

Prevalence of Bacterial Infections in Neonatal Intensive Care Unit and Genetic Characterization of Their Drug Resistance

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Abstract:

Out of the 120 blood samples collected from investigated neonates, 40 samples were positive for bacteria. Blood culture was carried out using BACT/ ALERT (3D 60) then the positive cultures were subculture on MacConkey, Blood and Sabouraud dextrose agar. Biochemical tests were used to identify the isolates, and the automated Vitek 2 system provided confirmation. The incidence of neonatal sepsis was predominantly due gram-negative bacteria (75%) mainly *K. pneumoniae* (60 %), following that *E. coli* (7.5 %). While all gram-positive bacteria (25 %) represented by *CONS*. As stated by the Clinical and Laboratory Standards Institute (CLSI) guidance, an antimicrobial susceptibility test using the disc diffusion method (Kirby-Bauer) was conducted to select multi drug resistant bacteria as (*ESBL*) producers. The *ESBL* genes responsible for the resistance were detected using PCR method. It was determined that among *ESBL* of *K. pneumoniae* and *E. coli*, the *bla*TEM and *bla*CTX-M genes were the predominant gene (100 %), followed by *bla*SHV (80 %).

Keywords: NICU, ESBL, Sepsis, *K. pneumoniae*.

Introduction:

The Severity of neonatal infection has a significant impact on neonatal death and morbidity rates. Sepsis causes 30 - 50% of neonatal deaths in underdeveloped nations. (Wu *et al.*, 2017).

Nearly one million of these deaths, mostly in low-income nations, are mainly related to infectious diseases such pneumonia, meningitis, and newborn sepsis. (Black *et al.*, 2010). Neonatal sepsis is classified into two primary types and is identified as a clinical illness in the first 29 days of

baby's life, that is accompanied by systemic symptoms of infection and the isolation of a microbial pathogen can be from the bloodstream. (*Edwards and Baker, 2004*).

Early onset sepsis: commonly manifests during the first 72 hours of life. Usually, the maternal vaginal tract is the source of infection.

Late onset sepsis: often manifests after 3 days of birth (*Patel and Saiman, 2010*).

Gram-positive, Gram-negative bacteria and as well as *Candida*, are the main causes of neonatal sepsis (*Sabeeh Jumah and Hassan, 2007*). Even in the same location, the variety of organisms that cause sepsis, differs from region to region and evolves through time. (*Shrestha et al., 2010*).

Before beginning antibiotic treatment, a blood culture should be performed in every case of suspected sepsis because it is the gold standard for the diagnosis of septicemia. The greatest indicator for antimicrobial therapy is a blood culture and sensitivity of the microbial pathogen. (*Prabhu et al., 2010*).

This study goals to determine the frequency of bacterial infection among Neonatal Intensive Care Unit (NICU) cases in Sohag. Specifically, we wanted to determine ESBL producing bacteria and detection of their resistant genes.

Materials and Methods: collection, isolation, and identification of samples.

This research was conducted on 120 neonates admitted with sepsis

symptoms and signs in NICU at Al-Jamaeia Alsharia Medical Center, Sohag City, over a period of 11 months between October 2020 and September 2021.

Blood cultures were collected at the time of admission from all cases (a total of 120 specimens). Next, a unilateral venipuncture used for drawn a 1–2 ml of blood under aseptic circumstances by cleaning the skin site with seventy percent alcohol and one percent povidone iodine followed by seventy percent alcohol again and then inoculated into a blood culture vials (BACT/ALERT® PF PLUS Aerobic medium) for incubation with a detection system Bact /Alert 3D (bio Merieux, France).

Positive cultures were subculture on MacConkey, blood agar, and Sabouraud dextrose agar, at 37°C and incubated for 24 – 48 hrs. Finally, manual biochemical tests and the Vitek® 2 system were used to identify bacterial isolates.

