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THE ANTIBIOTIC RESISTANCE OF BACTERIAL NEONATAL SEPSIS

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Abstract

Neonatal sepsis is a global disease that poses a management challenge for neonatal care groups. Its prevalence has risen in recent years, possibly as a result of the increased use of invasive procedures and the emergence of resistant organisms. The goal of this study is to determine the bacteria that lead to the development of sepsis in the newborn period so that early therapy can be used to reduce the incidence of sepsis as well as determine the antibiotic sensitivity. Common clinical variables (sociodemographic characteristics) were studied, as were bacteriological profiles and antibiotic susceptibility testing. The sepsis was confirmed in 54 (31.8%) neonates were admitted to the special care baby unit with clinical signs and symptoms. All bacterial isolates were identified using traditional methods (cultural, morphological, and biochemical characteristics), and the VITEK-2 compact system was used to confirm the results. Only 60 of the 100 samples revealed bacterial growth from isolates of a different genus, while only 40 of the 100 samples had no bacterial growth. 72 (71.6 %) were EOS, and 28 percent (28.3%) were LOS. The findings showed that the highest isolates was Staphylococcus epidermidis 26/60 (43.3%), followed by Klebsiella pneumoniae. 13/60 (21.7%). Staphylococcus aureus. 10/60 (16.7%), Pseudomonas aeruginosa. 8/60 (13.3%) and Escherichia coli 3/60 (5%). Furthermore, most Gram-negative bacteria showed highly resistance (100%) to Aztreonam, Ceftazidime, Piperacillin, Ceftriaxone and Gentamicin. As well as, most Gram-positive bacteria in this study were resistance (100%) to Penicillin, Cefoxitin, Vancomycin, Nirofuranation.

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Keywords: Neonatal sepsis, Bacteria, Antibiotic susceptibility, Risk factor.

Introduction

Neonatal sepsis is characterized as any systemic bacterial infection confirmed by a positive blood culture in the first month of life (Fanaroff et al .,2007) Despite advancements in hygiene, the advent of new and effective antimicrobial agents for treatment, and advanced diagnostic measures, neonatal septicemia remains one of the leading causes of mortality and morbidity. Infections affect up to 10% of babies in their first month of life, accounting for 30-50% of total neonatal deaths in developing countries (Al-Saady et al ., 2018) These neonatal deaths are mostly the result of infection, birth asphyxia, and the effects of p remature birth birth with a low birth weight (Movahedian et al ., 2006) It is well known that risk factors related to neonatal bacterial sepsis are complex; they include the interaction of maternal-fetal colonization, trans placental immunity and physical and cellular defense mechanisms of the neonate(Jumah et al ., 2007). Neonatal sepsis may be classified as either early-onset or late-onset. Early onset neonatal sepsis (EOS), which occurs during the first 72 hours of life, continues to be a major cause of illness and death in very low birth weight (VLBW)preterm infants (Stoll et al ., 2005) Microorganisms acquired from the mother before or during birth (vertically transmitted and prenatally acquired) cause EOS; hence, microorganisms from the maternal genital tract can play an important role in early infection (Kerur et al ., 2006) EOS is linked to the mother's acquisition of microorganisms, Organisms that colonize the mother's genitourinary tract (GUT) may cause trans placental infection or ascending infection from the cervix; the neonate acquires the microorganisms as it passes through the colonized birth canal at delivery (Klinger et al ., 2009). EOS is often associated with respiratory distress and pneumonia (Rajaratnam et al ., 2010) Gram-negative species are the most common pathogens associated with EOS (Stoll et al ., 2005). GBS, E. coli, and Coagulase-negative bacteria are the most frequently associated microorganisms with an early-onset infection. Staphylococcus aureus, Haemo philus influenzae, and Listeria monocytogenes, (Klinger et al ., 2009).

Gram-negative pathogens have been identified as the causative organisms in VLBW infants with EOS more frequently. Late onset sepsis (LOS) present during 7-28 days of age. In LOS, the infection is either nosocomial (hospital-acquired) or community-acquired, and neonates usually present with septicemia, pneumonia, or meningitis. Gram-positive bacteria are the most often isolated pathogens in VLBW infants diagnosed with LOS(Carolin et al ., 2012), Gram-negative bacilli that cause neonatal LOS are primarily E. coli, Klebsiella spp., Enterobacter spp., and Pseudomonas spp.

