Molecular identification of Multi Drug-Resistant *Klebsiella pneumoniae* isolated from neonates with sepsis in Egypt

Abstract

Neonatal sepsis is one of the most prevalent infections today, claiming the lives of almost one million newborns in developing countries. Our objective is to determine the bacteria that cause sepsis in newborns in Egyptian hospitals, as well as to discover their resistance profiles, allowing for the selection of appropriate drug combinations against multidrug resistant MDR bacteria. Nine hundred and eighty-nine blood samples were collected from neonates in Al Demerdash and Ain Shams University Specialized Hospital from March 2019 to March 20121 according to the standard protocols. Bacterial identification and antimicrobial susceptibility test AST were performed using VITEK® 2 system. The results revealed that 51.4 % of patients had positive blood cultures, with 60.3 % females and 39.7 %. Males. Sepsis with late onset was detected in 95% of positive cases, while sepsis with early onset was observed in only 5% of the total positive cases. According to the study, 98.3 % of *Klebsiella Pneumoniae* isolates were resistant to Ampicillin, 91% to Ampicillin/sulbactam, and 90% to Ceftazidime and Cefotaxime. Quantitative Real-time RT-PCR analysis of the $bla_{CTX\text{-}M\text{-}15}$ gene expression found that (69%) of MDR *K. pneumoniae* isolates expressed the gene at a significantly higher level than susceptible isolates, with a mean fold change of 10.0349 versus 1.8646. In conclusion, *Klebsiella pneumoniae* is the leading cause of neonatal sepsis in the majority of Egyptian hospitals' NICUs. Application of infection prevention and control strategies, in conjunction with appropriate antimicrobial stewardship programs, may be beneficial in overcoming this challenge.

***Keywords*:** *Klebsiella pneumoniae*, neonatal sepsis, multidrug resistant MDR bacteria, antimicrobial susceptibility test AST, Real-time RT-PCR, $bla_{CTX\text{-}M\text{-}15}$ gene expression, and treatment regimen.

1. Introduction

One of the most common diseases in developing countries is neonatal sepsis **Vergnano, 2005**. Neonatal sepsis has an incidence ranging from 1-5/1000 live birth to 49-170/1000 live birth **Cortese et al. 2016**. The mortality and morbidity rates vary according to the neonate's age, the type of bacteria causing infection, and the availability of effective antibiotics. Neonatal sepsis is classified into two subtypes based on the neonate's age at the time of infection. Early onset sepsis EOS develops within the first 72 hours after delivery when the infection is caused by the mother and spreads during birth via the maternal vaginal tract. Sepsis with late onset LOS develops after the first 72 hours but before the first 90 days. Thus, no newborn with neonatal sepsis can be excluded unless the neonate is older than 90 days **Pathak et al., 2018**. LOS has a higher mortality rate than EOS among neonates, reaching 52%. LOS is a term that refers to an infection that is acquired through the environment, specifically a newborn hospital-acquired infection **Cortese et al. 2016**. Risk factors for neonatal sepsis include low birth weight LBW babies, infants with functional impairment at delivery as indicated by a low Apgar score, and infants with mother perinatal risk factors premature rupture of membranes **Cortese et al. 2016; Murthy et al. 2019; Goswami et al.,**

