

Study of etiologic multi-drug resistant bacteria of neonatal sepsis in Al-Hilla city

Amal Talib Atiyah Al-Sa'ady¹, Habeeb S. Naher²

Abstract

131-blood samples were collected from neonates who were admitted to the preterm units and Neonatal Intensive Care Unit (NICU) in Babylon Hospital for Pediatric and Gynecology. Samples were cultured on many different media, 90(68.7%) of blood samples with positive culture. Among 96 bacterial isolates isolated from these cultures, 40(41.7%) gram negative bacteria versus 56(58.3%) gram positive bacteria. The most frequently isolated bacteria were 13(32.5%) *Klebsiella pneumoniae* and 11(27.5%) *pseudomonas aeruginosa* among gram negative. while among gram positive staphylococci have highest rate of isolation 39(69.6%) which included 16(41%) coagulase positive staphylococci and 23(59%) coagulase negative staphylococci (CoNS). Antibiotic susceptibility test performed by disc diffusion test for 28 antibiotics, most isolates have fully resistance to penicillins and cephalosporins. While fully sensitivity was shown in each imipenem and meropenem for both gram negative and gram positive bacteria.

Keywords: Multi-drug resistant bacteria; Neonatal sepsis; Al-Hilla

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Introduction

Neonatal Sepsis (Septicemia) is any systemic bacterial infection documented by a positive blood culture in the neonate [1]. Sepsis remains one of the important causes of neonatal morbidity and mortality all around the world [2]. Neonatal sepsis is categorized according to the time of onset of the disease into three types: Early Onset Sepsis (EOS), Late Onset Sepsis (LOS) and Late Onset Sepsis

(LLOS). EOS is presenting in the first 7-days after birth. It is congenital sepsis typically acquired during the intrapartum period often from microorganisms in the maternal genital tract. It has been reported that 85% of neonates with EOS show symptoms within the first 24hr of life that 5% have symptoms between 24-48hr and a very small percentage of neonates show symptoms between 48hr and 6 days of life. Premature neonates have the most rapid onset. A sudden onset and rapid



progression to septic shock often characterize these infections. There is high mortality rate ranging from 15-50% with EOS [3]. Most common microorganisms for EOS are Group B Streptococci (GBS), *E. coli*, *S. aureus*, *Enterococcus sp.*, and Chlamydia [4]. LOS is presenting 7 days after birth (7-28 days) of age, acquired from environment and has slower onset and a decreased mortality rate of around 10-20%. Common pathogens responsible for LOS are Coagulase Negative Staphylococci (CoNS), *Serratia sp.*, GBS, *Pseudomonas sp.* and *Candida albicans*. The third type of neonatal sepsis, LLOS occurs after 28 days of age mostly it is nosocomial infection. Common micro-organisms responsible for LLOS including *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *Candida sp.*, antibiotic-resistant Gram-negative rods and anaerobic Gram-negative rods [5]. Most neonatal sepsis is caused by bacteria, fungi and viruses. When pathogenic bacteria gain access into the blood stream, they may cause overwhelming infection without much localization (septicemia) or may get predominantly localized to the lung (pneumonia) or the meninges (meningitis).

Predominant organisms vary between geographical areas and time of onset of sepsis. Bacterial causes of syndrome may widely differ among countries according to some factors, mostly the environmental factors, such as, in developing countries the pathogens differ from those seen in developed countries [6]. Resistance to commonly used antibiotics is emerging and constitutes an important problem

worldwide. Although neonates admitted from the community may also carry resistant pathogens. The wide availability of over the counter antibiotics and the inappropriate use of broad spectrum antibiotics in the community may explain this [7]. Reports of multi-drugs resistant bacteria causing neonatal sepsis in developing countries are increasing, particularly in intensive care unit. Methicillin resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* are the most common, while, *Klebsiella spp.*, and *Enterobacter spp.*, are often reported in this context [8], this study was suggested and designed for detection the following aspects:

- Most common bacteria causing neonatal sepsis.
- The sensitivity of bacteria against the common use antibiotics.

Materials and methods

During the period of 8-months from first of March 2015 to October 2016, 131 blood samples were collected from neonates who were admitted to the preterm units and Neonatal Intensive Care Unit (NICU) in Babylon Hospital for Pediatric and Gynecology. Blood samples were inoculated directly into BHI broth supplemented w/0.05% SPS, LQ004 pediatrics (20 ml). The specimens were transported within one hour to the core laboratory and incubated for 24hr for culture. After incubation, the samples were inoculated on MacConkey agar and blood agar plates

and incubated aerobically while chocolate agar plate was incubated with 10%CO₂ in candle jar, then incubation at 37 C° for 3 days. Every day, the growth was checked. The bacterial isolates were diagnosed according to their characteristics depended on the morphological properties, Gram stain,

biochemical tests, in addition to Api kits and then compared with referential references [9, 10, 11]. Antibiotic susceptibility test was performed by using disc diffusion method on Muller-Hinton agar [12] with the antibiotic as in table-1.

Table 1.

Antibiotic Discs Used in the study.