Fungi, especially Candida spp., have been identified as one of the major pathogens of LOS in some areas (Leal et al ., 2012).

Materials and Methods

Study Design

This is case-control study conducted in the Department of Pediatric in AI-Batool Teaching Hospital ,Diyal Governorate ,Iraq for 5 month the period from (1 June/ 2021)to(30th October/ 2022).

Samples Culture

Blood samples drawn from newborns were placed in the Bact Alert device and incubated for different periods. The positive samples were dealt with according to the traditional isolation and diagnosis methods, which included blood agar and macConkey agar. The isolates were purified by selective media which included mannitol salt agar, EMB agar , and pseudo agar by streaking method. The agar plates were incubated for 24 h at 37°C (Stromberg et al ., 2015). Then, biochemical tests and diagnostic tests were performed for the bacteria under study. The Positive isolates were confirmed using VITEC system**.**

Bacterial Isolates

The total clinical isolates were 5 isolates of a different genus of bacteria from patients with septicemia which include(Staphylococcus epidermidis , Klebsiell pneumonia ,Staphylococcus aureus ,Pseudomonas aeruginosa and Escherichia coli) and all samples were entered into the Back Alert system to ensure that they were positive for bacterial growth and were incubated for various times. All isolates were diagnosed based on using conventional isolation and diagnosis methods and biochemical and bacteriological tests after cultured in the cultures media, these isolates gave a positive results for these tests. The Positive isolates were confirmed using VITEK 2 compact system .

Antibiotics Susceptibility Test

The sensitivity test procedure was done according to (CLSI , 2020) as the following steps: -

1. Mueller-Hinton agar plates were used for the use of rapidly growing species in the Kirby- Bauer method. The solvent was sterile in the plates and had a depth of around 4 mm.

2. Pure culture has been used as inoculum; 2-4 related colonies have been selected and transferred to around 5ml of standard sterile saline. To get an average number equal to 1.5x 10^8 CFU/ml, the turbidity of microbial suspension was compared with the turbidity of the McFarland Standard 0.5.
3. The sterile cotton swab was immersed into the standard inoculum, streaking was performed 3 times on the entire agar surface of the plate with the swab, rotary the plate between each line at 50 degrees. The inoculum had been allowed to dry with a lid in place for 5-10 minutes and after that, the antibiotics mentioned in the table (3-4) were distributed on the plates.
4. The plates were subsequently incubated at 37 ^oC and analyzed 18-24 h. Inhibition zones were measured, and the zones' diameters were reported to the nearest millimeter.

Results and Discussion

The bacterial isolated from neonates with different types of sepsis

From blood samples, the highest isolates was Staphylococcus epidermidis 26/60 (43.3%), followed by Klebsiella pneumonia 13/60 (21.7%). Staphylococcus aureus. 10/60 (16.7%), Pseudomonas aeruginosa. 8/60 (13.3%) and Escherichia coli 3/60 (5%), figure(1). The results revealed to statistical differences in gender with all bacterial isolates but there are significant differences in gender with Escherichia coli infected. Figure (1) showed the distribution of newborns participants according to types of bacteria and type of sepsis. It was seems that Neonates with EOS (0-7) days of age forming the highest rate (71.6%) of participants while Neonates with LOS (7-30) days of age (28.3%).

