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Neonatal Bacteremia Caused by Staphylococcus Epidermidis in Kirkuk City

Zahraa Ayid Ahmed, Ahmed Mahdi Hassan, Mustafa J. Jalal
Department of Biology, College of Science, University of Kirkuk, Iraq

Rabia Khalid Abdulsalam
College of pharmacy, University of Tikrit, Iraq

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Annotation: This study investigated cases of neonatal bacteremia and identified the causative agent in Kirkuk City. Eighty blood samples were collected from a cohort of neonates presenting with clinical signs of bloodstream infection who were admitted to the neonatal intensive care unit for sick and preterm infants. Staphylococcus epidermidis was isolated and identified based on morphological, biochemical, and physiological tests. Twenty-five (25) of the eighty samples (31.25%) yielded positive cultures. Antimicrobial susceptibility testing of selected isolates indicated susceptibility to vancomycin, amikacin, and teicoplanin, and demonstrated that penicillin was ineffective for the treatment of the bacteremia. The production of certain virulence factors by the isolates under study was also examined; the isolates differed in their ability to produce beta-lactamase enzymes and other virulence determinants.

Keywords: Neonatal sepsis, Newborns, Preterm infants, Staphylococcus epidermidis.

Introduction

Bacteremia caused by disruptions of mechanical and chemical barriers of immune system because of bacterial invasion and multiplication in blood stream (Cerra, 1985), and this case may result in septicemia and leads to septic shock, both are considered as severe disease especially in neonates and characterized by fever, skin rash, and blood clotting disorder as well as sever bleeding leading to death of neonates (Levinsonw et al., 2018). Many signs and symptoms appear in neonates with bacteremia like decreased breathing rate to 60 breaths/minute, deep

sleep, unregulated heart beats, decreased or increased body temperature, loss of consciousness, inability to breastfeed, and frequent seizures (Gotoff, 1996).

Neonatal bacteremia is classified according to the age of the children into:

A- Early-onset neonatal bacteria: which occurs during the first week immediately after birth due to the presence of bacteria in the mother's reproductive tract before or during birth, where symptoms appear within the first 72 hours of birth, as they are obtained vertically from the pregnant mother before or during childbirth, in this case, the germs in the mother's reproductive system are of great importance (Gebrehiwot *et al*., 2012) and also the presence of the germ in the cervix and the spread of the germ in the placenta through which the feeding vessels of the fetus pass or the settlement of these germs within the membrane lining the uterus and their multiplication during pregnancy (Sgro *et al*., 2011), premature birth of the mother or rupture of the membrane surrounding the fetus for a long time, where the germ can live inside the membrane surrounding the fetus, which ruptures before the date of birth, and in turn affects the fluid surrounding the fetus, where it is contaminated with the germ (Roymond *et al*., 2008), as well as multiple births without taking into account the intervals between births and the next, and the frequent infections in the mother's reproductive system without proper treatment, which are the most important causes(Faxelivs *et al*., 1998). Early neonatal bacteremia, this type of bacteremia is a very serious problem, especially among very low birth weight babies and triples the mortality rate (Klinger *et al*., 2020).

B- Late - onset neonatal bacteremia (LONB): After the first week of the child's life and caused by bacteria acquired from the hospital or the environment after birth, cases of children with acquired bacteremia have been documented significantly while in hospitals (Luthander *et al*., 2015).

It occurs after the first 72 hours of birth and is a major cause of infant mortality and is acquired from the care setting, the incidence rate ranges from 1.87 to 5.42 per 1000 births (Verma *et al*., 2018). Several studies have confirmed that the risk of late bacterial septicemia increases in infants who have diseases such as necrotizing enteritis, chronic lung disease, and children with these clinical issues often require more invasive care as they require prolonged periods of mechanical ventilation and venous ventilation during the treatment period in hospitals (Nguyen *et al*., 2020). Even after birth, the risk of this infection in children continues to be as it spreads rapidly by germs that originate from the environment (Brady, 2023).

Most LONB infections in newborns are classified as healthcare-related infections because they occur while infants are receiving central care, the most common symptoms being catheter-associated bloodstream inflammation, ventilator-associated pneumonia, and the most common germs causing LONB are *Staphylococcus epidermidis* (Downes *et al*., 2020).

The most important causes of Bacteremia in neonates

This infection may occur in babies after birth during their visit to the hospital, and the most common reasons are:

1. The presence of pathogenic germs, including bacteria, viruses, and fungi that reach the bloodstream and cause infection, including the bacterium under current study, and viruses such as hepatitis B virus and human immunodeficiency virus (Downes & Weiss *et al*., 2020).