Antibiotic	Symbol*	Conc. mcg*	Antibiotic	Symbol*	Conc. mcg*
Amikacin	AK	30	Imipenem	IPM	10
Amoxiclave	AMC	30	Kanamycin	K	30
Ampicillin	AMP	10	Meropenem	MRP	10
Aztreonam	AT	30	Methicillin	M	5
Bacitracin	B	0.04 U/disc	Nalidixic acid	NA	30
Carbencillin	CB	100	Norfloxacin	NX	10
Cefepime	CPM	30	Oxacillin	OX	1
Cefotaxime	CTX	30	Piperacillin	PIP	100
Ceftazidime	CAZ	30	Rifampicin	RA	5
Ceftriaxone	CTR	30	Tetracyclin	TE	10
Cephalothin	KF	30	Tobramycin	TOB	10
Chloramphenicol	C	10	Trimethoprim	TMP	10
Ciprofloxacin	CIP	5	Vancomycin	VA	30
Clindomycin	DA	10	Gentamicin	GEN	10

*According to the manufacturing company HiMedia/ India.

Results and discussion

A 90/131(68.7%) of blood samples with positive culture as in table 2, similar high rate was also reported by [13] in Baghdad (65.4%). Some other studies in Iraq reported low rate in positive blood culture [14, 15].

Table 2.

Numbers and percentages of blood samples with positive culture

Blood samples	Total number of samples	Growth (+)	%	Growth (-)	%
	131	90	68.7%	41	31.3%

Positive blood cultures remain the standard method for detecting neonatal sepsis and isolation of the pathogen in order to optimal choice and duration of antibiotic treatment. Therefore, finding of a negative blood culture frequently influences management of sepsis [16]. In spite of this, the negative blood cultures aren't necessarily mean no sepsis, because of many factors influence the yield from blood cultures included: non bacterial sepsis [17], the sensitivity lack of media to identify all bacterial species especially slow-growing or fastidious bacteria [18],

antibiotic pretreatment [16] and insufficient volume of blood sample for culture [19]. Among 96 bacterial isolates: 40(41.7%) gram negative bacteria versus 56(58.3%) gram positive, table 3. This finding is in accordance with that of other studies which showed that gram positive bacterial isolates were more common than gram negative [20, 14]. While another studies showed that gram negative bacteria were responsible in most cases of neonatal sepsis, such as [21].

Table 3.

Numbers and percentages of bacterial isolates isolated from blood samples

Blood Samples	Total Number Of Bacterial Isolates	Gram Negative Bacteria (%)	Gram Positive Bacteria (%)
	96	40 (41.7%)	56 (58.3%)

This variation among different studies can be attributed to various risk factors represented by children's age, gestational age, body weight, education and hygienic conditions [14]. Generally the spectrum of organisms causing neonatal sepsis in this study is different with those results being obtained from neonatal sepsis all around the developing countries, with gram negative bacteria being responsible in most cases. On the other hand, it was in disagreement with a worldwide increasing in the number of neonatal

sepsis caused by gram negative bacteria [22].

Gram negative bacteria

As shown in table 4, 40gram negative bacteria isolates were obtained from blood samples in descending order as follows: 13(32.5%) *Klebsiella pneumoniae*, 11(27.5%) *Pseudomonas aeruginosa*, 6(15%) *Enterobacter aerogenes*, 4(10%) *Escherichia coli*, 4(10%) *Citrobacter freundii*, 1(2.5%) *Acinitobacter baumannii*, 1(2.5%) *Klebsiella oxytoca*.

Table 4.

Gram negative bacteria isolated from blood samples.

No.	Bacteria	Number of Isolates	%
1	<i>Klebsiellapneumoniae</i>	13	32.5%
2	<i>Pseudomonas aeruginosa</i>	11	27.5%
3	<i>Enterobacteraerogenes</i>	6	15%
4	<i>Escherichia coli</i>	4	10%
5	<i>Citrobacter frundii</i>	4	10%
6	<i>Acinitobacter baumannii</i>	1	2.5%
7	<i>Klebsiella oxytoca</i>	1	2.5%
Total Number of Gram Negative Isolates		40	

Based on these data, predominant isolates were: 13(32.5%) *Klebsiella pneumoniae*. These results are in agreement with [13], while in agreement with [15]. This variation in the rate of isolation might be attributed to the hospital policy in management of such cases, drug over use; geographic climatic and hygienic factors may also be correlated with the relative variability of results among different areas [23]. It is not known whether these differences reflect true differences in pathogens across the world, reflect an epidemiological transition in some countries linked to the fact that most infected neonates in developing countries die at home before reaching the health facilities and they do not appear in the statistics [15]. In this study, *K. pneumoniae* ranks the first stage of isolation as a cause of neonatal sepsis which accounted for 13(32.5%) as shown in table 4. This finding was higher than [20, 13] who reported only 7% and 25%, respectively while it was lower than [24] who stated that the incidences were 38%. This finding was in agreement with [25] who found that *K. pneumoniae* plays as a major pathogen in neonatal sepsis and nosocomial infections. The possible

explanation for high rate of isolation perhaps because *K. pneumoniae* represents a member of coliform bacteria and intended to be an indicator of fecal contamination [25]. Although *K. pneumoniae* is found as normal flora of the mouth, skin, and intestines, it can cause destructive changes to human lungs if aspirated and it was well-established pathogens in NICU because it can cause upper respiratory tract infection, pneumonia, UTI, surgical wounds infection, diarrhea, conjunctivitis, osteomyelitis, meningitis, bacteremia and sepsis. In addition, it was the most common cause of outbreaks in the NICU during the 1970s [26].

In recent years, *K. pneumoniae* has become important pathogen in nosocomial infections. Because since once it infects the bloodstream, and can spread to every organ in the body. In addition, invasive devices such as respiratory support equipment, urinary catheters and the increase use of antibiotics put neonates at increased serious risk to it [27].