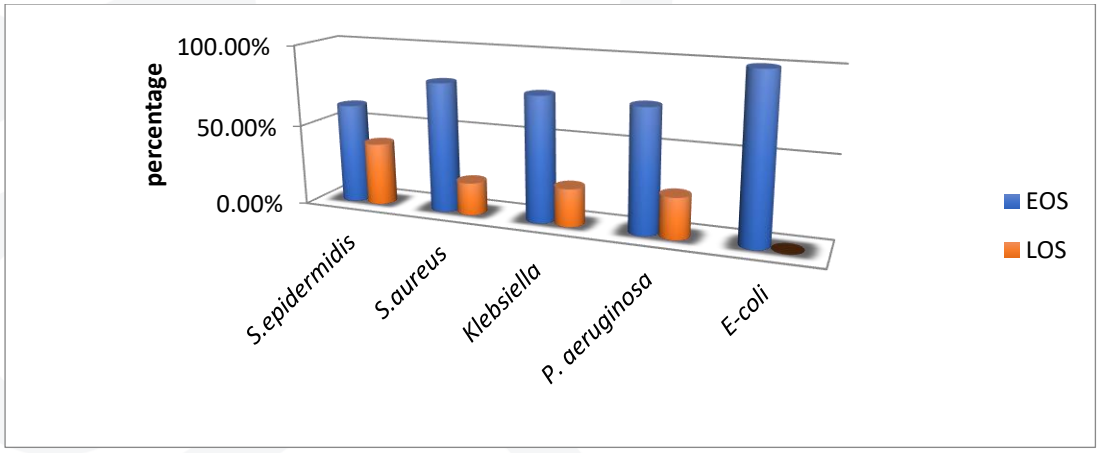

Figure 1 : The bacterial isolated from neonates with different types of sepsis which include (EOS: Early Onset Sepsis, LOS: Late Onset Sepsis)

The results of our current study revealed that S. epidermidis was the most common Gram-positive bacteria isolate, accounting for (43.3 %) of all isolates, and these findings are consistent with Shrestha S et al. (2010), which showed that the S. epidermidis was a major isolate, and disagree with Sharma et al. (2013) in India showed that S. aureus was the most prevalent. One explanation for the disparity may be the lack of compliance with infection prevention and control steps.

The klebsiella pneumoniae was the most common cause sepsis in Gram-negative bacteria isolate accounting for (21.7%) and this findings was similar with Gyawali N et al. (2013) and Shrestha et al. (2012), which showed that klebsiella pneumoniae isolate accounts the highest causes infection in neonates. According to Aftab et al. (2007) in a study of the Pakistani population, E. coli was the most common organism, followed by Klebsiella, this result differed with the results of our current study. It was evident from the results of our current study that the Gram-positive bacteria was more common than the Gram-negative bacteria, this results was agreement with Naderi-Nasab et al. (2007), they found that gram positive bacteria were more common than gram negative bacteria in nosocomial and acquired infections in NICUs of Mashhad, Iran, and disagreement with Shrestha S et al. (2007), which showed the majority of the isolate were gram-negative bacteria.

Geographical variations in the causative organism, as well as differences in the test climate, study population, and hand hygiene habits, which can explain the variation in the main isolate. The most common species linked to neonatal sepsis differ depending on when infections occur (Shrestha et al., 2013). As a result, awareness of the bacteriological profile of neonatal sepsis and appropriate antimicrobials for its treatment is critical in the fight against neonatal morbidity and mortality. The results of our study showed that early onset sepsis was highest rate (71.6) than late onset sepsis (28.3) for newborns. According to another report by (Aftab et al. 2006), it was showed that 42 % of all hospitalized neonates with sepsis in the NICU were EOS, the results of our current study were close to this result. The occurrence of EOS appears to be influenced by antibiotic resistance in common pathogens, the effectiveness of interventions used, and whether or not accurate information about the burden of sepsis and its effects is available.

This is attributed to the low weight for newborns as well as premature birth, it has a role in this infection and early neonatal infection is associated with ascending infection from the uterus. As well as the uterine environment surrounding the fetus before birth are all risk factors leading to the infection of newborns early onset sepsis. In addition, the immune system of premature newborns is less able to prevent and

destroy infection and as a result, they are more likely to develop early septicemia as shown in figure (1).

Antibiotic Susceptibility of bacterial isolates

Antibiotic Susceptibility of S. aureus

The current study's findings, as shown in Figure (3), suggested that 20 percent of the 10 isolates of Staphylococcus aureus were resistant to Ciprofloxacin, and its sensitivity was shown to be (80%).Sensitive to Vancomycin by(20%) and resistance of(80%).Sensitive to Gentamicin by(50%) and resistance of (50%).Sensitive to Amikacin(90%) and resistance of (10%).

