

Determination of Bacteria Causing Bacterimia in Infants with Unknown Origin of Pyrexia

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Abstract:

The research deals with Isolation and Identification of pathogenic bacterial species from pyrexia of unknown origin cases in infants and determination pattern of antibiotic profile of the most common isolating bacterial species.(150)blood samples were taken from infants below 36 months who were referred to Ibn-Sina Hospital in Mosul city suffering from pyrexia ,then the biochemical, morphological and cultural tests were done on the samples. The results showed positive blood culture for only (50) samples and the following bacteria were isolated: Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, viridians group Streptococci, Pseudomonas aeruginosa in percentage 68%, 16%, 10%, 4%, 2% respectively. Antibiotic sensitivity test performed for Staph.aureus as a high ratio isolated bacteria and the results showed that Gentamycin and Amoxiclav has great activity against Staph.aureus, the percentage of sensitivity were 82.35%, 73.52%, respectively. The strains showed high resistance against Ampicillin, and Co-trimoxazole, While it showed sensitivity against vancomycin at 32.35%.

تحديد الجراثيم المسببة لتجرثم دم الأطفال الرضع المصابين بحمى غير معروفة المصدر

الملخص:

تضمن البحث عزل وتشخيص الأنواع البكتيرية المرضية المسؤولة عن حالات الحمى غير معروفة المصدر في الأطفال الرضع. إذ جمعت 150 عينة دم من الأطفال الرضع الراقدين والوافدين لمستشفى ابن سينا التعليمي وأجريت عليها الاختبارات المجهرية والزرعية والكيموحيوية الملائمة ، وأظهرت النتائج ايجابية زرع الدم لـ 50 عينة فقط، إذ عزلت الجراثيم الآتية Staphylococcus aureus، Staphylococcus epidermidis ، viridians group Streptococci، Escherichia coli ، Pseudomonas aeruginosa ونسب (68%، 16%، 10%، 4%، 2%) على التوالي. ثم اجري اختبار الحساسية للمضادات الحيوية لعزلات جرثومة Staph.aureus لأنها عزلت بنسبة عالية ، وأظهرت مضادات Amoxiclav، Gentamycin فعالية عالية ضدها، إذ بلغت نسب الحساسية 82.35% ، 73.52% على التوالي. وأظهرت العزلات مقاومة كبيرة تجاه مضادات Ampicillin ، Co-trimoxazole، بينما أظهرت العزلات حساسية تجاه مضاد Vancomycin بنسبة 32.35%.

35.32

Introduction

Fever considered the chief cause for entrance the healthy infants into emergency of pediatric units due to suffering from fever without source (FWS) .although the reality that common causative of FWS due to viral infections for which no medical curing is applied, but They may also be resulted from harsh primary bacterial infection particularly in newborn

children shown to be at particularly high risk (Massin *et al.*, 2006)

The variance diagnosis of fever cases in newborn and young children may be resulted from contagious or non contagious causes. Nearly 12% to28% of newborns arriving to a pediatric emergency units with fever have severe bacterial infections(Ishimine.,2006) .

Bacteremia is the existence of feasible bacteria in the revolving human blood stream. Bacteria may arrive the blood stream and causes bacteremia from an presenting localize of infection or from a area having normal flora or by immediate impregnation of polluted instruments into circulating system. These germs are primarily refined from the circulatory

system during several minutes, in this way the bacteremia is quiet and temporal, when the immunity of human is interpenetrated, microbes diligence and stated in the blood and bacterimic signs would apparent during short time (Baraff *et al.*,1993).

Neonatal bacteremia endures a chief reason of infirmity and perishability amongst neonates particularly in evolving nations (Lindsay, 2006) . Wherefrom its prevalence is so higher in the evolved global (Vergnano *et al.*, 2005).

Escherichia coli and *Klebsiella pneumoniae* are most common diagnosed in resulting severe bacterial infections in newborns under than three months of age The most frequent gram positive pathogenic bacteria isolated from serious bacteremia cases in neonates are *Staphylococcus aureus* , group B *Streptococcus* and *Enterococcus* (Vergnano *et al.*, 2005).

Seriousness agents pioneering to newborn septicemia in evolving nations include extended fracture of membranes , miscarriage, birth suffocation , midwifery outside a hospitals, instruments utilized

in cutting and bandaging, the cord and motherly infections at the time of pregnancy (Ali *et al.*, 1994), while gram-negative bacteria dominated the causative organisms in the developing world (Mahapatra & Ghosh., 2002).the picture is different in Europe and North America where Group B Streptococcus is the prominent bacteria in neonatal infections (Fadero *et al.*, 2007) .