As shown in table 4, *Pseudomonas aeruginosa* ranks the second stage of isolation 11(27.5%). The high frequency for isolation of *P. aeruginosa*



may be related with this bacteria as a significant pathogen associated with both nosocomial and community-acquired infection. It is regarded as endemic organism causing serious nosocomial infections including meningitis, endocarditis, otitis media, chronic pulmonary colonization and pneumonia, conjunctivitis, UTI and osteomyelitis [28]. On the other hand, the hospital environment can represent an important source of *P. Aeruginosa* [29]. It is ubiquitous nature such as the ability to survive in the moist environment and resistance to many antibiotics make *P. aeruginosa* a common pathogen in NICU and associated with high mortality rates [30].

As shown in table 6. *Enterobacter aerogenes* ranks the third stage of isolation 6(15%). The increases colonization of these bacteria in neonates may be because it is a common commensal in the human gut and commonly recovered from the feces of neonates. Additionally, inadequate hygiene by staff and overcrowding of the NICU [31]. In a conclusion of study in India [5], the frequency of *E. aerogenes* isolation was under changing. During a period from (1995-1998), it represented the most common organism causing 35% of neonatal sepsis.

While a significant reduction in this incidence revealed after (2001) due to this bacteria dropped significantly with the emergence of Gram positive cocci especially CONS and *S. aureus*. *E. aerogenes* is reported to cause many outbreaks in NICU as documented in some literatures [32, 33].

With its capability to acquire antibiotics resistance rapidly during treatment [34]. In the literatures. The sources of infection in *Enterobacter spp.*, outbreaks varied from milk powder, thermometers, transducer head used in ICU, colonized patients, and water which were used to bathe the neonate [32]. In Fiji, an outbreak of *E. aerogenes* infection was reported in NICU where *E. aerogenes* was transmitted through direct injection of contaminated normal saline into the bloodstream of neonates, which was shared among the case patients [35].

Gram positive bacteria

As shown in figure 2, 56 gram positive isolates were obtained from blood samples. 39(69.6%) Staphylococci have highest rate of isolation followed by 10(17.9%) Streptococci, 5(8.9%) *Listeria monocytogenes* and 2(3.6%) *Bacillus cereus*. As detailed in table 5; among 39 isolates of Staphylococci, there are 16(41%) coagulase positive staphylococci and 23(59%) coagulase negative staphylococci (CoNS). Based on these data, the predominant staphylococcal group was CoNS. This result was in accordance with that results being obtained by [24] who reported that CoNS were the most common late onset sepsis pathogens accounting for In Al-Riyadh, Saudi Arabia. As the predominant nosocomial pathogen, CoNS presents a particular and continuing challenge for distinguishing between true infection and contamination. This result supports the view that CoNS should not be regarded as a skin contaminant in neonates. The high percentage of

CoNS suggests that may result from repeated handling of the neonates by personal and family with poor hygiene or it may be an over diagnosed entity as it is difficult to clearly definite from possible infections and probable contaminants even with the availability of guidelines [24]. Some other studies

have reported lower incidences: in the United States, CoNS represented 32.5% of Gram positive pathogens in late onset infections [36]. While in India, a study recorded that CoNS causes 4% of very low birth weight(VLBW) neonate infections [37].

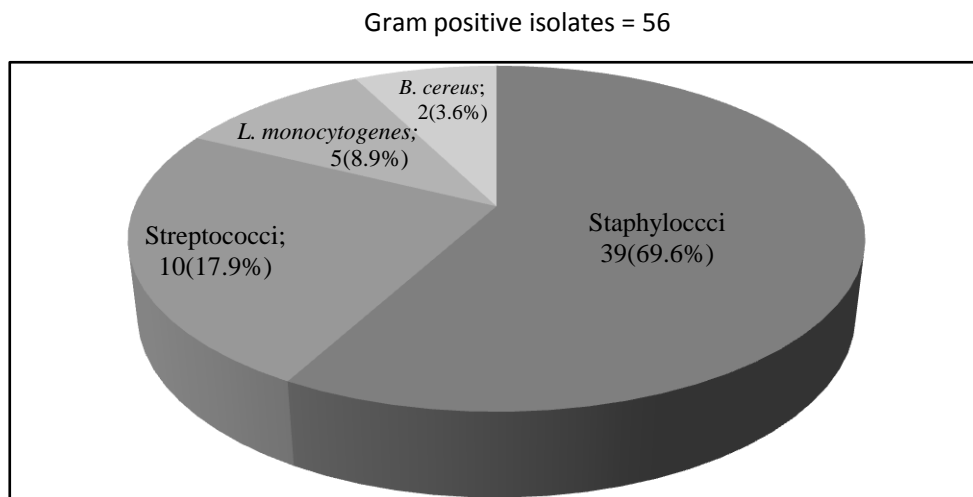


Figure 1.
 The percentages of gram positive isolates isolated in this study

Table 5.
 Distribution of coagulase positive and coagulase negative staphylococci