2- Central venous catheterization: caused by inserting intravenous catheterization for newborns who need continuous treatment through them or when they need parenteral nutrition, and it causes germs to enter the bloodstream when the catheters are contaminated with germs.

3- Respiratory infections such as bronchitis and pneumonia, which in turn lead to the spread of germs from the lungs to the bloodstream.

4-Acquired nosocomial infections that the child may be exposed to during the transmission of germs from other patients or from contaminated medical devices used and in direct contact with

them (Cameron & Trenti *et al* ., 2021) and may be related to the hospital environment when dealing with doctors, nurses and visitors, and it is one of the most important health problems that occur inside hospitals, and according to studies, bloodstream infections, burns, urinary and respiratory tract infections constitute the vast majority of nosocomial infections (Weber *et al* ., 2014)

The National Institutes of Health (NIH) has estimated that 60-80% of deaths that occur annually in children are caused by bacteremia (Kunz *et al*., 2022), which is one of the most serious diseases that threaten the lives of a large number of newborns if it is not treated in time with all effective methods (Cerra, 1985) and is increasing the number of children infected with bacteremia in recent years has risen from 621,000 in 2001 to 1 million in 2008 in the United States alone (Hall *et al*., 2021).

Staphylococcus epidermidis

It is one of the most important opportunistic pathogens that affect newborns or the elderly and immunocompromised children, and due to the difficulty of treating it due to its resistance to methicillin, it has made it the most prevalent Gram-positive bacteria (Jatsho *et al*., 2021, Meskin, 1998).

Staphylococcus cells are spherical in shape positive to Gram-stain, arranged in irregular clusters, and may be in pairs or singularly, immobile, and non-spore-forming, optional aerobic and anaerobic (Brooks, *et al*., 2007). Growing on normal mediums at a temperature of 37°C, the colonies exhibit a thin, shiny white to gray circular colony with a diameter of 2-3 mm depending on growing conditions and is able to tolerate concentrations of sodium chloride salt (NaCl up to 15%, catalase-positive, oxidase negative, and coagulation negative, and grows on the mannitol salt agar medium and is one of the most important normal flora for humans, as its presence is concentrated on the skin and is found in the oral cavity and upper respiratory tract (Forbes, *et al*., 2002).

This bacterium is involved in the occurrence and spread of infections associated with tissue and organ transplantation in the elderly and immunocompromised patients who take doses of immunosuppressive drugs (Kumur *et al*., 1997, Hassani, 2006), as well as having many virulence factors, the most important of which is presence of capsule, which play a very important role in resisting the body's defenses and antibiotics, and largely through the strength of the germ's adhesion to the body, where (Al-Zubaidi, 2000) indicated in his study that 20% of the isolates of this bacterium possess the capsule. It has the ability to produce the slime layer at the same time (Fiebelkorn *et al*., 2003).

Materials and Methods

Method of collecting blood samples

Eighty blood samples from newborns and premature babies were collected at the Children's Hospital in Kirkuk for the period from 1/1/2025 to 10/4/2025.

Blood culture and Subculture

Method listed by (Mahon *et al*., 2007), in brief, 2 ml of withdrawn blood from each neonate were injected into sterile Brain Heart Infusion bottles and incubated at 37˚ C for 48 hours, then positive culture were observed as coagulation of blood. Then positive blood cultures were streaked on Manitol salt agar and incubated at 37˚ C for 24 hours.

Phenotypic identification

It ensures the distinguishing of the developing colonies in terms of shape, size, height, edge shape, as well as their viscosity, where the colonies of *Staphylococcus epidermidis* bacteria are pink without changing the color of the medium.

Microscopic examination

Gram-staining technique was used to investigate the shape, color and arrangement of bacterial cells using a light microscope.

Biochemical identification

1-Catalase Test

This test was performed by mixing several young colonies in a drop of hydrogen peroxide solution at a concentration of 3% on the surface of glass slide, the positive result indicated by the appearance of O2 gas bubbles (Koneman *et al.*, 2006).

2. Oxidase Test

A portion of the pure colony was transferred with wooden to the surface of a filter paper saturated with the oxidase reagent, the appearance of a dark purple color within 5-10 seconds indicating the ability of the bacteria to produce the enzyme.

3. Urease Test

Christensen urea agar was inoculated with pure bacterial colony and then incubated at 37 o C for 24 hours, the positive result was inferred by the change in the color of the medium from yellow to pink (Collee *et al.*, 1996).