As newborn infant's bacteremia is a health destructible emergency, the understanding of grade of prevalence and antibiotic susceptibility profiles of dominated bacteria in a studied region benefits in determining a correct and efficient choice of antibiotics. Prevalence of either the gram-positive or gram-negative pathogens is effected by geographical site and differences in periods of isolation , so the antibiotic sensitivity profile effected by location and period (Nwadioha *et al.*, 2009).

The objectives of our research were to estimate the incidence of bacteremia in newborns below than 36 months of age arrived to the pediatric incidental medical units of Ibn-Sina and al- salam hospitals and to identify the most frequent etiological agents of bacteremia in infants who had fever without a known

origin and determination antibiotic sensitivity profile of the common bacterial isolates.

Materials & Methods

Collection of specimens:

One hundred fifty blood specimens were gathered in April to October 2012 from newborn children under 36 months who were expedited to Ibn-Sina and al-salam Hospitals in Mosul city suffering from fever and which not given any antibiotic. One milliliter venous blood samples were collected in screw cupped tubes containing brain heart infusion broth without anticoagulant materials under aseptic conditions and transported directly to the laboratory and incubated at 37c for 2-7 days, after the incubation time bottles culture which showed signs of growth at any time during the seven days were sub-cultured on to blood agar and chocolate agar and Macconkey agar plates and incubated at 37c for 24h (Murry *et al.*, 2003).

Identification of bacterial isolates:

The growing bacterial isolates on the culture plates were identified by bacteriological techniques including

colony characteristics, Gram staining and biochemical characters (Brooks *et al.*,2007) .

Antimicrobial sensitivity Test

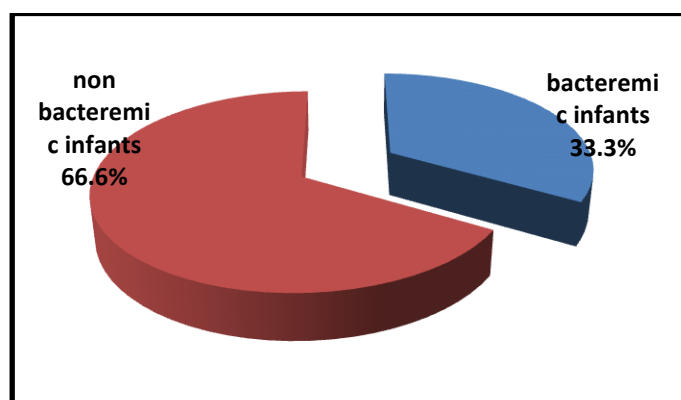
Antibiotic sensitivity test was determined by disk diffusion procedure as referred by the National Committee for Clinical Laboratory Standard NCCLS. The antibiotics tested were ampicillin (AM)(10µg), amoxicillin/clavulanic (AMC) (20/10µg), cephalexin (30µg) (CE), cephtriaxone (30µg) (Cph), amikacin (30µg) (AN) gentamicin (10µg) (GN), tobramycin (10µg) (TO), ciprofloxacin(5µg))(CIP), vancomycin (30µg) (VA), erythromycin (15 µg) (Er), clindamycin (2 µg) (DA), co-trimoxazole(30µg) (COT). Antimicrobial agents disks were obtained from Bioanalyse co., Turkey. The explanatory standards for resistance / sensitive of each antibiotic we were depended on criteria determined by the NCCLS (NNIS,2004).

Results & discussion

Our study showed that 33.3 %(50/150) of the all infants being examined were bacteremic, whereas they exhibited positive blood culture, while

66.6%(100/150) were negative for bacteremia as shown in figure 1.

Figure-1- Rate of bacteremia in infants



The study found that 50 infants (33.3%) have bacteremia (figure 1) and this result was higher compared to some previous studies done in Iraq, namely; maznee (19.4%) (Hasson *et al.*, 2002) , Najaf (23%) (Shams Al-Deen .,2001). Also in the present study the percentage of bacteremia in infants were higher than many studies such as (Nwadioha *et al.*, 2009; Gomez *et al.*,2010) which indicated that percentage of bacteremia in infants were 18.2%,2.2%.1.4% respectively .

This dissimilarity can be correlated with variations in sample number, the geological demographic investigated and

/or to environmental circumstances .The occurrence of fever in the feverish infant at 80.6% percentage can be none of bacterial origin, so most of the examined newborn infant with fever had no origin of contagion displayed with self-limiting viral infection (Brooks *et al.*,2007).