Bacteria			Number and Percentages of Isolates	Total Number of Staphylococci = 39
Coagulase Positive Staphylococci	1	<i>S. aureus</i>	16/39(41%)	
Coagulase Negative Staphylococci	2	<i>S. epidermidis</i>	6/39(15.4%)	
	3	<i>S. haemolyticus</i>	6/39(15.4%)	
	4	<i>S. saprophyticus</i>	4/39(10%)	
	5	<i>S. lugdunensis</i>	3/39(7.7%)	
	6	<i>S. xylosus</i>	1/39(2.6%)	
	7	<i>S. warneri</i>	1/39(2.6%)	
	8	<i>S. caprae</i>	1/39(2.6%)	
	9	<i>S. gallinarum</i>	1/39(2.6%)	
Total number of coagulase negative staphylococci = 23/39(59%)				

It must always take into account that CoNS are considered as non pathogenic until their implication as nosocomial agents with emergence of multi-drug resistance which are responsible for high mortality rates worldwide [38]. The emergence of CoNS populations with heterogeneous resistance to oxacillin causes a great difficulty to detect them in clinical routine laboratories [39]. With advances in neonatal medicine and technology at the present time, there are more premature infants surviving at younger gestational ages and at lower birth weights [40].

It is believed that increased risk of nosocomial infection in this population is due to host factors such as relative immaturity of their immune system, along with dependence on technology, particularly the use of indwelling catheters, and prolonged hospital stays [41]. A detailed look at the distribution of CoNS isolates was shown in table 5, in descending order of percentages as following; 6/39(15.4%) for each *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*, followed by 4/39(10%) *Staphylococcus saprophyticus*, 3/39 (7.7%) *Staphylococcus lugdunensis*, and finally, 1/39(2.6%) for each *Staphylococcus xylosum*, *Staphylococcus warneri*, *Staphylococcus caprae* and *Staphylococcus gallinarum* (figure 2). This finding was in agreement with other studies about predominant of *S. Epidermidis*. *S.epidermidis* is a particular concern for patient with catheters or other surgical implants because it forms as a virulence factor that occurs most commonly on

intravenous catheters and on medical prostheses [42].

In general looks for table 5 and figure 2, *S. aureus* was the highest incidence among staphylococci since it accounted 16/39(41%). And it was recorded as predominant bacteria among all isolates of Gram positive in the current study. This finding can be explained as human seems to have weak resistance to surface *S. aureus* colonization, so the bacteria are easily colonize in the nose and on the skin. The isolation rate of *S. aureus* depends on several factors like virulence of isolates, health status of patients and effect of environmental conditions [43]. This finding was similar to that in other studies which noticed that *S. aureus* became the dominant organism causing LOS after 2001, primarily due to an increase in LOS exclusively in low birth weight (LBW) and VLBW neonates and the resultant longer hospitalization with using of invasive devices and catheters which make neonate more vulnerable to the infection [5].

A recent study by showed that *S. aureus* accounted for (33%)of bacteria isolated from blood stream [14]. Whereas, another studies reported low isolation rate of *S. aureus* [37, 24]. As compared to other bacteria, *S. aureus* was more resistant to the action of disinfectants, tolerant to desiccation and they keep viable for long time in the environment after having been discharged from patients, resistant to many antibiotics especially methicillin (MRSA) which facilitated their widespread in environment, and their ability to survive in adverse

environments for a very long time and some strains can even withstand temperature up to 60°C for 30 minutes and survive at NaCl concentrations as high as 15 percent [44]. Additionally, It's adaptive power to antibiotics and to competition with other bacteria made *S. aureus* one of the most potent pathogens, and thus it can cause a range of infectious diseases from mild conditions, such as skin and soft tissue

infections, to severe, life-threatening infections like pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis, these infections may spread through many ways, contact with pus from an infected wound, skin-to-skin contact, and contact with objects such as towels, sheets and clothes. Deeply penetrating *S. aureus* infections can be severe [45].