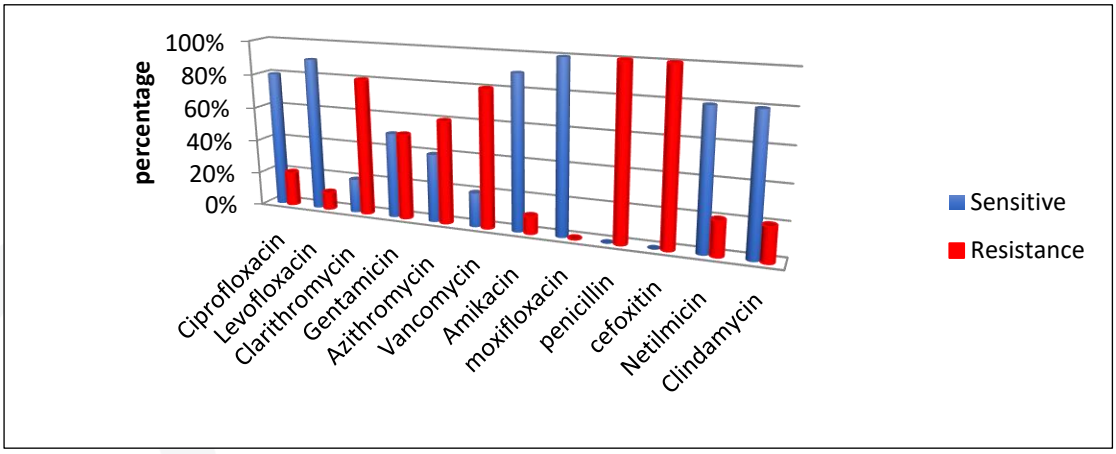

Figure 2 : The percentages of antibiotic resistance and sensitive of S. aureus isolates.

The results of the current study showed that S. Aureus isolates were resistant to vancomycin by 80% and sensitive to it by 20%, this finding disagreed with Hanna (2008) that showed all S. Aureus strains were susceptible to Ceftriaxone and Vancomycin and it showed sensitivity to ciprofloxacin, gentamicin, and amikacin, 80%,50% and 90% respectively, These findings are close to those of Marwah et al. (2015) they found that the majority of S. Aureus isolates from neonatal sepsis were cephalosporin-resistant but sensitive to aminoglycosides. As shown in Figure (2).

Antibiotic Susceptibility of S.epidermidis

The current study's findings, as shown in Figure(4), revealed that of the 26 isolates of S. epidermidis tested, 73.10 percent of them were resistant to Ciprofloxacin(73.10%) and its sensitivity was shown by(27 %). Ofloxacin resistance of (53.80 %) and sensitive to it by (46.20%). Gentamicin resistance by (76.90 %) and sensitive to it by(23.10).

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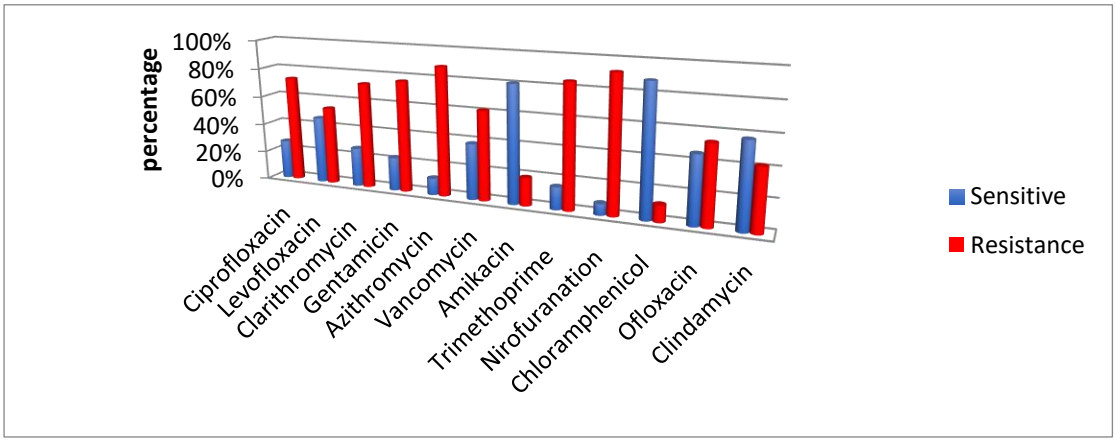

Figure 3 : The percentages of antibiotic resistance and sensitive of S. epidermidis isolates.