4. DNase agar test

It was prepared by dissolving 4.2 g of the medium prepared by Oxoid in a liter of distilled water, adding a little Toluidine blue dye, adjusting the pH at 7.3, sterilizing the sealer (Macfaddin, 2000) and using it to detect DNase-producing bacteria.

5. Blood hemolysis test

The Blood agar base was prepared according to the manufacturer's instructions, and blood was added to it by 5%, stirring gently, and then poured into sterile petri dishes (Chessbrough, 2006).

6. Investigation of Lecithinase Enzyme Production

The isolates under study were inoculated by streaking method and then incubated at 37 °C for 24 hours, the results were read by observing bright areas around the developing colonies and observing white deposits around or under the colonies, evidence of lecithinase enzyme production.

7. Investigation of Lipase Enzyme Production

The isolates under study were inoculated by streaking method and then incubated at 37° C for 24 h, the lipase-degrading colonies were surrounded by a wide range of transparent zones (Collee *et al.*, 1996).

8. Protease Enzyme Investigation

The bacterial isolates were also inoculated by the streaking method and incubated at a temperature of 37 °C for 24 hours, and it was found that the isolates producing the Protease that decomposes complex proteins into simple amino acids observed to form a clear zone around the growth line of the bacteria, indicating the production of the enzyme by the bacteria (Collee et al., 1996).

9. Investigation of the production of beta-lactamase enzymes:

Rapid Iodometric method was used as follow:

1- The bacterial isolates were cultured on MacConkey agar and incubated at a temperature of 37 °C for 24 hours.

2- Several colonies were taken from MacConkey agar by a Loop and transferred to a test tube

containing 100 microliters of Penicillin G solution and incubated the tubes at a temperature of 37 C for 30 minutes.

3- 50 microliters of the prepared starch solution were added to the tube and the tubes were Shaked.

4. 20 microliters of the prepared iodine solution were added to all tubes and Shaked well for one minute.

5- The result is positive when the dark blue color changes to white one minute after adding the solutions.

6- The test is repeated when a positive result is delayed (more than ten minutes).

10. Nutrient Broth Medium

Use for the purpose of activating bacterial isolates and also use the slant agar medium to preserve bacterial isolates.

11-Antibiotics Sensitivity Test

The susceptibility test of isolated bacteria to several antibiotics was performed using the modified (Kirby–Bauer *et al*., 1966) method approved by the World Health Organization (Vandpittee *et al*., 1991). Muller Hinton medium (Oxoid) was used and antibiotic disks were used according to the concentrations used globally as stated in the recommendations of the World Health Organization, a suspension of young bacteria was prepared in a phosphate saline solution and the concentration of bacteria was fixed at 10^8 cells/cm^3 using 0.5 standard McFarland tube, and the sterile cotton swab was immersed in the suspension and the excess suspension was disposed of by wiping the swab on the inner walls of the tube and then spreading the suspension on the surface of the medium and leaving the dishes at room temperature to dry, the antibiotic disks were fixed to the surface of the dishes using sterile forceps, then the petri dishes were incubated at 37°C for 24 hours and the inhibition zones was measured for each antibiotic disk.

Results and Discussion

Isolation

80 samples were collected from newborns aged (1-40) days who were admitted to the hospital and the results showed that 25 neonates (31.25%) were infected with bacteremia while other 55 (68.75%) did not show infection, and this is a result consistent with the results of (Mane *et al*., 2010) which obtained a percentage of positive samples estimated at (26.9) %.

Identification

Early diagnosis of the disease leads to increased effectiveness in treatment, reduces other clinical consequences of the disease, and reduces the costs associated with diagnosis and treatment, as it is possible to start treatment during the early stages to avoid negative consequences later on, and the diagnosis can be summarized with four clinical signs observed on the affected child, which are a rise in body temperature to more than 38 degrees Celsius. The heartbeat accelerates to more than 90 beats per minute and the breathing rate increases to more than 20 breaths per minute (Bone *et al*., 1992).

The bacterium was diagnosed by microscopic examination of the bacterial colonies stained with Gram stain where they appeared in clusters and the colonies appeared circular in shape white or oblique to a gray or shiny yellow color (Al-Salihi 2020).