Figure-2 :percentage of gram-positive to gram-negative bacterial strains

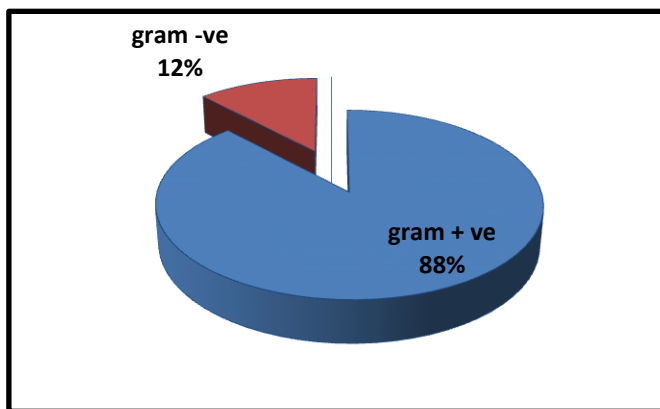


Figure-2- represent the ratio of gram-positive to gram-negative bacteria. Gram-positive bacteria constitute about 88% (44/50) versus 12% (6/50) of gram-negative

In our research, 50 feverish infants were bacteremic being due to infection with gram-positive bacteria (88%) 44 and gram-negative bacteria (12%) 6 as described in figure 2. Our data were approximate to research data being stated by (Hasson *et al.*,2002; ShamsAL-Deen

2001), who reported that the percentage of bacteremia caused by gram-positive bacteria were higher than the percentage of gram-negative bacteria. However, the results were in contrast with other studies(Nwadioha *et al.*, 2009) , who showed that percentages of the gram-negative isolates were more than the gram-positive bacteria. Different data are described in such researches and the variation may be due to several different danger agents such as infant lifetime, pregnancy lifetime, body weight, scholarship and sanitary situations or may due to differences in pattern of bacterial colonization in hospitals and different community habitats.

Table (1) represents the types and rates of pathogens being determined in bacteremia cases which include gram-positive bacteria such as *Staph. aureus* 34(68%), *Staph .epidermidis* 8(16%), *Strep.viridans* 2(4%), while gram-negative bacteria represented by *E.coli* 5(10%), *P.aeruginosa* 1(2%).

Table (1) : rates of bacterial strains isolated from infant bacteremia cases.

Bacterial species	Percentage of isolation
<i>Staphylococcus aureus</i>	%68
<i>Staphylococcus epidermidis</i>	%16
<i>Escherichia coli</i>	%10
Viridians streptococci	%4
<i>Pseudomonas aeruginosa</i>	%2

Staph.aureus was prevalent isolate amidst gram-positive strains which constituted about (68%) among all isolates (Table 1). The prominence of *Staph.aureus* in this study is similar to finding from the following studies (Fadero *et al.*, 2007 ; Hasson *et al.*, 2002),However ,this results contrasts with the studies (Nwadioha *et al.*,2009: Gomez *et al.*, 2010).

This variation in microbial pattern of neonatal bacteremia is in keeping with

the varying nature of bacterial isolates within the same and different geographic region. *Staph.aureus* was also the major isolate in inborn neonates with infection. Thus, a part from possible community and nosocomial acquisition of *Staph.aureus* in both inborn and out born neonates, some of the mothers may harbor the organism in their genital tract as demonstrated in a study from Saudi Arabia (Fadero *et al.*, 2007) .

Bacteremia caused by *Staph.aureus* in infants considered a important health care risk factor because it responsible for high infection occurrence rates on admission, also this bacteria is more infestation amidst gram-positive pathogens and an significant bacteria that able to cause lethal bacteremia, this may be due to its virulence strategies and its capability to resist several antimicrobial agents especially Methicillin Resistant *Staph.aureus* ,which responsible for its widespread in environment and hospitals quickly .

Staphylococcus aureus considered the second dominant bacteria in resulting in late- reliable bacteremia in newborns whose have very low weights. Miserable innovative host protection strategies , the

exigency for central venous catheters, extended entire parenteral feeding ,and the administration of steroids or antibiotics all above responsible for high prevalence and dangerous of *Staphylococcus* infection in preterm birth newborns (Stoll *et al.*, 2002) .

In our study *Staphylococcus epidermidis* represent the second pathogenic bacteria among gram positive strains (16%) of all strains (Table 1), which covenanted with the two studies (Hasson *et al.*, 2002 ; Kloos & Bannerman 1994) which found that *Staphylococcus epidermidis* isolation percentages among newborns with bacteremia were 16.3%,16% respectively. The raising isolation percentage of this bacteria may be due to the irrational administration of broad spectrum antimicrobial agents and to the virulence factors which produced from these pathogens such as of specific pili and fimbriae and slime production especially in newborns whose immune system were immature (Kloos & Bannerman 1994) .

Staphylococcus epidermidis apparent to be the chief pathogenic bacteria universally and responsible for

incidence of disease occurrence rates and decrease percentages in newborns and infants. These bacteria are represented as the most publicity pathogens correlated with bacteremia occurrence in newborns (Hasson *et al.*, 2002) .

