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THE ROLE OF MULTIDRUG RESISTANT ACINETOBACTER BAUMANNII IN NEONATAL SEPTICEMIA AND PROMISING BIOMARKER FOR SCREENING SEPSIS

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Abstract

Acinetobacter baumannii is a Gram-negative Coccobacillus that causes outbreaks of nosocomial infections, specially in intensive care units. *Acinetobacter baumannii* blood infection in the neonatal intensive care unit patients create a great problem in hospital settings, In this study, we determined the epidemiology, risk factors, and results of blood stream infection (BSI) caused by *Acinetobacter baumannii* in neonates. and screening level of SAA to diagnosis neonatal sepsis.

Acinetobacter baumannii from November 2019 to March 2020. 29 of *A. baumannii* were identified from 120 specimens of neonates from Neonatal Care Unite, among of these 16 (55.17%) were male and 13 (44.83%) were female. Main risk factor is Low Birth Weight, 21 (72.41%) neonates low birth weight (< 2500g) and 8 (27.58%) had weight more than 2500g.

We shown in present study A significant increase SAA in the presence of *Acinetobacter Baumannii* infection. main two antibiotics that effective on *Acinetobacter baumannii* infection is imipenem and meropenem.

Key words : *Acinetobacter baumannii*, Neonatal sepsis, SAA.

Introduction

Neonatal sepsis refers to an infection involving bloodstream in newborn infants less than 28 days old. It continues to remain a leading cause of morbidity and mortality among infants, especially in middle and lower-income countries (Seale, *et al.,* 2014). It is the 20% of neonates develop sepsis and approximately 1% death due to sepsis (Madavi, *et al.,* 2015 ; Begum, Fatema 2020) The organisms that give neonatal sepsis vary over time and change from region to region, it can even alteration from hospital to hospital (Gessner, *et al.*, 2005).

Neonatal sepsis is divided into 2 groups based on the time of presentation after birth: Early-onset sepsis (EOS) and Late-onset sepsis (LOS).

EOS refers to sepsis in neonates at or before 72 hours of life, and LOS is defined as sepsis occurring at or after 72 hours of life. Although, some experts use 7 days as the cutoff date (Aku, *et al.,* 2018)

Early-onset sepsis (EOS) is generally caused by the transmission of pathogens from the female genitourinary system to the newborn or the fetus. These pathogens can ascend the vagina, the cervix, and the uterus, and can also infect the amniotic fluid. Neonates can become infected in utero or during delivery as they pass through the vaginal canal.

Late-onset sepsis (LOS) usually occurs via the transmission of pathogens from the environment after delivery, such as contact from healthcare workers or caregivers. LOS may also be caused by a late manifestation of vertically transmitted infection. Infants that require intravascular catheter insertion, or other invasive procedure that disrupts the mucosa, are at increased risk for developing LOS. Preterm neonates are at higher risk for sepsis/infection than term neonates, as they tend to require more invasive procedures than term neonates (Simonsen, *et al.,* 2014)

Infection is one of the major causes of neonatal mortality in developing countries. Premature neonates are at high risk of infection due to underdeveloped innate

immunity, fragile skin and lack of protective maternal antibodies (Thomas, *et al.*, 2018)

Acinetobacter baumannii are aerobic Gram-negative, catalase-positive, oxidase-negative coccobacilli which have the ability to survive in the hospital environment for prolonged periods. (Nazir, 2019)

During the last two decades, *Acinetobacter baumannii* has become a pathogen of increased clinical importance due to its remarkable ability to cause outbreaks and its ability to accumulate mechanisms of antimicrobial resistance rapidly, leading to multi-drug resistance (Shamsizadeh, *et al.*, 2017).

Acinetobacter baumannii has emerged worldwide as an important hospital-acquired infection (HAI) causing pathogen. *Acinetobacter baumannii* can colonize the human skin and gastrointestinal tract and thereby can cause HAIs (Gales, *et al.*, 2001).

Furthermore, this study has been performed in Iraq to Determine the prevalence of *Acinetobacter baumannii* bacteremia in neonates as important pathogens in neonatal blood stream infection and Assess the precision of SAA in sepsis diagnosis in children admitted in Neonatal Care Unit as a marker for bloodstream infection in infants undergoing a sepsis.

Materials and Methods

Sample collection

The samples were obtained 120 samples collected from Neonatal Care Units (NCU)/ Children Welfare Teaching Hospital /Medical city / Baghdad, during the period from November \ 2019 till March \ 2020. Blood samples were collected in a sterile tubes containing nutrient broth from patients. All samples we put in Brain heart infusion and incubate 24 hours before cultured by spreading on different media (MacConkey agar and Blood agar).