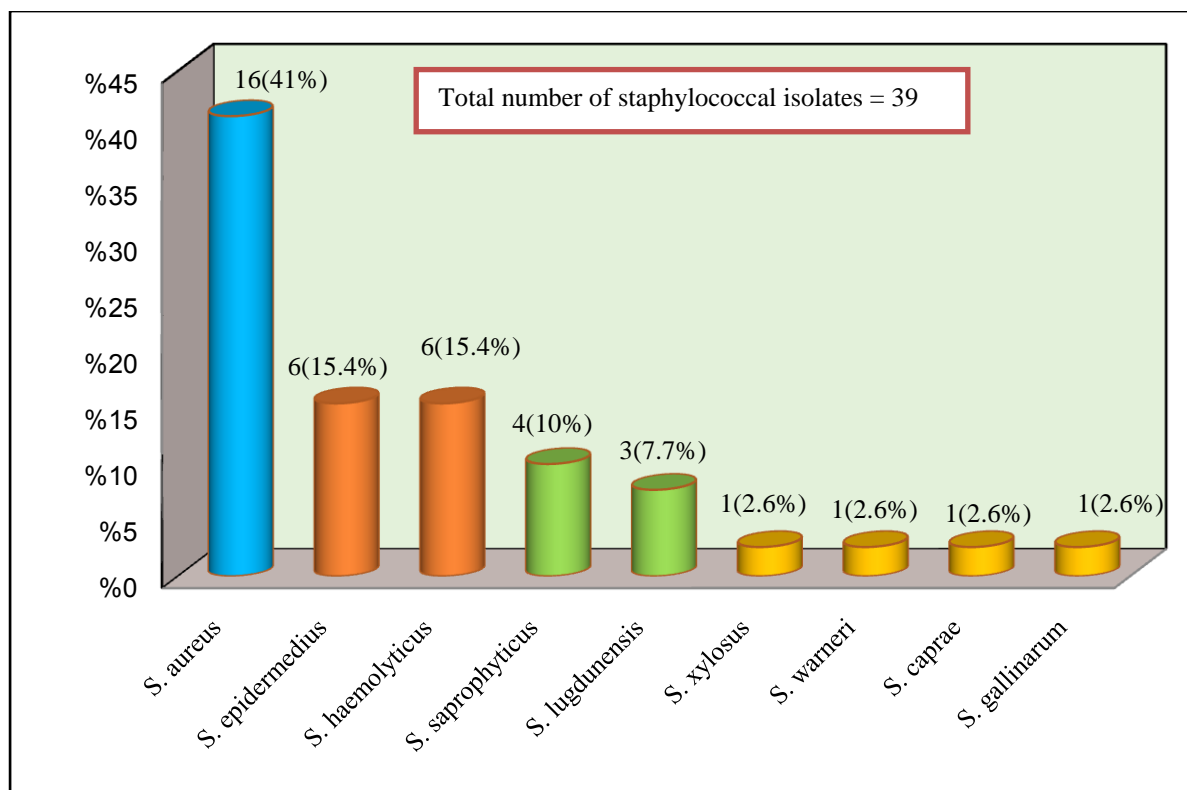


Figure 2.
 Numbers and percentages of staphylococcal isolates

Table 6, shows the numbers and percentages of streptococcal isolates which included; 4(40%) *Enterococcus faecalis*, 3(30%) *Streptococcus agalactiae*, 2(20%) *Streptococcus pyogenes*, and finally,1(10%) *Streptococcus pneumoniae*. Based on

these data, *E. faecalis* and *S. agalactiae* are the most common species of streptococci in neonatal sepsis and this finding was in agreement with other studies which have been reported *Enterococcus spp.* as an important pathogen in neonatal sepsis [46].

Table 6.
 Numbers and percentages of streptococcal isolates

No.	Bacteria	Number and Percentage
1	<i>Enterococcus faecalis</i>	4/10(40%)
2	<i>Strptococcus agalactiae</i>	3/10(30%)
3	<i>Strptococcus pyogenes</i>	2/10(20%)
4	<i>Strptococcus pneumoniae</i>	1/10(10%)
Total number of streptococcal isolates = 10		

On the other hand, an outbreak was indicated of primary bloodstream infections caused *E. faecalis* at NICU of a cardiology hospital in Brazil [47]. These outbreaks are an indication of patient-to-patient transmission due to the immunosuppression caused by underlying conditions and therapeutic procedures e.g., central venous catheterization which causes direct contact between neonate's bloodstream and the environment [48]. Although *E. faecalis* is normal flora of the gastrointestinal and genitourinary tract, recently, it has emerged as important nosocomial pathogen with high mortality infections; endocarditis, bacteremia, UTI, sepsis, pneumonia and meningitis, and it was reported as the second-most commonly isolated agent of nosocomial infections in the United States during a period of 1986-1989 [49]. The widespread of *E. faecalis* as a nosocomial pathogen can be explained by its possession of various survival and virulence factors. Survival factors including: ability for competition with other bacteria, tolerance of hard nutritional conditions, survive in very harsh environments e.g., extreme alkaline pH(9.6), salt concentrations,

bile salts, detergents, heavy metals, ethanol, azide, and desiccation [50], survive over long periods on inanimate objects such as thermometers and stethoscopes, growth in the range of (10–45)°C and survive at temperatures of 60°C for 30 min [51]. While, virulence factors including: suppression of lymphocytes action, biofilmand possession of lytic enzymes. Additionally, high levels of antibiotic resistance contribute to *E. faecalis* pathogenicity especially since vancomycin resistant *E. faecalis* (VREF) is becoming more common. In the assessment of the rate of isolationin a recent study, it is important to take the time of onset of the infection into consideration, this factor affects the rate of isolation of *E. faecalis* in neonates,, this bacteria accounted 4% for early onset sepsis (EOS) and 17% for late onset sepsis (LOS) [36].

In the current study, *S. agalactiae* (GBS) ranks second order as a causative agent of neonatal sepsis, this was in contrast to other studies where showed that this bacteria was reported as the main cause and it was isolated from 70% blood samples [46].While another study found that GBS



accounted for most pathogen in 22.6% EOS [36]. Other studies showed low frequency of isolation of *S. agalactiae* [37, 24]. This variation in the rate of isolation may be because of the differences in maternal health, hygiene conditions during pregnancy and delivery, and hospital policy between countries and between different regions in the same country. Maternal colonization of the lower genital tract with GBS during pregnancy increases the risk of neonatal sepsis by vertical transmission. Approximately 20–30% of healthy women are colonized rectovaginally with GBS, and 50–70% of neonates to these women will themselves become colonized. Group B streptococcus GBS is recognized as the most frequent cause of early onset neonatal infection. GBS early onset infection which occurs with pneumonia and respiratory failure complicated by bloodstream infection and sepsis. These cases result from ascending infection through the placental membranes to initiate intrauterine infection, or by aspiration of infected vaginal fluids during the birth process [52].