Escherchia coli isolates represented the most common cause of infection among the gram negative bacteria and this result agreed with many studies indicated prevalence of this bacteria in etiology of bacteremia in children and infants (Gomez *et al.*,2010; Ireghu *et al.*, 2006) .

Table(2): Percentage of resistance and sensitive isolates of *Staph. aureus*

Antimicrobi alagent	Symbol	Concentration disk / µg	sensitive isolate (%)NO	Sensitive isolate (%)NO	Resistanc e isolates (%)NO
Ampicillin	AM	µg 10	(0)	(5.88)2	(94.11)32
Amoxicillin- clavulnicacid	AMC	µg 30	(0)	(73.52)25	(26.47)9
Co- trimoxazol	COT	µg 30	2.94)1	(14.7)5	(82.35)28
Cephalexin	CE	µg 30	(44.11)15	(52.94)18	(2.94)1
Cephatrixone	Cph	µg 30	(41.14)17	(55.88)9	(2.94)1
Amikacin	AN	µg 30	(26.47)14	(64.7)22	(8.82)3
Gentamycin	GN	µg 10	(11.76)4	(82.35)28	(5.88)2
Ciprofloxacin	Cip	µg 5	(26.47)4	(64.7)22	(8.82)3
Erythromycin	Er	µg 15	(67.64)23	(20.58)7	(11.76)4
Vancomycin	VA	µg 30	(52.94)18	(32.35)11	(14.7)5
Tobramycin	TO	µg 10	(32.35)11	(55.88)19	(11.76)4
Clindamycin	DA	µg 2	(41.17)14	(55.88)19	(2.94)1

The antibacterial potency and spectrum for 12 selected antimicrobial against the isolated *Staphylococcus aureus* from bacteremia in infants are summarized in table 2. The isolates revealed high resistance rates toward ampicillin and co-trimoxazol whereas the strains susceptibility to many antibiotics such as gentamycin , amoxillin-clavulnicacid , amikacin, ciprofloxacin

the isolates showed the same susceptibility rate against three antibiotic (cephatrixone, clindamycin, tobramycin) at percentage (55.88)% .

The risk of raising resistance to antibiotics consider significant problem to the health care centers and to community hygiene after 60 years of irrational and incorrect uses of penicillin

antibiotics in health care centers, raising rate of antibiotic resistance among bacterial strains which leads to decrease the benefit of antibiotics administration for treatment of bacterial infections especially in hospitals (NNIS,2004) .

Bacteria become resistance to antibiotics through the development of specific defense mechanisms which render the antibiotic ineffective like preventing the antibiotic from binding with and entering the cell , or pumping it out of the cell after entrance , producing enzymes that inactivate the antibiotics (i.e., β -lactamases 6 aminoglycosides modifying enzymes),altering the internal binding (target) sites of the antibiotics [i.e,alteration in the PBPs or ribosomal proteins] (Willey *et al.*, 2011) . Some of these mechanisms can be transmitted to other cells through conjugation, which increase the dissemination of antibiotic resistance between bacterial pathogens (Philippon *et al.*, 2002) .

The prevalence of *Staph.aureus* bacteremia in hospital and communities has abstractly developed during the elapsed decenniums(Steinberg *et al.*,1996) . With the arising of methicillin resistance, *Staph.aureus* has againd

more interest as bacteremia including methicillin-resistance *Staph.aureus* (MRSA)are predisposing for high levels of health care center disbursements (Abramson *et al.*, 1999).the raising incidence of antibiotic resistance responsible for casing of earnest attention, commanding an international proposal to its administering. The world health organization and the European commission have apprehend the significance of monographing the rising and deciding of resistance and the requirement for application of restriction approaches (Williams & Heymann., 1998).

Staph.aureus has increasly acquired resistance to several antibiotics which used in recurrence cases . The common notable pattern of this projecting is methicillin-resistant *Staph.aureus* (Oteo *et al.*, 2004) .

Early routine blood culture and antimicrobial sensitivity tests in neonates at risk of bacteremia is away to cut the emergence of multi-drug resistant bacteria and to provide the effective treatment at short period of time before expansion of infection.

It is quite obvious from the results presented in this study that there is a high level of multi-drug resistance among *Staph.aureus* isolated from infants suffering from bacteremia in Mosul city ,Iraq.

Uncontrolled and irrational usage of antimicrobials is definitely one of the major causes of the emergence of multi-drug resistant bacteria especially *Staphylococcus aureus* bacteria ,as it is known to have several resistance mechanisms, whether they are chromosomal or plasmid-mediated.

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