Isolates of S. epidermidis bacteria showed resistance to ciprofloxacin, gentamycin and ofloxacin and this results finding agree with Abdul-Rahman, S, (2019) that showed resistance against ciprofloxacin, gentamycin and ofloxacin were (100%) as shown in Figure (3). Antibiotic resistance is difficult to compare between the countries because neonatal sepsis epidemiology is highly variable as seen in some countries (KOSOVSKI et al., 2019).

The most common cause of neonatal sepsis is S. aureus and methicillin-resistant strains S. aureus (MRSA), which are the common bacteria have three aminoglycoside-mutating enzymes that are encoded by plasmids. These enzymes act by inhibiting antibiotics such as gentamicin, as well as causing resistance to this group due to a shift in the S30 subunit to which the antibiotics were attached, while Chloramphenicol inhibits protein synthesis since it is an inhibitory antibiotic due to the fact that it binds to the S30 subunit, Enzymatic inhibitions are the most well-known public resistance mechanism in bacteria. This mechanism is based on a variety of techniques for altering the structure of antimicrobial agents, including hydrolysis, which is a type of reaction that occurs primarily with Beta lactam agents Bhullar et al., (2012).The explanation for the continuous rise in antibiotic resistance may be due to widespread use of these antibiotics by humans, which leads to the emergence of new strains with high resistance to antibiotics (Llarrull et al., 2009).

Antibiotic Susceptibility of Pseudomonas aerginosa

Pseudomonas aerginos isolates showed resistance to Ceftazidime (12.50 %)and sensitive by (88%). Ticarcillin resistance by(62.50%) and sensitive to it (37.50%).

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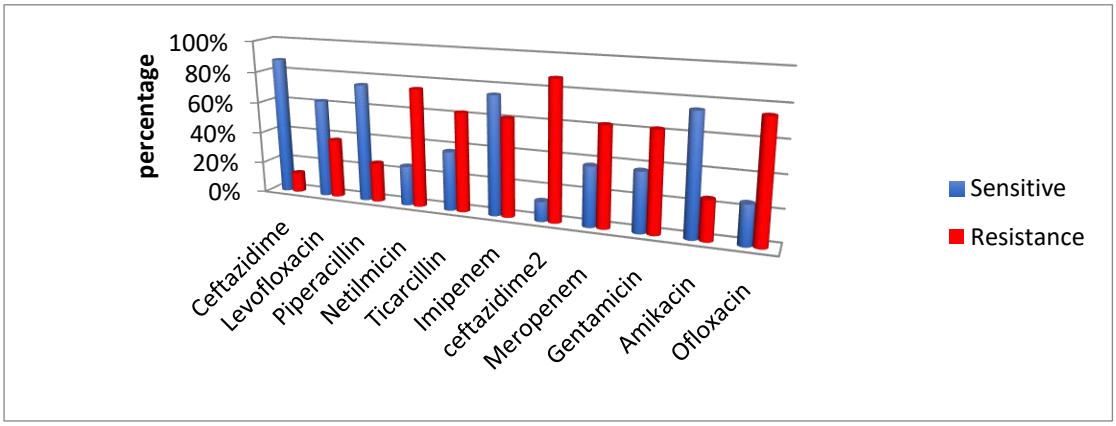

Figure 4 : The percentages of antibiotic resistance and sensitive of Pseudomonas aerginosa isolates.

The current study found that Pseudomonas aeruginosa isolates were sensitive to ceftazidime. These findings are close to those of Sami (2018), who found that all Gram-negative bacteria were resistant to Ceftazidime (77.4%), but all Pseudomonas aeruginosa isolates were sensitive to Ceftazidime by (100%), It showed resistance to Ticarcillin by 62.50% and was sensitive to it by 37.50% , According to Köksal et al. (2001) from India, that showed Gram-negative bacteria were resistance to Amoxicillin, Ticarcillin, Cefotaxime, Ceftazidime, Ceftriaxone, and Gentamicin, and only with 57.14 % of E. coli sensitive to Gentamicin, these results were consistent with the current study. The variation in resistance may be due to sample sources, as well as environmental and test conditions. Based on this studies and previous studies, it was determined that Pseudomonas aeruginosa resist to antibiotics due to its ability to alter membrane permeability represents a variety of mechanisms, it manufactures wide-narrow beta-lactamase enzymes and biofilm formation, as well as its own R-resistance plasmids that carry various antibiotic resistance genes (Hong et al., 2016). As shown in Figure (4).