In this study, the reported incidence of *Listeria monocytogenes* was 5(8.9%), figure 3-1. This incidence was higher than other studies [46, 14]. This variation can be attributed in part to hygienic policy, feeding regulation and industrial clean-up efforts. Listeriosis is an infection caused by *L. monocytogenes*, it is relatively rare and occurs primarily in neonates and immunocompromised patients [57]. Pregnant women may be acquired listeriosis by ingestion of *listeria* and can carry it asymptotically in their

genitourinary tract or vagina. Maternal infection with *L. monocytogenes* can result in chorioamnionitis, premature labor, spontaneous abortion, or stillbirth. Fetal Listeriosis can occur via descending transplacental transmission, ascending transmission through ruptured amniotic membranes and vertical transmission can also occur via passage through an infected birth canal [53]. In the present study, the lowest isolation rate of gram positive bacteria was 2(3.6%) for *Bacillus cereus*, figure 2. This rate of isolation was low in contrast with results by a study in Netherlands, where detected an outbreak of systemic infections caused by (37%) *B. cereus* occurred in NICU. The assessment of the origin of infections with *B. cereus* is often difficult because it's a ubiquitous in the air, water, soil, feces and other environments, furthermore, the lack of awareness of its toxicity in neonates may mistakenly be overlooked and considered as a contamination without clinically relevant [54]. Nonetheless, many published reports described numerous environmental reservoirs for *B.cereus* in hospitals include air filtration and ventilation equipment, fiber-optic bronchoscopy equipment, linens, gloves, hands of staff, intravenous catheters, alcohol-based washing solutions, specimen collection tubes, balloons used in manual ventilation and reused towels [54]. On the other hand, epidemiologic investigations of outbreaks that have occurred, a recent study in U.S. investigated *B.cereus*–positive tracheal aspirates from neonates on ventilators in NICU and *B. cereus* isolates were



characterized in 33.3% of neonatal samples [55]. Nosocomial infections by *B. cereus* was considered a serious issue because of the hospital environment may be contain it's endospores which are highly resistant to harsh environmental conditions and common methods of decontamination, such as heating, desiccation, chemical exposure and radiation. Furthermore, alcohol-based hand washing solutions used in NICU are not sporicidal [56]. Despite *B.cereus* is a common cause of

food poisoning, it is increasingly being acknowledged as an opportunistic pathogen can cause systemic infections and localized infections.

The pathogenicity and virulence of *B. cereus* are related to production of several toxins and enzymes include necrotizing enterotoxin, emetic toxin, hemolysis and phospholipases. In addition to production of biofilm which can play a major role in attachment of this bacteria to medical instruments [57].

Disc diffusion test (ddt)

Antibiotic susceptibility tests performed by disc diffusion test for 28 antibiotics were detailed previously in table 2.

Gram negative bacteria

The percentages of antibiotic resistance in Gram negative bacteria, table 6, shows that 100% resistance was reported against KF, while no resistance was observed in both IPM and MRP for all Gram negative bacteria (full sensitivity), This finding is in agreement with a study demonstrated that Enterobacteriaceae has 100%

sensitivity to carbapenems [55], whereas another study emphasized that infection with carbapenem-resistant Enterobacteriaceae or carbapenemase-producing Enterobacteriaceae is emerging as an important challenge in health-care settings infections have caused high rates of morbidity and mortality in particular among persons with prolonged hospitalization [54].

Table 7.

Percentages of antibiotic resistance in gram negative isolates by disc diffusion Test in this study.

Bacterial isolate		<i>P. aeruginosa</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>E. aerogenes</i>	<i>C. frundii</i>
Antibiotics						
Penicillins	AMP	--*	100	92.3	100	75
	PIP	63.6	50	53.8	50	25
	CB	100	100	61.5	83.3	61.5
β-lactamase inhibitor combinations	AMC	100	100	84.6	83.3	61.5
Cephems	KF	100	100	100	100	100
	CPM	90.9	50	76.9	66.7	50
	CTR	81.8	100	53.8	33.3	0
	CTX	81.8	100	53.8	16.7	0
	CAZ	36.4	25	0	0	0
Carpapenems	IMP	0	0	0	0	0
	MRP	0	0	0	0	0
Monobactams	AT	90.9	25	92.3	66.7	25
Aminoglycosides	GEN	90.9	75	30.8	16.7	25
	AK	45.5	25	0	0	0
	TOB	45.5	25	30.8	16.7	25
Tetracyclins	TE	72.7	25	15.4	33.3	50
Fluoroquinolones	CIP	45.5	25	15.4	0	0
	NX	45.5	25	0	0	0
Quinolones	NA	81.8	25	23.1	33.3	25
Folate pathway inhibitors	TMP	--	75	61.5	66.7	50
Phenicols	C	90.9	50	0	16.7	25
Ansamycin	RIF	100	100	92.3	100	75

*(--) test was'nt performed for this bacteria.

On the other hand, many antibiotics were revealed high levels of resistance: >70% for each AMP and RIF; >60% for each CB and AMC. Similar findings were reported by [13]. In the present study, low levels of resistance <50% were recorded against several antibiotics: NX, CIP, CAZ and TOB. This observation is comparable with [58]. In a recent study, CIP was the most effective drug against the tested Gram positive and Gram negative bacteria [68]. CIP and NX were relatively new class of quinolone

antibiotics and the use of which has recently become very common, particularly in general practice, in addition quinolones have also been found to be effective by other investigators in the treatment of multidrug resistant gram negative infections in various patients, including premature and extremely low birth neonates [58]. As detailed in table 3-6, most types of gram negative bacteria have high resistance >70% for more than three groups of antibiotics, included, *P.aeruginosa*, *K.*



pneumoniae, *K. Oxytoca*, *E. aerogenes*, and *E. coli*. That means, these bacteria have multi-drug resistance. Development of antibiotic resistance in Iraq is often related to the availability of antibiotics out of hospitals which encourage self-medication [59]. Published series reported severe gram negative neonatal infections with all organisms being multi-resistant. The detection of multi-drug resistant isolates may further limit therapeutic options. The high prevalence of gram-negative neonatal infections associated with resistance to the commonly used antibiotics indicated that the infection was most probably nosocomial in origin [60].

