram-positive and Gram-negative bacteria. It was discovered that 184 isolates were Gram-negative bacteria and 311isolates were Gram-positive bacteria, which accounted for the majority of isolates (indicating that they are the primary cause of septicemia). These findings contradicted the results of several prior studies, which indicated that gram - negative bacteria were more prevalent in newborn sepsis (**Joshi et al. 2000; Aftab and Iqbal, 2006**). The results showed that Gram-positive isolates were significantly more common in LOS neonates than in EOS.

A total of 467 culture-positive sepsis cases, 101 (21.6%) were *K. pneumoniae*, the majority of which were isolated in LOS, 23 (4.5%) *E. coli* were detected in LOS, and *Pseudomonas aeruginosa* 13 (2.8%) were found in LOS. **Ghonaim et al. (2021))** found similar findings, indicating that *K. pneumoniae* was the predominant isolate (31.6 %) among newborns with confirmed sepsis. *E. coli* was also found to predominant in other study done by **Mondal et al. (1991)**. Another study by **Bhat et al. (2011)** discovered that 90.8 % of organisms in India were gram-negative, with *Pseudomonas* (33.2 %), *Klebsiella* (31.2 %), and *E. coli* (4.4 %). However, **Pais et al. (2012))** reported that the most frequent organism in EOS was *Pseudomonas* (11.46 %). On the other hand, **Al-Matary et al. (2019))** found that *Staphylococcus spp.* were the most common bacteria found in blood cultures, followed by *Klebsiella* spp. Similarly, (**Alrafiaah et al. 2016)** observed that the majority of bacteria responsible for newborn sepsis were gram-positive, with CoNS accounting for 35% (15/43) of total isolates. Another study in Ethiopia reported by (**Mulat et al. 2013)** found that the majority of isolates (69%) were Gram-positive bacteria (with coagulase negative staphylococci being the most common isolates) followed by 31% Gram-negative bacteria. On the other hand, **Shehab El-Din et al.** (**2015)** reported that coagulase-negative staphylococcus was the most prevalent pathogen in more than 50% of the positive blood samples. Moreover, **Köstlin-Gille et al. (2020)** reported that *E. coli*, is the most prevalent isolate followed by Coagulase negative streptococci and group B streptococci in *EOS* While Coagulase negative streptococci is the most prevalent isolate followed by *Staphylococcus aureus in* case of LOS. This difference might be explained by geographical and temporal variances (**Lamichhan and Mishra 2019**). It is difficult to compare antibiotic susceptibility profiles between countries, as the epidemiological profile of newborn sepsis varies considerably depending on the usage and accessibility of over-the-counter antibiotics (**Jumah and Hassan 2007**).

The current study showed that multidrug-resistant *E. coli* isolates were prevalent in neonatal sepsis cases. We found that 7/23 (30%) of the *E. coli* isolates showed high resistance 22% - 96% to Ampicillin, Ampicillin /Sulbactam, Piperacillin/Tazobactam Cefazolin, Cefoxitin, Ceftazidime, Cefotaxime, Cefepime, Meropenem, Amikacin, Gentamicin, Tobramycin, Ciprofloxacin, Levofloxacin, Nitrofurantoin, and Trimethoprim + Sulfamethoxazole according to AST -VITEK 2. The large diversity in the antimicrobial susceptibility pattern of first- and second-line drugs shown in this study might be attributed to physicians' prescribing patterns, which are influenced by predicted (rather than confirmed) resistance and antibiotic availability. Regarding other studies of antimicrobial susceptibility, the results were consistent with those of (**Flannery et al. (2021))** who reported that 721 infants with *E. coli* infection admitted to 69 neonatal intensive care units across the United States from 2009 to 2017, 66.8 % of isolates from blood, cerebrospinal fluid, and urine specimens were resistant to ampicillin, 16.8 % to aminoglycosides, and 5.0 % had an extended-spectrum beta -lactamase. Due to the well-established limitations of blood cultures in terms of turnaround time and sensitivity, a novel rapid and effective method for detecting and identifying *E. coli* in blood samples from neonates with suspected sepsis is required. This study demonstrates the feasibility of using real-time polymerase chain reaction (qRT-PCR) to quantify the $bla_{\text{CTX-M-15}}$ gene in MDR *E. coli* isolates from newborns with confirmed sepsis. Our PCR results were comparable to those obtained by (**Jordan et al. 2000)**, who conducted a PCR investigation with sufficient sensitivity on clinical samples from septic newborns, employing a preculturing method to increase the number of bacteria in the sample and facilitate bacterial identification. Our findings indicated that MDR *E. coli* isolates (XR1-XR17) carrying the $bla_{\text{CTX-M-15}}$ gene were the most often encountered ESBL-producing isolates among newborn sepsis clinical isolates. The q-RT-PCR results revealed that all $bla_{\text{CTX-M-15}}$ amplified products expressed at a high level in MDR *E. coli* isolates (30 %). The $bla_{\text{CTX-M-15}}$ gene expression was significantly different between resistant and susceptible *E. coli* isolates, with a mean fold change of 8.0458 versus 1.7581. $bla_{\text{CTX-M-15}}$ was identified as the most prevalent gene among *E. coli* and *K. pneumoniae* in Riyadh (Saudi Arabia) (**Ohlsson et al. 1986**). A study reported by (**Moghaddam et al. 2014))** showed that 27 (27%) of the bacterial isolates were ESBL producers with the highest frequency for *K. pneumoniae* (47.4%) and *E. coli* (17.9%). The current results revealed that 73.9 % of *E. coli* isolates (XR1-XR17) had a significant higher level of $bla_{\text{CTX-M-15}}$ gene expression than the sensitive isolates (21.7 %). The mean value of the $bla_{\text{CTX-M-15}}$ transcript level was 8.0458-fold higher than that of the sensitive isolates (1.7581) and there was a significant difference between ESβL-producing and non-ESβL-producing *E. coli* ($p > 0.05$). Numerous authors have reported various rates of ESBL production. **Ananthkrishnan et al. (2000)** revealed a 58.06 % prevalence of *E. coli* express ESBLs. In a study conducted in Nagpur, India, 58.3 % of *K. pneumoniae* and 50% of *E. coli*

were found to produce ESBLs, which is fairly comparable to the current findings (**Mane et al. 2010**).

5. Conclusions

In this study bla $_{CTXM15}$ -positive *E. coli* is noted in neonates with confirmed sepsis in Egyptian Hospitals. The results indicated that *E. coli* was found to be the second most common infection causing LOS in newborns. It is important to highlight the high prevalence of MDR *E. coli* isolates expressing *CTX-M-15* consecutively, providing high-level resistance to multiple drugs. There was an unacceptably high level of antibiotic resistance, particularly to frequently used antibiotics. As a result, the choice of antimicrobial therapy and the subsequent prognosis of septicemia are heavily influenced by the clinician's ability to correctly identify bacteria and accurately describe their susceptibility to antibiotics. By combining infection prevention and control strategies with appropriate antimicrobial stewardship programmes, this challenge could be overcome.

Ethical approval

This research was approved by the Ethical Committee, faculty of medicine, Ain Shams University. Ethical approval number FWA000017585.

Conflict of Interest

The authors declared that they have no known conflicting financial or personal interests that might seem to have influenced the work presented in this study.

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