Antibiotic Susceptibility of Escherichia Coli

Escherichia coli isolates showed resistance to Cefotaxime by (33.30 %) and sensitive to it by (67 %). Meropenem resistance by(0 %) and (100 %) sensitive to it. Gentamicin resistance by(33.30 %) and its sensitivity (66.70%). Amikacin resistance by(0%) and its sensitivity (100%).

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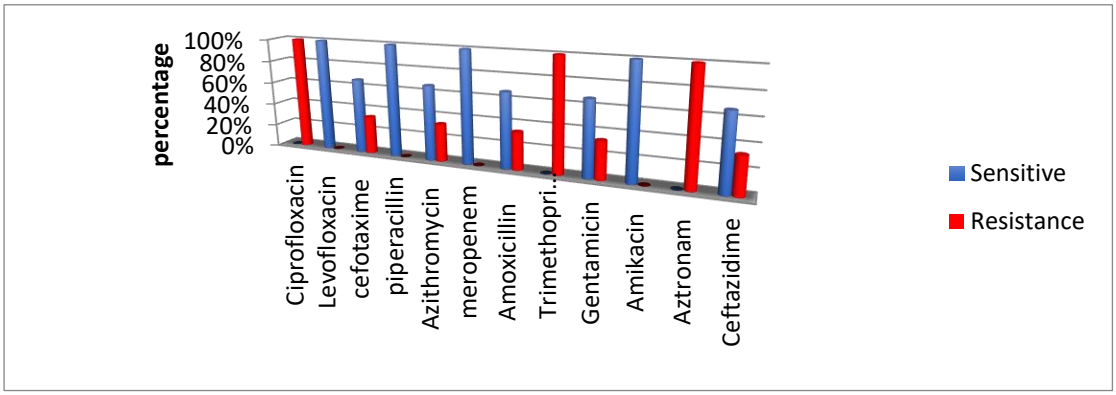

Figure 5 : The percentages of antibiotic resistance and sensitive of Escherichia coli isolates.

The isolates of Escherichia coli showed sensitive to Meropenem, Cefotaxime and Gentamicin with high sensitivity to Amikacin, and this results are agree with Lebea and Davies reported in (2017) that showed all isolated of E. coli strains were sensitive to Meropenem and Cefotaxime, with high sensitivity to Amikacin (65%) and Piperacillin (92.8%) from South Africa. These results were disagree to the results reported by El-Din (2015) from Egypt, that found most Gram-positive bacteria isolated were 100% resistant to Penicillin and Cefoxitin, but 100% sensitive to Moxifloxacin. Results of antimicrobial sensitivity tests for all isolated Gram-negative bacteria against certain antibiotics showed variable sensitivity patterns in antibiotic resistance which is now a worldwide problem. Studies of multidrug-resistant bacteria that cause neonatal sepsis in developing nations are increasing, especially in NICU (Aletayeb et al., 2011). There was no total resistance to all antimicrobials displayed by any isolated bacteria in this study. E. coli isolates may have several antibiotic-resistant mechanisms, such as the ability to form biofilms. They may also be able to customize Efflux pumps and alter the antibiotic's target locations (Liedo et al., 2009).

E. coli produces beta-lactamase enzymes, which are an effective way for bacteria to avoid beta-lactam antibiotics. B- Lactamase enzymes are encoded by genes found in the majority of Gram-negative bacteria genera (Chuma et al., 2013) **(**Figure 5). The antibiotic susceptibility trend varies in different studies as well as at different times in the same hospitals (Lebea and Davies, 2017).

Furthermore, the emphasis in Baqubah city in the last ten years has been on antibiotic resistance, as evidenced by the current study and other studies such as (Khder, 2008, Aziz et al., 2014, Bakir and Ali, 2016, Abdulrahman et al., 2018,Motib et al., 2020), whose bacterial resistance to antibiotics has been increasing in recent years. This is a real crisis and there are many explanations for it, including: many antibiotic prescriptions are written in clinical settings without first investigating the infectious germ and without conducting an antibiotic sensitivity test; the patient does not take

or follow the entire prescription exactly as prescribed; and the patient does not take or follow the entire prescription exactly as directed, and if she or he begins to feel better, they stop to take the antibiotic before the infection is fully eradicated, than the bacteria are more likely to develop drug resistance. Many medicines are available on the market (pharmacy) that are poor quality, poor hygiene and sanitation, and are stored at high or low temperatures (Salah, 2017).