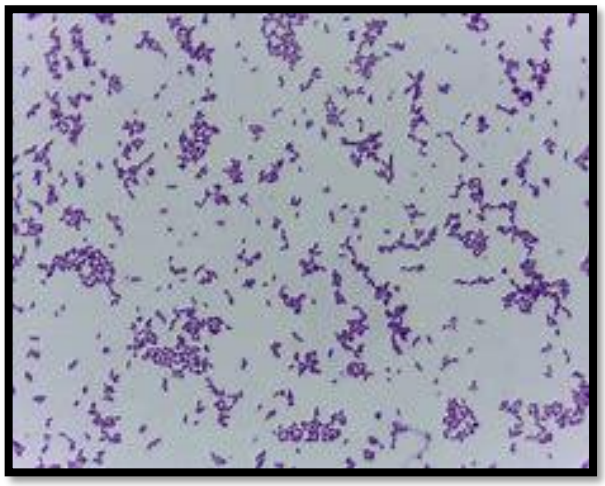

Figure (1) shows the *Staphylococcus epidermidis* bacterium under the microscope after staining with Gram stain.

Staphylococcus epidermidis was cultured, the results indicate that this bacteria was not fermented for mannitol and biochemical tests were performed as shown in Table (1) where (25) isolates (31.25%) were obtained and this result is similar to the findings of (Zhiling *et al*., 2013) who explained that this bacterium constitutes the majority of positive isolates of Gram stain isolated from the blood in newborns.

Table (1) shows the most important biochemical tests used in the diagnosis of the bacterium.

S. epidermidis	Chemochemical Tests
+	Catalase •
-	Oxidase •
+	Urease •
-	Mannitol •
+	Glucose •
-	Coagulase •
-	Dnase •
-	Hemolysis •
-	Motility •

The API STAPH diagnostic system was used to confirm the diagnosis and purity of the microbial isolation and to identify the type of *S. epidermidis* as shown in the figure (2).

Figure (2): API system result of *S. epidermidis*.

Investigation of the production of beta-lactamase enzymes

The ability of bacterial isolates to produce beta-lactamase enzymes has been revealed by the rapid iodine method, which is one of the most accurate and best methods that give correct and clear results (Dubin, 1999).

The ability of the bacterium to produce these enzymes was revealed by the appearance of white color immediately after the addition of the iodine reagent due to the ability of the enzyme beta-lactam to hydrolyze the beta-lactam ring of penicillin and the production of penicillic acid, which in turn reduces iodine to iodide, causing the appearance of the white color represented by the origin complex – iodine. (Koneman *et al* ., 2006).

The results of the detection showed that 20 isolates (80%) were producing the enzyme and similar to what they obtained by (Jarlov and Hoiby, 1998) in their study, indicating that (80-90) % of their isolates were producing the enzyme beta-lactames.

Virulence factors that *Staphylococcus epidermidis*

This bacterium has a set of virulence factors that enable it to easily reach the bloodstream and thus be able to damage the targeted organs (Uslan *et al*., 2007).

Production of Lecithinase and Lipase

The present study showed that *Staphylococcus epidermidis* produces Lecthinase enzymes by 64% (16 isolates), that is consistent with the findings of (Kirkan *et al*., 2005) which show that this bacterium produces this enzyme by 70% of isolates.

The result show that only 13 isolates (52%) were lipase producing isolates, as this enzyme works to give the bacterium a high pathogenicity through its hydrolysis of fat, thus facilitating the penetration into the skin tissues of the infected organ down to the subcutaneous area, and this finding was in agreement with with the result of (Gupta *et al* ., 1980).

Protease Investigation

The production potential of this enzyme was investigated and the percentage was 72% with 18 isolates, which is in agreement with (Brooks *et al*., 2001) who confirmed the ability of clinically isolated of *Staphylococcus epidermidi*s to produce the Protease enzyme, and thus differ slightly from the study of (Hamada, 2008) which obtained 58% of its total isolates.

Table (2) The Bacteria Production of virulence factors

Virulence factors	*Staphylococcus epidermidis*
Lipase	13(52%)
Lecithinase	16(64%)
Protease	18(72%)
B-Lactamase	20(80%)

Antibiotic sensitivity

Early and correct antibiotic treatment reduces the risk of acute illness and reduces deaths among children due to bacteremia and reduces the appearance of multidrug-resistant bacteria in hospital intensive care units (Vaudaux *et al*., 1994).

In this study, *Staphylococcus epidermidis* was resist at 40% to oxacillin in children with bacteremia, previous work by (Jain *et al*., 2004) show that this bacteria resist to this antibiotic at 66%. While the results show that this bacterium was 100% sensitive to Teicoplanin and Vancomycin, a result that is completely consistent with the results of (Mane *et al*., 2010) and with the results of the study (Zhiling *et al*. 2013). While it resists both Erythromycin and Tetracycline at (15 isolates (60%) and 8 isolates (32%), respectively, which are agreed by Zhiling *et al*. (2013), which obtained an antibiotic resistance rate of 86.6% and 38.4%,

respectively. It differed with (Khalaf, 2008) where the resistance to the Erythromycin in his study was 19%.