Bacterial isolation and identification

All bacterial isolates were examined for gram stain and conventional biochemical tests which include: Oxidase test, Catalase test, Kligler iron agar (KIA), Indole production test, Motility test, Urease production test, Citrate utilization test, Identification and confirm results by API 20E and VITEK 2 System.

ELISA technique

Blood sample must be centrifuged for 15 min. taken serum for screened level of nCD64, SAA by ELISA technique, To assess the markers for their early diagnosis neonatal sepsis.

Antibiotic susceptibility testing

The antibiotic susceptibility test were determined by the disk diffusion method on Mueller Hinton agar plates with the following antibiotics: Imipenem, Ciprofloxacin, Meropenem, Ampicillin sulbactam, Amikacin, Levofloxacin, Gentamicin,Ceftriaxone, Cefotaxime, Piperacillin, Amoxicillin clavunic acid and Azithromycin.

Results

Blood specimens from 120 neonates, 77 (64.17%) were male and 43 (35.83%) female; age ranged between 1 day to 27 days. Acinetobacter spp. was isolated from 29 samples, All isolates was *Acinetobacter baumannii*.

All *Acinetobacter baumannii* isolates appeared as Gram-negative coccobacilli and occasionally arranged in diplococcic (figure 1). All isolates showed negative results for oxidase test, motility test, indole production test and urease production test, while the isolates gave positive results to catalase test and citrate utilization test. Kligler iron agar developed an alkaline slant, no change bottom, H2S negative without gas production. Also when *Acinetobacter baumannii* isolates were cultured on MacConkey agar, they appeared as small, pale and non lactose fermenter colonies, while on blood agar they appeared as opaque, creamy and non-hemolytic colonies. As showen in (Fig. 2).

Of the total 29 babies who had *Acinetobacter baumannii* enrolled for the study all has one or more than one risk factors for neonatal sepsis.

Of the babies 16 (55.17%) were male and 13 (44.83%) were female with significant difference (0.04). 11 (3.86 ± 0.44) neonate were found with EOS and 18 (13.40 ± 1.05) found with LOS. Approximately 21 (2157.14 ±62.32) neonates with *Acinetobacter baumannii* had low birth weight (< 2500g) out of 8 (2725.00 ±55.90) had weight more than 2500g. 1 (2800.0 ± 0.00) of them had low WBC count (<5000), 15 (8007.0 ± 461.62) with neonatal WBC count (5000-11000%) and 13 (17800.0± 2675.53) had high WBC count with a

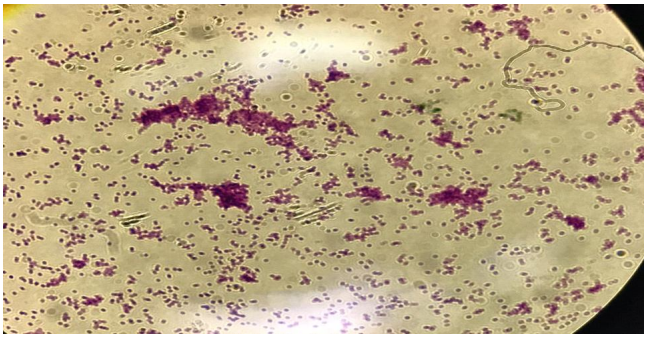

Fig. 1: Gram stain for *Acinetobacter baumannii.*

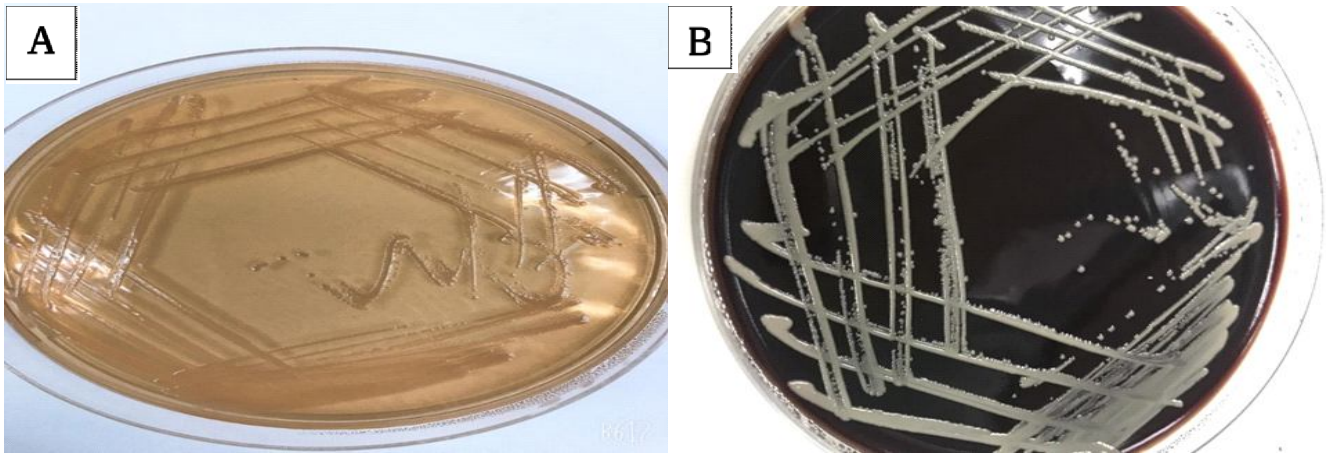

Fig. 2: A- colony in macConkey agar B- colony in blood agar.

significant difference ($P \leq 0.01$) (Table 1).