2020. The type of bacteria and its resistance to antibiotics are limiting factors in LOS treatment. The most common drug resistant gram-negative bacteria associated with LOS are *Klebsiella pneumonia*., *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella spp.* whereas the most common gram-positive bacteria include mainly *Staphylococcus aureus*, *Coagulase-negative staphylococci* CONS, and *Streptococcus pneumoniae.* The challenge is not confined to bacterial infection or infection rate, but also to appropriate treatment that is safe and circumvents bacterial resistance, where the incidence of bacterial resistance is rapidly increasing, particularly in developing countries due to miss-use of antibiotic **Mohsen et al. 2017**. In recent years, the frequency of *Klebsiella pneumoniae* has increased in the hospital environment. *Klebsiella pneumoniae* develops resistance to a variety of drugs and has been proven to affect the hospital microbiome **Gray et al. 2012; Mohsen et al. 2017**. As a result, *Klebsiella* emerges as the predominant pathogen linked with hospital-acquired infections **Malik, 2010**. *Klebsiella* resistance to penicillin derivatives and third-generation cephalosporins as first-line therapies for neonatal sepsis **Hammoud et al., 2017; Basavaraj et al., 2013**. This resistance is dependent upon the expanded spectrum beta lactamase activity of newly acquired plasmid bearing the $bla_{CTX\text{-}M\text{-}15}$ gene across *Klebsiella* species **Soge et al., 2006; Almogbel et al., 2021**. This has been reported in various countries, resulting in a significant level of resistance to penicillin derivatives and third generation cephalosporins, particularly cefotaxime.

In Japan, **Shibata et al., (2006)** isolated 1,456 gram-negative bacterial strains resistant to cephalosporin using the polymerase chain reaction PCR. The results demonstrated that all bacterial strains harbored $bla_{CTX\text{-}M}$ genes. Class A beta lactamase genes $bla_{CTX\text{-}M}$ and bla_{SHV} may be identified in one-step reaction using the multiplex real time polymerase chain reaction (PCR) established by **Roschanski et al., (2014**). Extension of the spectrum of the ESL beta-lactamase detection by real-time PCR has been reported in other studies (**Randegger and Hachler 2001; Chia et al., 2005)**.

Third-generation cephalosporin susceptibility tests show that they are no more effective than the currently prescribed antibiotics benzylpenicillin and gentamicin in treating sepsis **Downie et al. 2013**. Due to resistance to penicillin derivatives and third generation cephalosporins, the use of these antibiotics in neonatal sepsis is limited, suggesting the use of a combination of antibiotics to overcome this resistance **Abdul-Jabar, Abd, and Abdulamir 2020**. The combination of amikacin and cefotaxime, as well as piperacillin/tazobactam and cefotaxime, resulted in a significant increase in Klebsiella sensitivity, particularly with the amikacin combination **Giamarellou et al. 1984**. The combination of amikacin and cephalosporins increases Klebsiella's 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 **Wooley et al., 1984; Krause et al., 2016**. As a result, the availability of combinations will enable more clinical studies to circumvent bacterial resistance and reduce neonatal mortality. The current study investigated that Cefotaxime MIC and fold change in blaCTX-M-15 gene expression were shown to be strongly correlated, in Egyptian neonatal intensive care units NICUs where *Klebsiella pneumoniae* is a primary cause of newborn

sepsis. To overcome CTXM-15 resistance, it is recommended that amikacin and cefotaxime be included in the combination therapy, followed by piperacillin/Tazobactam and cefotaxime.

2. Materials and methods

2.1 Sample Collection:

Between March 2019 till March 2021, 989 blood samples have been collected from 902 neonates diagnosed of having sepsis, either EOS or LOS, at NICU of Ain Shams Specialized hospital and EL-Demerdash hospital. The hospital's Ethical Committee approved the study. Infants were divided into two groups according to the time at which sepsis was detected: EOS identified within 72 hours of life and LOS diagnosed after 72 hours of life. The gold standard for diagnosing septicemia is a blood culture and should be conducted to the neonates before initiating antibiotic therapy. One millilitre of blood was plated on a culture medium. A blood culture was checked for 72 h before being declared sterile. Within 12-24 hours, it is now possible to detect growth using the BACTEC or BACT/ALERT culture systems, which detect bacteria at a concentration of 1-2 CFU/ml. Blood samples obtained by indwelling catheters or lines are prone to contamination.

2.2 Bacterial Culture and Growth:

Blood samples were cultured on different types of media including nutrient agar, MacConkey's agar, Mannitol salt agar and Brain Heart infusion agar. The plates were incubated at 37°C for 24-48 h. Following incubation, the bacterial colonies were monitored, and the suspected colonies were selected for further identification.