Based on the guidelines of CLSI, antibiotic sensitivity tests were done using disc diffusion method (Kirby Bauer). The antibiotics used for Gram-negative bacteria was beta lactam group, as ampicillin (10ug), amoxicillin/sulbactam (20 ug), piperacillin/tazobactam (100/10ug), cefoxitin (30 ug), cephazolin (30 ug), ceftriaxone (30 ug), ceftazidime (30 ug), cefepime (30 ug) and meropenem (10 ug); fluoroquinolones, as ciprofloxacin (5 ug) and levofloxacin (5 ug); aminoglycosides, such as amikacin

(30 ug) and gentamicin (10 ug) and sulfamethoxazole /trimethoprim (1.25/23.7 ug). The antibiotics used for Gram-positive bacteria involved cefoxitin (30 ug), ampicillin (10 ug), erythromycin (15 ug), vancomycin (30 ug), gentamicin (10 ug), doxycycline (30 ug), tetracycline (30 ug), clindamycin (2 ug), tigecycline (15 ug), rifampicin (10 ug), linezolid (30 ug), levofloxacin (5 ug), ciprofloxacin (5 ug) and SXT (1.25/23.7 ug). These discs totally manufactured by Oxoid, England. The CLSI recommendations were used to interpret the inhibition zones of various antibiotics. (CLSI, 2020).

Extended Spectrum β -lactamase detection by Phenotypic studies:

Test of Double Disc Synergy (DDST)

Amoxicillin/Clavulanic acid (20/10ug), Ceftazidime (30ug) and Ceftriaxone (30ug) discs were used for DDST. At center the discs containing clavulanic acid were positioned at a distance of 1.5 cm. Inhibition zone toward the Clavulanate disc developing after 24 hours at 37°C Incubation considered an ES β L positive organism (Jacoby and Medeiros, 1991).

Phenotypic Confirmatory Disc Diffusion Test (PCDDT):

In this test, Muller-Hinton agar plates were loaded with bacterial suspensions matched with 0.5 McFarland turbidity standards, just around 108 CFU/ml, as advised for routine disc diffusion susceptibility testing. Ceftazidime (30 ug), ceftazidime /clavulanic acid (30/10

ug), cefotaxime (30 ug), and cefotaxime /clavulanic acid (30/10 ug) discs were located on the plate's surface on the surface of the plate after inoculation and allowed to dry. The plates were then incubated in air at 37 °C for 18–24 h. After growth, the diameters of the zones surrounding the discs were measured and noted. The zone diameter around the discs containing cephalosporin plus clavulanic acid increased by around 5 mm compared to the discs containing cephalosporin alone, indicating the production of ES β L. (CLSI, 2014).

Molecular studies

Molecular studies were done in the Research Institute of Animal Health in GIZA for determination the genetic type of β -lactamase using PCR.

Results:

From 120 samples were collected., 40 samples (18 females and 22 males) were positive for bacteria. Among the 40 proven sepsis neonates, 6 (12.8) had early-onset sepsis (EOS) and 41 (87.2) had late-onset sepsis (LOS). Gram negative bacteria was the majority of the culture isolates represented by 75 % while gram positive isolates were 25 %. *Klebsiella pneumoniae* (60 %) constituted the most prevalent of gram-negative isolate, followed by *E. coli* (7.5 %), *Enterobacter cloacae* (5 %) and *pseudomonas aeruginosa* (2.5 %). While all gram-positive isolates were *CoN Staphylococcus* (100 %) (Figure 1), (Table 1).

Antimicrobial susceptibility test

Gram-negative bacteria were more sensitive to Amikacin and levofloxacin (60 %), followed by meropenem (53%) while they were less sensitive to SAM, Cefoxitin, ceftriaxone, TZP and Ceftazidime (20 %), and markedly resist to Ampicillin and Cephazolin (**Figure 2**). All of gram-positive bacteria were sensitive to levofloxacin (100 %), followed by Ciprofloxacin and Tigecycline (90%) and Gentamicin, Clindamycin, linezolid and Tetracycline (80 %) while they were less sensitive to SAM, Cefoxitin, SXT and Erythromycin (40 %), and markedly resist to Ampicillin (**Figure 3**).

ESBL detection with PCDDT and DDST as screening methods.

The ESBL producing organisms were detected by PCDDT and DDST It showed positive in 5/24 (20.8 %) of *K. pneumoniae* and 1/3 (33.3 %) of *E. coli* (**Figure 4 and 5**).