These results were consistent with the findings of (Omar, 2009) who found that the rate of resistance to the tetracycline is 33%. as well as with the findings of (Mahmood, 2010) and the results of the study (Al-Jumaili, 2008) which reached 37%.

As for its resistance to Clindamycin, it was close to (Ahmed, 2008) as it achieved a resistance rate of 42.85% to the antibiotic while the resistance of the bacterium in our study was 32% with only (8) isolates.

It was also found that this bacterium is 72% sensitive to Gentamicin, and this finding is consistent with the result of (Sudharshan *et al*., 2013), which is identical to the findings of Al-Saadi (2001) and Al-Nuaimi (2002) for the resistance of the *Staphylococcus epidermidis* to this antibody by 75%.

All isolates *of S. epidermidis* showed 100% resistance to penicillin and ampicillin, and these results were consistent with (Al-Douri, 2009), as they shows that they exceeded 97%. This proves that the bacterium *Staphylococcus epidermidis* has a mec gene which encodes the enzyme Penicillinase, in turns breaks the *ß.lactam* ring of the antibiotic and interferes with the synthesis of peptidoglycan, a component of the bacterial cell wall (medigan et al., 2001).

As for the Tobramycin resistance, it was high resistance at 68%, which is completely consistent with the results of (Kamarulzaman, 2001) who also obtained high resistance to the same antibiotic.

The reasons for bacterial resistance to aminoglycosides are due to the presence of antibiotic modulatory enzymes such as cellular enzymes that act on anti-aminoglycosides such as Aminoglycosid acetyl Transferase (AAC), Aminoglycosid Acetyl Phosphotransferase (APH), and Aminoglycoside Adenyl Transferase (ADD)(Pool, 2005).

Staph. epidermidis shows resistance to ciprofloxacin at 28%, and this result is agreed with the previous work by (Abid AL-Rashedi, 2009), while not agree with the study of (Esraa, 2010) which shows that this bacteria resist at 9.1% towards the ciprofloxacin. While all isolates were sensitive to amikacin and this in agreement with (Al-Taii, 2007).

Table (3): Antibiotics susceptibility profile of *Staph.epidermidis*.

Isolates	OX	VA	TOB	CL	P	AMP	GEN	TET	ERY	ON	CIP	FRIEND
1	R	S	R	R	R	R	R	R	R	S	R	S
2	R	S	R	R	R	R	R	R	R	S	R	S
3	R	S	R	R	R	R	R	R	R	S	R	S
4	R	S	R	S	R	R	R	R	R	S	R	S
5	S	S	S	S	R	R	R	S	S	S	R	S
6	S	S	S	S	R	R	R	S	S	S	R	S
7	S	S	S	S	R	R	R	S	S	S	S	S
8	R	S	R	R	R	R	R	R	R	S	R	S
9	R	S	R	R	R	R	R	R	R	S	S	S
10	R	S	R	R	R	R	S	S	S	S	S	S
11	S	S	S	S	R	R	S	S	S	S	S	S
12	R	S	R	S	R	R	R	R	R	S	S	S
13	R	S	R	S	R	R	R	S	R	S	S	S
14	R	S	R	S	R	R	R	S	R	S	S	S
15	S	S	R	S	R	R	R	S	R	S	S	S
16	S	S	R	S	R	R	R	S	R	S	S	S
17	S	S	R	S	R	R	R	S	R	S	S	S
18	S	S	R	S	R	R	R	S	R	S	S	S
19	S	S	R	S	R	R	S	S	S	S	S	S

20	S	S	S	S	R	R	S	S	S	S	S	S
21	S	S	R	R	R	R	R	R	R	S	S	S
22	S	S	R	R	R	R	S	S	S	S	S	S
23	S	S	S	S	R	R	R	S	R	S	S	S
24	S	S	S	S	R	R	S	S	S	S	S	S
25	S	S	S	S	R	R	S	S	S	S	S	S
Resistance	40%	0%	68%	32%	100%	100%	72%	32%	60%	0%	28%	0%

Ciprofloxacin=CIP,Oxacillin=OXA,Tetracyclin=TET,ERY=Erythromycin,Ampicillin=AMP,Penicillin=P,Clindamyci=CLI,Gentamicin=GEN,Tobramycin=TOB,Vancomycin=VAN,Ciprofloxacilin=CIP ,Teicoplanin= TEI, Amikacin=AMI.

Conclusion

It is included from current study that is staphylococcus epidermidis that was isolated from blood (septisemia) it has high resistance to antibiotics and in addition possesses virulance factors It increased ability to infection.

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