2.3 Bacterial Identification using ViteK2:

A 5-ml of positive bacterial culture was centrifuged at 6,000 xg for 5 min to pellet bacterial cells. VITEK Densichek bioMe'rieux was used to adjust the turbidity of the bacterial suspension to match the McFarland 0.5 standard in a 0.45% sodium chloride solution to achieve the desired turbidity. This was followed by manually loading the VITEK 2 ID-GNB card, the AST-NO09 card, and the bacterial solution. With software release 2.01, the VITEK 2 system automatically reported the results.

2.4 Antimicrobial susceptibility testing

The VITEK 2 system was used to determine the antimicrobial susceptibility of K. pneumoniae isolates. Standard strain including *Klebsiella Pneumoniae* ATCC 13883 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 MB 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 blaCTX-M-15 gene using Real time RT-PCR

2.5.1 RNA isolation

The RNeasy Mini Kit Qiagen, Germany, GmbH, Cat. no. 74104 was used to extract RNA in line with the manufacturer's instructions with minor modifications. Subcultured the overnight inoculum in 10 mL of 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. 700 µl of Buffer RW1 was added. Centrifugation was done for 1 min. at 10000 rpm. The flow-through was removed from the spin basket. 500 µl of RPE Buffer was added to the spin column and spun for 1 minute at 10000 rpm. The flow-through was discarded. To elute the RNA, 50 µl RNase-free water was added and centrifuged at 10000 rpm for 1 minute. To eliminate DNA contamination from the RNA, TURBO DNase 4 U was added and incubated at 37°C for 30 minutes.

2.5.2 Primer Design

QRT-PCR was conducted with the 16S-23S ITS and *blaCTX-M-15* primers **Table 4**, which amplifies a 281-bp fragment. All primers were synthesized commercially Willowfort Birmingham Research and Development Park, Birmingham. **Table 4** shows the primer sequences specific for the selected candidate gene for *K. pneumoniae* strains.

2.5.3 Quantitative real-time reverse transcriptase PCR QRT-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 Stratagene 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 Sample collections

Nine hundred and eighty-nine blood samples were 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 were 509100 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	1142.3	19139.5	20239.7	**0.0422**
Female	59560.2	1557.7	29260.5	30760.3	
Total	**989100**	**26100**	**483100**	509100	

3. 2 Total microorganisms isolated from neonatal positive cases with sepsis

The total number of Gram-negative bacteria detected was 184, including 5 from EOS cases and 179 from LOS cases. There were 311 Gram-positive bacteria in total, 21 of which were from EOS cases and 290 from LOS cases. Only fourteen *Candida albicans* were identified from LOS cases. The total number of isolates of *K. pneumoniae* from LOS cases was 101, whereas the total number of *E. coli* isolated from LOS cases was 23 (**Table 2)**. Sepsis with a LOS was detected in 95% of positive cases, whereas sepsis with an EOS was detected in only 5% of positive cases. Gram-negative organisms were predominant with *K. pneumoniae* being most frequent followed by *E. coli*, and *Acinetobacter baumanii*. There was highly significant difference between gram reaction results and infection occurrence at $p > 0.05$.

Table 2: The Frequency of microorganisms identified from newborn sepsis positive cases between 2019 and 20121 using ViteK2 system.