Genotyping of ESβL producers by polymerase chain reaction (PCR):

All proven ESβL isolates were analyzed by PCR to determine the probable type of ESβL gene which is responsible for resistance. It was found that *bla*TEM and *bla*CTX-M genes are the major types of ESβLs detected 100% (**Figure 6 and 7**). *bla*SHV gene was the second 83.3 % (**Figure**).

Table 1. Prevalence rate of bacterial species isolated from neonatal blood samples.

Bacterial isolates (Total= 40)	No. (%)	Early onset No. (%)	Late onset No. (%)
Gram +ve			
<i>CoN Staphylococcus</i>	10 (25 %)	6 (60 %)	4 (40 %)
Gram -ve			
<i>K. pneumoniae</i>	24 (60 %)	0 (0 %)	24 (100 %)
<i>E. coli</i>	3 (7.5 %)	0 (0 %)	3 (100 %)
<i>Enterobacter cloacae</i>	2 (5 %)	0 (0 %)	2 (100 %)
<i>Pseudomonas aeruginosa</i>	1 (2.5 %)	0 (0 %)	1 (100 %)
Total	40 (100 %)	6 (15 %)	34 (85 %)

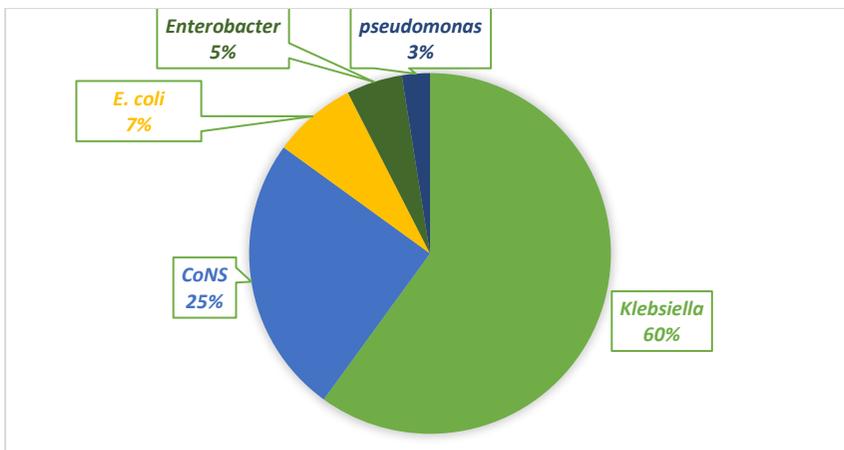


Figure 1. prevalence rate of bacterial species isolated from neonatal blood samples.