A total of 29 *Acinetobacter baumannii* were examined for their susceptibility to Antibiotics (Table 2). Overall, susceptibility of *Acinetobacter baumannii* to antibiotics was low ranging between 0% and 41.37%. High degree of resistance was observed to the various antibiotics used. The study shown Imipenem and Meropenem most effective antibiotics against *Acinetobacter baumannii* infection.

The serum levels of SAA were measured by ELISA technique in terms of mean ± SE and p-value.

(Table 3) revealed the serum level SAA in circulation that determining by ELISA method. SAA was (20.66 ± 2.07) in neonates with septicemia and 4.12 ± 0.77 in control. The serum level of neonatal sepsis with *Acinetobacter baumannii* and control group differ significantly p>0.0001.

Discussion

Neonatal sepsis is characterised by bacteraemia and clinical symptoms caused by microorganisms and their toxic products. Gram negative bacteria are the commonest causes of neonatal Sepsis. The resistance to the commonly used antibiotics is alarmingly high. The major reason for emerging resistance against antibiotics is that doctors often do not take blood cultures before starting antibiotics (Najeeb, *et al.*, 2012).

In our study, showed the *Acinetobacter baumannii* was commonest microorganism cause sepsis (24.1 %), This percentage was similar to the studies which were revealed (8.3%) (Vinodkumar and Neelagund, 2004) and (12.3%) (Arora and Jaitwani, 2006).

Regarding the gender, current study 16 (55.17%) were male and 13 (44.83%) were female, several studies

Table 1: Characteristic and risk factors for *Acinetobacter baumannii* infection in neonates.

Studied groups Items	(n=29)	Chi-Square of % and Test of means	P-value
Sex			
Male	16 (55.17%)	4.538 *	0.0461
Female	13 (44.83%)		
Age (days) (mean ± SD)			
≤ 3 days EOS	11 (3.86 ± 0.44)	2.399 **	0.0001
>3 days LOS	18 (13.40 ± 1.05)		
Birth weight (g) (mean ± SD)			
≤ 2500 gram LBW	21 (2157.14 ±62.32)	220.50 **	0.0001
>2500 Over weight	8 (2725.00 ±55.90)		
WBCs (mean ± SD)			
< 5000% Low	1 (2800.0 ± 0.00)	11996.0 **	0.0014
5000 – 18000 Normal	15 (8007.0 ± 461.62)		
>18000 High	13 (17800.0± 2675.53)		

Table 2: Antimicrobial susceptibility pattern of *Acinetobacter baumannii* isolates to various antibiotics (n=29).

Antibiotic	Symbol	Sensitive (S)%	Intermediate	Resistant (R) %	Chi-Square (X^2)
Imipenem	IPM	12 (41.37%)	3 (10.34%)	14 (48.27%)	9.72 **
Ciprofloxacin	Cip	2 (6.89%)	3 (10.34%)	24 (82.75%)	13.58 **
Meropenem	MEM	12 (41.37%)	5 (17.24%)	12 (41.37%)	9.06 **
Ampicillin sulbactam	SAM	1 (3.45%)	4 (13.79%)	24 (82.75%)	14.17 **
Amikacin	AK	4 (13.79%)	1 (3.45%)	24 (82.75%)	13.46 **
Levofloxacin	LEV	8 (27.59%)	4 (13.79%)	17 (58.62%)	8.95 **
Gentamicin	CN	3(10.34%)	5 (17.24%)	21 (72.41%)	11.48 **
Ceftriaxone	CRO	2 (6.89%)	0 (0.00%)	27 (93.10%)	14.72 **
Cefotaxime	CTX	1 (3.45%)	2 (6.89%)	26 (89.65%)	14.33 **
Piperacillin	PC	0 (0.00%)	0 (0.00%)	29 (100%)	15.00 **
Amoxicillin clavunic acid	AMC	0 (0.00%)	0 (0.00%)	29 (100%)	15.00 **
Azithromycin	AZM	4 (13.79%)	2 (6.89%)	23 (79.31%)	12.84 **
Chi-Square (X^2)	----	10.063 **	6.251 **	12.831 **	----

** ($P \leq 0.01$).

Table 3: Comparison between difference groups in SAA conc.