Organism Isolated	**Frequency**			
	Early Onset EOS	Late Onset LOS	Total	%
Gram-negative organisms	**6**	**179**	**185**	**36.3%**
Klebsiella pneumoniae	0	101	101	19.8%
E. Coli	0	23	23	4.5%
Burkholderia cepacia	1	1	2	0.3%
Citrobacter	1	1	2	0.3%
Acinetobacter baumanii	2	40	42	8.2%
Pseudomonas aeruginosa	0	13	13	2.5%
Enterobacter	1	0	1	0.19%
Neisseria	1	0	1	0.19%
Gram-positive organisms	**20**	**290**	**310**	**60.9%**
CoNS	2	249	251	49%.3%
Staphylococcus aureus	3	29	32	6.28%
Streptococcus viridans	4	9	13	2.5%

Enterococci	9	2	11	2.1%
Bacillus spp.	1	1	2	0.39%
Diphtheroid	1	0	1	0.19%
Fungi	**0**	**14**	**14**	**2.75%**
Candida albicans	0	14	14	2.7%
Total	**26**	**483**	**509**	

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 98.3 % of *Klebsiella pneumoniae* isolates were resistant to Ampicillin, 91% to Ampicillin/sulbactam, and 90% to Ceftazidime and Cefotaxime (**Table 3)**. *K. pneumoniae* isolates were reported to be multidrug resistant in 66/101 (65%).

Table 3. Antibiotic susceptibilities of *Klebsiella pneumoniae* isolates isolated from neonatal sepsis cases as determined by the VITEK 2 system.

Antibiotic	***Klebsiella pneumoniae***	
	Number	*%*
Ampicillin	100	99%
Ampicillin /Sulbactam	90	89%
Piperacillin/Tazobactam	75	74%
Cefazolin	80	79%
Cefoxitin	81	80%
Ceftazidime	91	90%
Cefotaxime	91	90%
Cefepime	80	79%
Meropenem	30	30%
Amikacin	76	75%
Gentamicin	80	79%
Tobramycin	81	80%
Ciprofloxacin	70	69%
Levofloxacin	65	64%
Nitrofurantoin	67	66%
Trimethoprim + Sulfamethoxazole	68	67%

3.1 RT-PCR amplification

The target gene *bla*$_{CTX\text{-}M\text{-}15}$ and the reference gene 16S-23S ITS rDNA was chosen for RT-Q-PCR analysis in *K. pneumoniae*. Primers for the *bla*$_{CTX\text{-}M\text{-}15}$ and 16S-23S ITS rDNA 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. 1**). **Table 4** summarizes the PCR primer sequences and amplicon sizes for selected candidate genes, amplified from *K. pneumoniae* isolates.