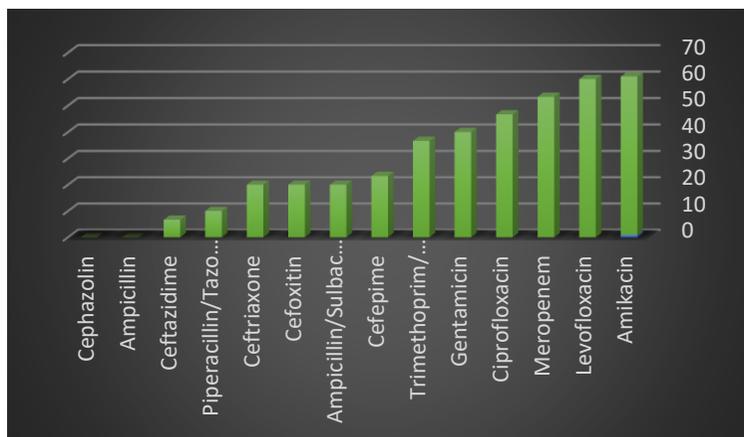


Figure 2. Antibiotic sensitivity rate of Gram-negative bacteria

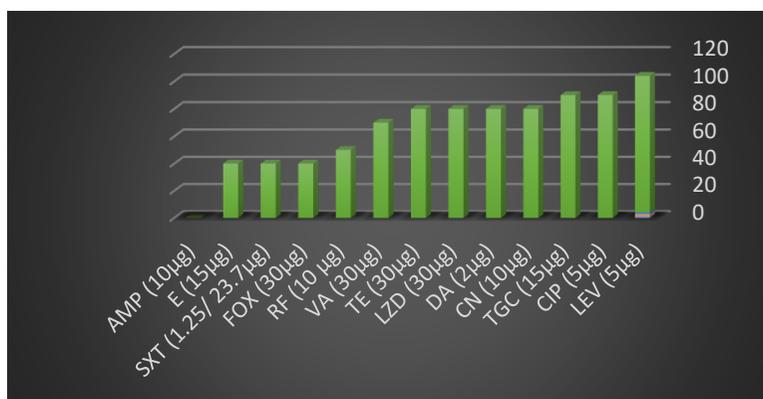


Figure 3. Antibiotics sensitivity rate of Gram-positive bacteria

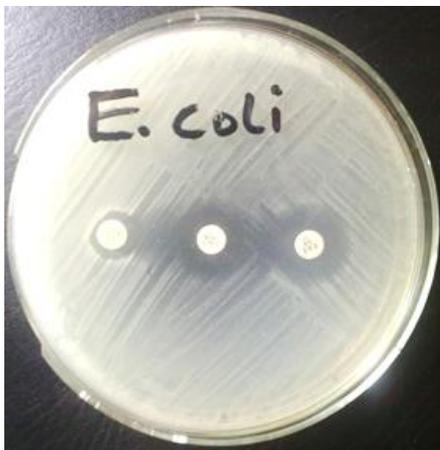


Figure 4. Double Disc Synergy Test (DDST) for ESBL confirmation on *E.*



Figure 5. Phenotypic Confirmatory Disc Diffusion

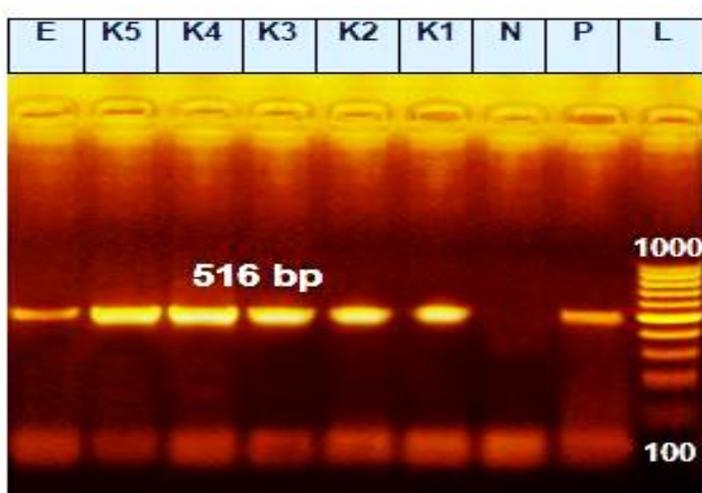


Figure 6. Agarose gel electrophoresis of amplified DNA of *bla* TEM showing PCR amplification of 5 isolates with *bla* TEM (516 bp). Lane L: DNA marker; Lane P: Positive control; Lane N: Negative control; Lane E: *E. coli* isolate Positive for TEM, Lane K1 to K5: *K. pneumonia* isolates positive for TEM.

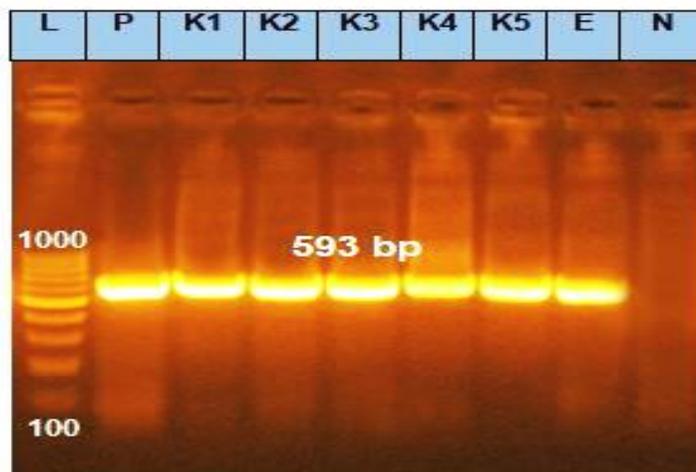


Figure 7. Agarose gel electrophoresis of amplified DNA of *bla CTX-M*, showing PCR amplification of 5 isolates with *bla CTX-M* (593 bP). Lane L: DNA marker; Lane P: Positive control; Lane N: Negative control; Lane E: *E. coli* isolate Positive for CTX-M, Lane K1 to K5: *K. pneumonia* isolates positive for CTX-M