Group	Mean ± SE of SAA
G1: Acineto	22.56 ± 4.04 a
G2: Other bacteria	16.80 ± 3.64 a
G3: Control	4.12 ± 0.77 b
LSD value	9.829 **
P-value	0.0010

Means having with the different letters in same column differed significantly. ** ($P \leq 0.01$).

agreement with this study. In Taiwan (Wei, *et al.*,2015) they revealed male (57.47 %) and female (42.55 %). In Palestine (AL Jarousha, *et al.*, 2008) similar study that revealed (62.5%) male and (37.5%) female.. While there is an incompatible study in Taiwan (Lee, *et al.*, 2017) revealed the infected neonates female (57.5%) more than male (42.5%).

Regarding birth weight, the main risk factor is low birth weight in present study revealed approximately 21 (72.41%) neonates with *Acinetobacter baumannii* had low birth weight (< 2500g) out of 8 (27.58%) had weight more than 2500g. also there is study that discovered septicemia in low birth weight most common, (73.2%) neonates with *Acinetobacter baumannii* had low birth weight (< 2500g) out of (26.8%) had weight more than 2500g (Ulu-Kilic, *et al.*, 2017). Another study proved that low birth weight exposed to septicemia rather than neonates that weight more than 2500 g (Akter, *et al.*, 2015).

Regarding the EOS and LOS, in our study There are 11 (37.9%) neonate were found with EOS and 18 (62.1%) found with LOS. Several studies that are compatible with present study were revealed infected LOS (60%) more than EOS (40%) (Al Jarousha, *et al.*, 2009). While there is an opposite study that revealed septicemia was more common for the neonates in the first 7 days of life (early onset), EOS was (70%) and LOS (30%) (Akter, *et al.*, 2015).

In current study showed the level of serum SAA that determining by ELISA method. SAA in neonatal sepsis with *A. baumannii* were 22.56 ± 4.04 versus 16.80 ± 3.64 in neonatal sepsis non infected with *A. baumannii*, but SAA was 4.12 ± 0.77 in control group. There is agreement study in India (Krishnaveni, *et al.*, 2016) that provide higher proportion of the neonates with sepsis showed raised SAA levels than those without sepsis, and the level correlated well to the severity of the condition. SAA protein levels were significantly higher in the neonates with a positive blood culture than in the neonates with clinical signs of sepsis but with a negative blood culture. In Egypt study that revealed SAA is a sensitive and specific sepsis biochemical marker among critically ill children (Yahia, *et al.*, 2019).

In present study shown 100% resistant to Piperacillin and Amoxicillin clavunic acid and the second you are more resistant is Ceftriaxone (93.1%) and Cefotaxime (89.65%), followed Ampicillin sulbactam (82.75%), Amikacin (82.75%), Ciprofloxacin (82.75%), Azithromycin (79.31%), Gentamicin (72.41%), Levofloxacin (58.62%), Imipenem (48.27%) and Meropenem (41.37%) respectively. Imipenem and meropenem were found as the most active agents against *Acinetobacter baumannii*, Also there are Similar study in north India that revealed Imipenem and meropenem were most effective antibiotic to treatment *Acinetobacter* infection. (Nazir, 2019).

In the current study isolated *Acinetobacter baumannii* showed high level of resistance to most of

the antibiotic compared with other studies done in different places (Mishra, *et al.,* 1998; Shete, *et al.,* 2019).

Our study were disagree with the study conducted in Dhaka that revealed more than 96% of isolates were resistant to 3rd generation cephalosporin, followed by gentamycin 84.37%, ciprofloxacin 78.12%, amikacin 75%, piperacillin-tazobactum 59.37%, imipenem and cotrimoxazole 53.12%, levofloxacin 40.62% and colistin 3.12%. Ampicillin and 1st and 2nd generation cephalosporin found 100% resistant (Akter, *et al.,* 2015)

Conclusions

Neonatal septicemia is an important cause of morbidity and mortality. This is due to infection by both Gram positive and gram negative bacteria most of which are multidrug resistant especially in the hospital environment.

This study suggests Blood culture is the gold standard to diagnose the neonatal sepsis. Blood culture is significantly correlated with the risk factors of neonatal sepsis.

Acinetobacter baumannii is one of the emerging cause of nosocomial infection in NCU. Due to of this bacteria develop antibiotic resistance. The most effective antibiotic for *Acinetobacter baumannii* isolates was imipenem and Meropenem.

A significant increase of nCD64 and SAA in the presence of *Acinetobacter baumannii* compared in patients with other bacteria and healthy subjects. CD64 expression on neutrophils is a helpful biomarker for early diagnosis of sepsis since it has a good diagnostic performance.

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