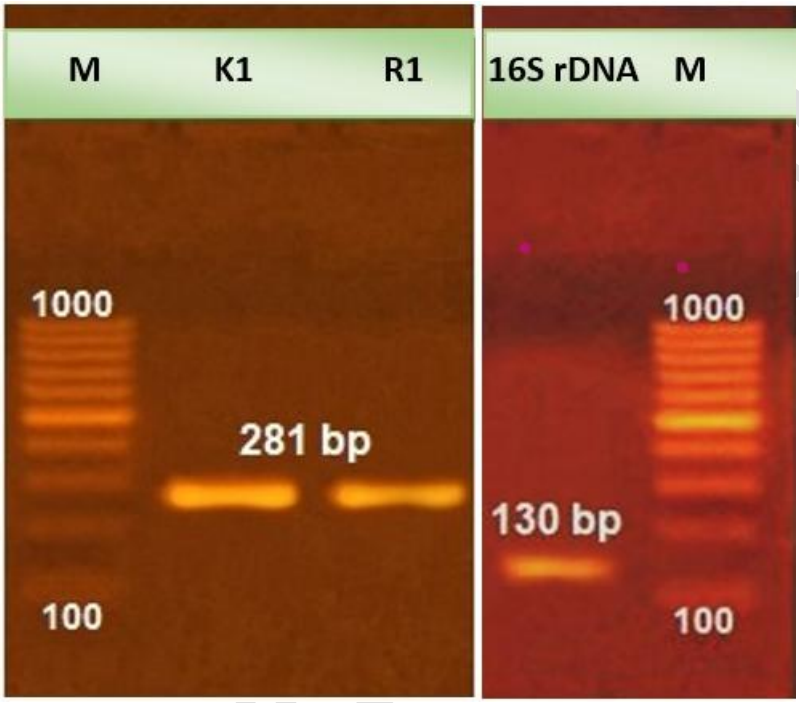

Fig. 1. Agarose gel electrophoresis showing the RT-QPCR products amplified from *K. pneumoniae.* A: PCR products 281 bp of *bla*$_{CTX\text{-}M\text{-}15}$ gene amplified from *K. pneumoniae* K1 and reference strain *K. pneumoniae* ATCC 700603 R1. B: PCR product 130 bp of 16S-23S ITS rDNA gene. M: 100 bp DNA Ladder.

Table 4. Primers used for the specific amplification of *K. pneumoniae***.**

Name	Gene	Primer sequence 5'-3'	Size of PCR amplicon	Reference
Pf	*16S-23S ITS*	ATTTGAAGAGGTTGCAAACGAT	130 bp	Liu *et al.*, 2008
Pr1		TTCACTCTGAAGTTTTCTTGTGTTC		
CTX-M15	*blaCTX-M-15*	CGGAAAAGCACGTCAATGGG	281 bp	GenBank: DQ302097.1 Liu *et al.*, 2009
CTX-M15'		GCTGTCGCCCAATGCTTTAC		

3.2 RT-QPCR

A high degree of confidence in real-time PCR quantification was achieved with PCR efficiencies ranging from 95.0% for *bla*$_{CTX\text{-}M\text{-}15}$ to 98.00% for the 16S-23S ITS sequence (**Table 2** & 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 5**, which provided relative estimate of the expression of *bla*$_{CTX\text{-}M\text{-}15}$ copies belonging to each sample relative to the house keeping gene 16S-23S ITS as described under materials and methods. Gene expression levels of the *bla*$_{CTX\text{-}M\text{-}15}$ gene are significantly higher in resistant *K. pneumoniae* isolates (KR1-KR23) than in susceptible ones (K2-K10) with a mean fold change of 10.0349 vs 1.8646.

Table 5: Results of real-time PCR, Ct, and fold change of *bla*$_{CTX\text{-}M\text{-}15}$ in sensitive and resistant *K. pneumoniae* strains.

Gp ID.	Sample ID.	*K. pneumoniae*		
		16S-23S ITS	*bla*$_{CTX\text{-}M\text{-}15}$	
		Ct	Ct	Fold change
Control	K1	18.30	21.84	-
Sensitive	K2	19.31	22.76	1.0644
	K3	18.55	21.94	1.1096
	K4	19.29	22.18	1.5692
	K5	20.33	23.08	1.7291
	K6	20.68	23.33	1.8532
	K7	18.42	20.90	2.0849
	K8	21.18	23.37	2.5491
	K9	19.71	21.74	2.8481
	K10	20.66	22.60	3.0314
	Mean	**19.79**	**22.43**	**1.8646**
Resistant	KR1	20.30	19.41	21.5557
	KR2	21.07	20.28	20.1122
	KR3	20.10	19.38	19.1597
	KR4	20.12	19.43	18.7654
	KR5	18.55	18.04	16.5642
	KR6	19.42	19.10	14.5203
	KR7	17.94	17.75	13.2691
	KR8	19.05	18.94	12.5533
	KR9	22.32	22.30	11.7942
	KR10	21.40	21.67	9.6465
	KR11	19.62	19.90	9.5798
	KR12	18.71	19.00	9.5137
	KR13	22.08	22.58	8.2249
	KR14	20.61	21.31	7.1602
	KR15	20.73	21.42	7.2100
	KR16	19.15	19.82	7.3107
	KR17	19.33	19.95	7.5685
	KR18	19.22	20.00	6.7740
	KR19	19.50	20.30	6.6807
	KR20	20.74	21.56	6.5887
	KR21	18.51	19.42	6.1903
	KR22	20.29	21.16	6.3643
	KR23	21.03	21.97	6.0629
	Mean	**19.99**	**20.20**	**10.0349**