On the other hand, the variety of antibiotic susceptibility of gram negative bacteria worldwide may attributed to that generally it is not an easy task to compare antibiotic resistance between countries because the epidemiology of neonatal sepsis is extremely variable. Few studies compare antibiotic susceptibility over time in the same unit, but where data are available they show increasing resistance to commonly used antibiotics. As with many bacteria, the recommended treatment has changed as the organism has developed resistances. The choice of a specific antimicrobial agent depends on local susceptibility patterns and on the part of the body that is infected. For patients with severe infections, a prudent approach is the use of an initial short course (48-72hrs) of combination therapy, followed by a switch to a specific mono-therapy once the susceptibility pattern is known for the specific patient. *Klebsiellaspp*, with

the ability to produce extended spectrum β -lactamases are resistant to multiple antibiotics and current evidence implicates a plasmid as the source of the resistant genes [31]. *P. aeruginosa* is naturally resistant to a large range of antibiotics and may demonstrate additional resistance after unsuccessful treatment, in particular, through modification of a porin. It should usually be possible to guide treatment according to laboratory sensitivities, rather than choosing an antibiotic empirically. If antibiotics are started empirically, then every effort should be made to obtain cultures, and the choice of antibiotic used should be reviewed when the culture results are available [58].

Gram positive bacteria

The percentages of antibiotic resistance in gram positive bacteria are detailed in table 8. Gram positive bacteria have 100% resistance against each AMP, AMC and KF. All isolates have high resistance $\geq 80\%$ for CAZ, may be because these antibiotics are the most commonly used in the therapy of bacterial infections compared with other β -lactam antibiotics [33]. The resistance to these antibiotics was not attributed only to production of β -lactamases, but could be due to drug extrusion through efflux pumps or the decreased affinity of the target PBPs or decreased permeability of the drug into the cell. These organisms not only survive penicillin therapy but can also protect penicillin-susceptible bacteria from penicillin by releasing the free enzyme into the infected tissue or pus [44].

Table 8.

Percentages of antibiotic resistance in gram positive isolates by disc diffusion test in this study.

Antibiotic	Bacterial isolate	<i>Staphylococci</i>					<i>Streptococci</i>			<i>L. monocytogenes</i>	<i>B. cereus</i>
		<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. haemolyticu</i>	<i>S. saprophyticus</i>	<i>S. lugdunensis</i>	<i>E. faecalis</i>	<i>S. agalactiae</i>	<i>S. pyogenes</i>		
Penicillins	AMP	100	100	100	100	100	100	100	100	100	100
	OX	81.3	33.3	50	25	33.3	75	66.7	0	40	50
	MET	75	50	66.7	25	33.3	75	33.3	0	20	0
β -lactamase inhibitor combinations	AMC	100	100	100	100	100	100	100	100	100	100
Cephems	CPM	93.8	0	50	50	66.7	100	66.7	50	20	50
	CTX	100	50	100	50	66.7	100	33.3	0	0	0
	CTR	100	50	66.7	50	33.3	100	33.3	50	20	0
	CAZ	100	90	83.3	75	80	100	100	100	80	100
	KF	100	100	100	100	100	100	100	100	100	100
Carbapenems	IMP	0	0	0	0	0	0	0	0	0	0
	MRP	0	0	0	0	0	0	0	0	0	0
Glycopeptides	VA	6.3	0	0	0	0	0	0	0	0	0
Aminoglycosides	GEN	75	83.3	83.3	75	66.7	75	100	50	40	0
	AK	25	33.3	33.3	25	0	50	0	50	20	0
	K	100	100	66.7	75	66.7	100	66.7	100	20	0
	TOB	81.3	83.3	83.3	50	100	50	100	50	40	50
Macrolides	E	75	50	50	25	33.3	75	33.3	50	0	0
Tetracyclins	TE	25	50	0	50	0	50	33.3	50	20	0
Fluoroquinolones	CIP	25	33.3	0	0	33.3	25	0	0	0	0
	NX	25	16.7	0	0	0	25	0	0	0	0
Lincosamides	DA	25	33.3	16.7	25	0	25	33.3	0	20	0
Phenicols	C	0	0	0	25	0	25	33.3	0	0	0
Ansamycin	RIF	31.3	33.3	33.3	25	33.3	25	33.3	0	0	0

In the present study, fully sensitivity (100%) was found against each of IMP, MRP and VA for all isolates (except one isolate of *S. aureus*). The very high sensitivity in IMP and MRP can be attributed to the fact that Carbapenems are broad-spectrum antibiotics, and its β -lactam rings are resistant to hydrolysis by most β -lactamases [55].