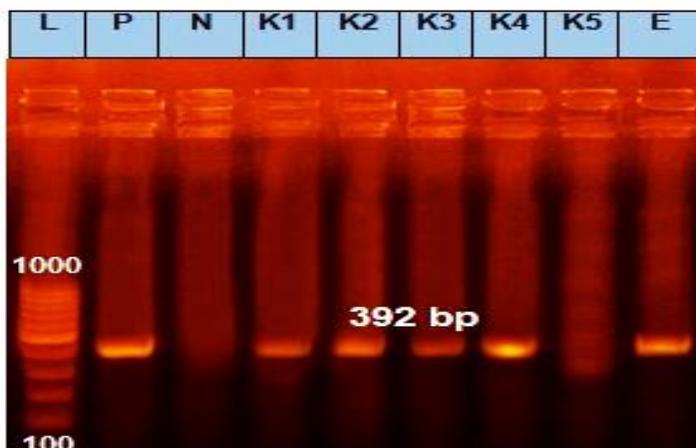


Figure 8. Agarose gel electrophoresis of amplified DNA of *bla SHV*, showing PCR amplification of 5 isolates with *bla SHV* (392bP). Lane L: DNA marker; Lane P: Positive control; Lane N: Negative control; Lane E: *E. coli* isolate Positive for SHV, Lane K1 to K4: *K. pneumonia* isolates positive for SHV, Lane K5: *K. pneumonia* isolate negative for SHV

Discussion

Neonatal sepsis has non-specific clinical signs and symptoms, which

makes early identification challenging. Accordingly, blood culture urgently required and still be

the optimum method for diagnosis of neonatal sepsis in spite of it is time consuming.

During the trial period, 120 newborns were enrolled with clinically suspected neonatal sepsis, and blood cultures were performed to confirm the diagnosis.

Furthermore, positive blood culture results vary significantly across studies due to different study designs or techniques. In this trial, positive sepsis was confirmed by (39.1 %) of blood cultures. This is comparable to the finding of previous studies in Egypt (33.25%) (*Awad et al., 2016*) and (33 %) which stated in (*Kabwe et al., 2016*) while be less in (20.7%) (*Pokhrel et al., 2018*) and (15%) (*Guerti et al., 2011*).

The majority of studied neonates was late onset sepsis (87.2 %), a comparable result (71.2 %) (*Yadav et al., 2018*) had been reported, and were males slightly higher than females (51.1 % males- 36.1 % females). This could be due to the wider prevalence of community acquired infections among newborns.

Incidence of neonatal sepsis was predominantly due gram-negative bacteria (75%) mainly *Klebsiella pneumoniae* (60 %), followed by *E. coli* (7.5 %). While all gram-positive bacteria (25 %) represented by *CONS*.

Klebsiella pneumoniae predominated among the causal Gram-negative pathogens in other Egyptian research like (*El Badawy et al., 2005; Fahmey., 2013*) and

other different countries (*Macharashvili et al., 2009; Chiabi et al., 2011; Kohli-Kochhar et al., 2011; Leal et al., 2012; Li et al., 2013*). In almost all research, *Klebsiella pneumoniae* was the primary *Klebsiella* species found.

Klebsiella isolates showed high sensitivity for aminoglycosides and quinolones antibiotics while resistance to penicillin's group, *Staphylococcus* isolates showed resistance to penicillin's and aminoglycosides mostly due misuse of antibiotics in hospitals that leads to this significant resistant while sensitive to quinolones and rarely used antibiotics like nitrofurantions, *E. coli* and *pseudomonas* species showed moderate sensitivity to 3rd generation cephalosporins, aminoglycosides and quinolones while resistance to classic penicillins, This finding is consistent with a study of (*Kohli-Kochhar et al., 2011*).