Discussion

Septic shock, disseminated intravascular coagulation, and other life-threatening disorders may accompany neonatal sepsis. Sepsis often triggers a systemic inflammatory

response, with 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**). These cytokines stimulate neutrophils, leading them to release toxic mediators and damaging the endothelium and alveolar epithelium (**De Freitas et al., 2018; Wohlrab et al., 2018**), ultimately culminating in disruption to the alveolar-capillary barrier and acute respiratory distress syndrome (ARDS) as a secondary deficit of pulmonary surfactant (PS) (**Timmons, 2006; Hornik et al., 2012**). Neonatal sepsis, a potentially fatal illness, manifests clinically in a non-specific manner and requires rapid empirical antibiotic treatment. Due to the fact that extended-spectrum betalactamases genes contribute to the failure of therapy with extended-spectrum cephalosporins, extended-spectrum betalactamase (ESβL) resistant bacteria and particularly Enterobacteriaceae are becoming an extensive global concern (**EFSA, 2011; Liebana et al., 2013**). ESβL/AmpC-producing Enterobacteriaceae have been suggested for active and passive surveillance in Europe and other non-European countries (**EFSA, 2012; EU Commission 2013**). Numerous countries already have national programmes in place. As a result, quick and accurate screening approaches for large numbers of samples are crucial (**Lupo et al. 2013**).

In this study, a total of 509 out of 989 (51%) culture positive sepsis, including 101(19.8%) *K. pneumoniae*, were identified, with the majority of them being detected in LOS. No statistically significant difference was seen between findings of Gram staining and incidence of infection. Results showed the presence of Gram-negative and Gram-positive bacteria, as well as *Candida albicans*. Our results showed that Gram-positive isolates were found to be considerably more prevalent in LOS neonates than in EOS, however, *Klebsiella pneumoniae* was the most frequently isolated organism (19.8%) among Gram-negative bacteria. Similar results were obtained by **Ghonaim et al., (2021)** who reported that *K. pneumoniae* was the dominant isolate (31.6%) among neonates with confirmed sepsis. Other studies from India showed different organism profile as well, it was found that 90.8% of organisms were gram-negative, with the most frequent being Pseudomonas (33.2%), Klebsiella (31.2%), Acinetobacter (14.4%), and *E. coli* (4.4%) (**Bhat et al., (2011**). In a study by **Pais et al., (2012)** found that the most prevalent organism was pseudomonas in EOS (11.46%). This discrepancy might be explained by variations in location and time (**Lamichhan and Mishra 2019**).

Our study discovered a high incidence of multi-drug resistant (MDR) *Klebsiella pneumoniae* isolates among newborn sepsis cases. We found that most of the *K. pneumoniae* isolates showed high resistance 30% - 99% 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. These isolates (66/101) had the $bla_{CTX\text{-}M\text{-}15}$ gene, was consistent with studies from Greece and China (**Shen et al., 2020; Xie et al., 2020; Galani et al., 2021**). Regarding the other studies of susceptibility to antimicrobials, **Fursova et al., (2021)** reported that MDR *Klebsiella pneumoniae* isolates associated with severe infections in the neuro-ICU, $bla_{CTX\text{-}M}$ (n = 28) were detected using the Vitek-2 instrument.

Due to the well-known limits of blood cultures in terms of processing times and sensitivity, a novel quick and accurate approach for detecting and identifying *K. pneumoniae* in blood samples from neonates with suspected sepsis is required. This study demonstrates the potential of real-time polymerase chain reaction (QRT-PCR) for quantifying the $bla_{CTX\text{-}M\text{-}15}$ gene in (23/33, 69%) *K. pneumoniae* isolated from neonates with confirmed sepsis. Our PCR results were similar to that obtained by **Jordan et al. (2000); Kalathia et al. 2021)** who conducted PCR investigation with adequate sensitivity on clinical samples from septic

newborns, utilizing a preculturing method to increase the quantity of bacteria in the sample and facilitate bacterial identification.