Vancomycin (VA) is a glycopeptide antibiotic and it is considered a last resort medication for the treatment of septicemia caused by gram positive bacteria due to early observations of its nephrotoxicity and ototoxicity as well as its intravenous dosing requirements [43]. A use kept alive for many years by the fact that compound had to be



given intravenously and thus provided bacteria fewer opportunities to evolve resistance, and the fact that organisms were relatively slow to evolve/adapt to it, even in experiments. On the other hand, low levels of resistance < 40% was recorded for CIP, NX, RIF, C and DA. These findings were in agreement with other studies which have stated that fluoroquinolones (CIP, NX) are effective antibiotics which can inhibit bacterial growth by effecting DNA maintenance, therefore, many types of Gram positive bacteria were sensitive to it [59]. In many studies, DA was showed low levels resistance at rate and the possible explanation that presence the action of virulence factors that found in some isolates and absent in others, also differences in source of samples, conditions of tests used and type of techniques. All these factors may lead to differences in resistance levels [41]. Also low resistance to RIF can be attributed to the fact that development of rifampin resistance which required long period of time for organism to evolve resistance [43] or because rifampin should not be used alone for antimicrobial therapy but combination with other agent in order to reduce the rate of resistance. So, rifampin combined with newer fluoroquinolones (e.g. gatifloxacin, norfloxacin, moxifloxacin, and levofloxacin) have enhanced activity against G+ve bacteria [51]. Most other antibiotics revealed a variable levels in resistance according to the type of bacteria: OX, ME, CPM, CTX, CTR, GEN, TOB, E, TE, DA, and K, table 8.

As detailed in table 3-7, *S. aureus*, *S. epidermidis*, *S. haemolyticus*, *S. saprophyticus*, *S. lugdunensis*, *E. faecalis* and *S. agalactiae* have resistance >70% for more than three groups of antibiotics, which means that

these bacteria were multi-drug resistant. *S. epidermidis*, *S. haemolyticus*, *S. saprophyticus*, and *S. lugdunensis*, are members of CoNS which were considered nosocomial agents with emergence of multiresistance [38]. Healthcare-associated infections caused by these bacteria are responsible for high mortality rates worldwide. The emergence of heterogeneous resistance to oxacillin of CoNS populations resulted in a great difficulty to detect and treat them in clinical routine laboratories [42]. On this account, vancomycin has been widely used and it is a major cause for the emergence of glycopeptide-resistant CoNS [39].

In this study, *S. aureus* and *E. faecalis* have highest levels of resistance for most antibiotics. This results was in agreement with results of other study which was reported that all isolates of *E. faecalis* had high levels of resistance to AMP, CIP, GEN, penicillin, streptomycin, teicoplanin and VAN [60]. In recent years, enterococci have become important nosocomial pathogens. The most important characteristics of this bacteria include their inherent resistance to several antimicrobial agents and their ability to acquire resistance determinants. Resistance against such diverse groups of β -lactams, macrolides, aminoglycosides, and glycopeptides continues to evolve. The emergence of glycopeptides resistant enterococci is a serious nosocomial problem with important implications for hospital infection control. Although it's geographic distribution is worldwide, the epidemiology appears to differ within and across regions. In this study, *S. aureus* isolates were highly resistant to most antibiotics, especially, β -lactams which is mostly due to either production of β -



lactamases or lack of penicillins receptors on cell wall and/or alteration in their permeability to β -lactam antibiotics preventing the up taking of them [56]. However, in the present study, the high percentage of resistance to the combination amoxicillin-clavulanic acid (CAM) can be attributed to the antibiotic spectrum of the combination which is determined by the companion penicillin, not the β -lactamase inhibitor. Thus, some β -lactamases that resistant to amoxicillin, can also resistant to clavulanic acid [61]. The resistance to cephalosporins mediated by cephalosporinase production.

Furthermore; β -lactamase produced by staphylococci excreted into the surrounding environment by which the hyper production of β -lactamase will give longer validity and surviving to this bacterium, because the hydrolysis of β -lactams takes place before the drug can bind to PBPs in the cell membrane. In the present study, Level of resistance in *S. aureus* against OX was >80%. This percentage was higher than those obtained by [62]. This variation can be assign to the popular administration of antibiotics without physician's consulting and it's availability in the hospitals and pharmacies and due to differences in source of samples. Staphylococcal resistance to either oxacillin or methicillin occurs when the organism including an altered PBP (PBP2A) [57]. As shown in table 3-7, 75% of *S. aureus* isolates were methicillin resistant (MRSA). This finding was higher than other studies, in UK, methicillin-resistance percentages are (39.6%, 40.1%, 39.3%, 39.2%) for years (1999, 2000, 2004, 2005)

respectively [44]. In US, a recent study reported 62% (MRSA) during a period (1999-2005) [63]. MRSA is a bacterium responsible for several difficult to treat infections in humans. It is any strain of *S. aureus* that has developed, through the process of natural selection, resistance to β -lactam antibiotics, which include the penicillins and the cephalosporins. MRSA is especially troublesome in hospitals, prisons and nursing homes, where patients with open wounds, invasive devices, and weakened immune systems are at greater risk of infection than the general public [59]. In the current study, 6.3% one isolate only of *S. aureus* was vancomycin resistant (VRSA), table 3-7. this finding was in contrast with other studies in Iraq were reported full sensitivity rate (100%) to vancomycin and no VRSA isolates. This variation may be attributed to the fact that disk diffusion procedure can not differentiate isolates with reduced susceptibility to vancomycin from susceptible isolates even when incubated for 24hr.

Additionally, vancomycin resistant *S. aureus* (VRSA) strains may produce only subtle growth around a vancomycin disk [63]. The widespread use of vancomycin makes resistance to the drug a significant worry, Vancomycin resistance evolved in more common pathogenic organisms during the 1990s and 2000s, including vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) [64].



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