Antibiotic Susceptibility of Klebsiella Pneumonia

Klebsiella Pneumonia isolates showed resistance to Amikacin (23.10 %) and sensitive to it by(76.90 %). Ceftriaxone resistance by (84.60 %) and sensitive to it by(15 %). Ceftazidime resistance by (92.30%) and sensitive by (7.70 %).

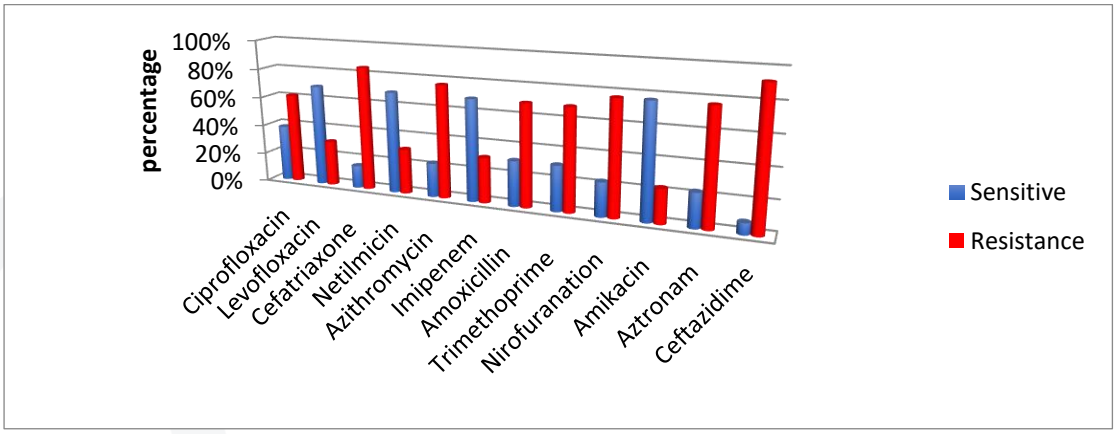

Figure 6: The percentages of antibiotic resistance and sensitive of Klebsiella Pneumonia isolates.

The isolates of klebsiella pneumoniae showed resistance to ceftazidime and cefatriaxone and these results are in agreement with Köksal et al. (2001) from India which showed that Gram-negative bacteria were resistant to ceftazidime, cefatriaxone, Ticarcillin, and showed sensitive to amikacin by 76.90% and resistance by 23.10%, and this finding are agreement with Farshed and Emamghoraishi (2010) which shown that most Gram positive and Gram negative bacteria were totally sensitive to amikacin. The ability of the Enterobacteriaceae family to produce beta-lactamase enzymes and its resistance to beta-lactamase group is attributed to its ability to produce beta-lactamase enzymes and its resistance by several mechanisms, including reducing antibiotic permeability into the cell, analyzing antibiotics with beta-lactamase enzyme, and reducing affinity to the enzyme. Penicillin-Binding Proteins are proteins that bind to penicillin (Latteef et al., 2017), bacterial resistance to the group of quinolones anti-quinolone resistance in Klebsiella spp, is mediated by

flow pumps, which cause multiple antibiotic resistance. The widespread use of this antibiotic has resulted in a high rate of bacterial resistance. As shown in Figure (6).

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Conclusion

This study showed the high prevalence of S. epidermidis as Gram-positive bacteria and K. pneumoniae as Gram-negative bacteria among suspected neonatal cases. Overall isolates showed maximum sensitivity towards aminoglycosides and quinolones, emergence of antibiotic resistance among bacterial isolates from neonatal sepsis is a major cause for treatment failure, higher morbidity and mortality. Proper antibiotic guidelines and its effective implementation could be milestone for revolution in the field of antibiotic resistance control. The epidemiology of neonatal sepsis, causative risk factors and antibiotic resistance pattern of pathogens may be used to develop guidelines for management of neonatal sepsis.

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