The results of this study revealed that *K. pneumoniae* isolates (KR1-KR23) with $bla_{CTX\text{-}M\text{-}15}$ gene were the most common ESBL-producing strains among neonatal sepsis clinical isolates. The Q-RT-PCR products showed that all $bla_{CTX\text{-}M\text{-}15}$ amplified products showed high level of gene expression in MDR *K. pneumoniae* (69%) isolates. There was a significant difference in the $bla_{CTX\text{-}M\text{-}15}$ gene expression between resistant and susceptible strains of *K. pneumoniae*, with a mean fold change of 10.0349 versus 1.8646. In Riyadh (Saudi Arabia), it was found that $bla_{CTX\text{-}M\text{-}15}$ was the most common gene among *E. coli* and *K. pneumoniae* (**Ohlsson et al. 1986**). Another study by **Moghaddam et al. (2014)** showed that 27 (27%) isolates were ESBL producers with the highest frequency for *Klebsiella pneumoniae* (47.4%) and *Escherichia coli* (17.9%). In accordance to our results, **Eskandari-Nasab et al. (2018)** revealed that the RF of the $bla_{CTX\text{-}M}$ gene varied between 7.7% and 100% in Tabriz, Mashhad, Tehran, and Zahedan, with an average RF of 56.7 % among clinical isolates of *K. pneumoniae* expressing ESβLs. In contrast, **Taheri et al. (2010)** reported that coagulase-negative staphylococcus was the most prevalent pathogen in 58 % of the positive blood samples.

To evaluate the expression of the $bla_{CTX\text{-}M\text{-}15}$ gene in MDR *K. pneumoniae* isolates, the two-step quantitative reverse transcription-PCR was used as recommended by **Fey et al. (2004)**. The data were normalized to the transcription level of the 16S-23S ITS rRNA gene constitutively expressed in *K. pneumoniae*. Calibration curves for each gene were produced using serially diluted cDNA prepared from *in vitro*-obtained RNA standards (**Maillet et al. 2006**). The slope of each calibration curve was used to compare the amount of $bla_{CTX\text{-}M\text{-}15}$ gene copies in *K. pneumoniae* isolates in comparison to the susceptible isolates. The results revealed that the *K. pneumoniae* isolates (KR1-KR23) had a significant higher level of $bla_{CTX\text{-}M\text{-}15}$ gene expression. The mean value of the $bla_{CTX\text{-}M\text{-}15}$ transcript level was 10.0349-fold higher than that of the sensitive isolates (p <0. 01). Similar results were obtained by **Ghonaim et al. (2021)** who reported that about 58.3% of *K. pneumoniae* isolates had the *CTX-M* gene and there was a significant difference between ESβL-producing and non-ESβL-producing *K. pneumoniae* ($p > 0.05$).

Conclusions

In this study, *CTX-M-15*-positive *K. pneumoniae* isolates were recovered from neonates with confirmed sepsis in Al Demerdash and Ain Shams University Specialized Hospital, Egypt. It is alarming to note the high rate of *K. Pneumoniae* isolates with simultaneous production of *CTX-M-15* conferring high-level resistance to multiple drugs, including Ampicillin, Ampicillin /Sulbactam, Piperacillin/Tazobactam Cefazolin, Cefoxitin, Ceftazidime, Cefotaxime, Cefepime, Meropenem, Amikacin, Gentamicin, Tobramycin, Ciprofloxacin, Levofloxacin, Nitrofurantoin, and Trimethoprim + Sulfamethoxazole. There was an unacceptably high level of antibiotic resistance, particularly to frequently used antibiotics, and appropriate use of presumably susceptible antibiotics would have a considerable impact on the outcome of the disease.

Ethical approval

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

Supporting information S1

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