In the current research, the ES β L-producing bacteria were detected by PCDDT, DDST and PCR. Its showed positive in 5/24 (20.8 %) of *K. pneumoniae* and 1/3 (33.3 %) of *E. coli*.

The predominance rate of ES β L-producing *K. pneumoniae* is showing similarity (21 %) to another study in our country (*Ahmed et al., 2013*) and (25.6) in pervious study in Saudi Arabia (*Tawfik et al., 2011*). While greater incidence of ES β L-producing *K. pneumoniae* (55%) was stated in Riyadh (*Al-Agamy et al., 2009*)

The incidence of ES β L-producing *E. coli* (33.3 %) was comparable to the previous studies from 35 to 42 % in United States (*Ajao et al., 2013*) and also India (*Taneja et al., 2010*) (55.68%). Higher rate was reported in Egypt (54.5%) (*Abdallah et al., 2015*), but all of *E. coli* isolates were ES β L-Positive as in Saudi Arabia (*Al-Agamy et al., 2014*), Ethiopia (*Legese et al., 2017*) and Senegal (*Camara et al., 2017*).

This study confirmed that the *bla*CTX-M, *bla*TEM, and *bla*SHV are the most common ES β L genes. The majority of β -lactamase genes in *K. pneumoniae* isolates were *bla*TEM and *bla*CTX-M, followed by the *bla*SHV gene. The occurrence rate of *bla*TEM, *bla*CTX-M and *bla*SHV in *K. pneumoniae* isolates was 100%, 100%, 80%, respectively. This finding is higher than the results in Tanzania that showed *bla*CTX-M 98%, *bla*TEM 85.7% and *bla*SHV 71.4% (*Silago et al., 2021*).

In contrast the prevalence rate of *bla*SHV, *bla*TEM, *bla*CTX-M in Saudi Arabia was 89.1%, 70.9 % and 36.4 % respectively (*Tawfik et al., 2011*).

The incidence of ES β L-genes in *E. coli* showed 100 % for *bla*TEM, *bla*CTX-M and *bla*SHV. While reported in pervious study in Egypt as *bla*SHV (61.2%) followed by *bla*TEM (38.78%) and *bla*CTX-M comes (20.41%) (*Zaki et al., 2019*).

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شروع العدوى الميكروبية في وحدة رعاية الاطفال المبتسرين والتوصيف الجيني لمقاومتها للمضادات الحيوية

الملخص العربي

من بين 120 عينة دم تم جمعها من حديثي الولادة الذين تم فحصهم سريريا (49 أنثى و71 ذكرا)، كانت 40 عينة (17 أنثى و23 ذكرا) ايجابية للبكتيريا، وتبين أن غالبية حديثي الولادة الذين شملتهم الدراسة (87.2%) كانوا من النوع الثاني للعدوى الميكروبية الذي يحدث بعد مرور 72 ساعة من الولادة. وأظهرت نتائج هذه الدراسة أيضا ان البكتيريا المسببة للعدوى الميكروبية عند حديثي الولادة هي البكتيريا سالبة جرام وبالتحديد لوحظ أن بكتريا كلبسيلا نوموني هي المسبب الأكبر للتسمم الوليدي لدى الأطفال حديثي الولادة (60%) تليها بكتيريا الإشريشيا كولاي (7.5%) في حين أن جميع البكتيريا موجبة الجرام (25%) كانت ممثلة في بكتريا المكورات العنقودية سالبة التخثر. وأظهرت الدراسة أيضا ان البكتيريا المعزولة كانت مقاومه لمعظم المضادات الحيوية الكلاسيكية والتي تستخدم عامة كخط أول وثاني في العلاج المبدئي للتسمم الوليدي ما قبل ظهور نتيجة مزرعة الدم مثل البنسلين بكافة تركيباته واشكاله في الوقت الذي ظهرت استجابة هذه البكتيريا لمجموعات مضادات حيوية مثل الكينولول والامينوجليكوزايد. وأخيرا تم استخدام تقنية تفاعل البلمرة المتسلسل لتحديد توزيع الجينات المقاومة للمضادات الحيوية الشائعة بين العزلات في هذه الدراسة. وجد أن جينات TEM وCTX-M كانوا النوعين الرئيسيين من الجينات المقاومة (100%) تليها SHV (80%).