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Research Article

BACTERIOLOGICAL STUDY OF SEPTICAEMIA IN PRETERM NEONATES AND THEIR ANTIBIOGRAM

***Mallika Reddy K, Saroj Golia, Sujatha Karjigi K and Sam Ahuja**
Department of Microbiology, DR. B.R. Ambedkar Medical College, Kadugondanahalli Bangalore-45
**Author for Correspondence*

ABSTRACT

The aim of our study is to analyse the hospital data on bacteraemic septicaemia in preterm neonates with reference to gestational age, birth weight, pathogens involved and their antibiogram**.** Over one year period, samples were collected from 116 preterm neonates who had clinical symptoms of sepsis. 0.5ml of blood was collected from them with aseptic precautions into a blood culture bottle and incubated for 37^0c, for 7 days. Subcultures were made on blood agar and MacConkey agar plates. Organisms were identified and antibiotic sensitivity test of isolates were performed. In Our study out of 116 suspected samples of septicaemia only 54 samples showed growth. Incidence of bacteriological septicaemia is 53.7% in males and 46.2% in females. The time of isolation of the organism was predominantly seen in early onset sepsis .Preterm neonates are more susceptible to infection as their immune status is yet to develop .In our study preterm neonates with gestational age less than 28 weeks and birth weight less than 1005 grams had high rate of infection .The predominant organism to be isolated was *Klebsiella* spp, followed by MSSA, *Escherichia.coli*, CONS ,*Enterococcus* spp ,MRSA,*Pseudomonas* spp,*Citrobacter* spp, *Enterobacter*spp and *Acinetobacter* spp. Most of the isolates showed resistance to Pencillin G, Ampicillin, Cotrimoxazole, while all of these isolates were sensitive to Meropenem, Vancomycin and Azetreonem. In developing country like India infection is common feature in preterm neonates. Blood culture is the gold standard method of diagnosis of sepsis. This study concludes low birth weight and lesser gestational age play key role in causing infection of preterm neonates. Since isolated organisms are showing resistance too many antibiotics, choosing appropriate antibiotic becomes very important in treating these patients.

Key Words: *Preterm, Blood Culture, Gestational Age*

INTRODUCTION

Babies born before 37 weeks of gestation are termed as premature neonates (Kliegman, 2008) Birth of premature babies has been escalating steadily and alarmingly over the past two decades. Neonatal sepsis is a clinical syndrome, an infant 28 days of life or younger characterized by systemic signs of infection and accompanied by bacteraemia in the first month of life (SurgCdrNarayan and SurgCdrMathai, 2000).

Preterm neonates are frequently subjected to invasive procedures that increase probabilities of infection. Although there is reduction in neonatal sepsis due to aggressive enteral feeding, shorter duration of invasive ventilation because of surfactant use, better hand hygiene practices, and better protocols for handling vascular lines still septicaemia is most common cause of mortality in NICU (Neonatal Sepsis Protocol For Indian Neonatal Intensive Care Unit; Mathur, 2009; Fanaroff, 1998). These babies have a longer stay in hospital which gives rise to nosocomial infection while advances in neonatal intensive care have resulted in improved survival of preterm infants, mortality is as much as threefold higher for VLBW Infants who develop sepsis than for those without sepsis (Stoll, 2002; Heath, 2003). Septicaemia is a major cause of morbidity and mortality in such newborns. The bacteriological profile of neonatal sepsis is constantly under change with advances in the early diagnosis and treatment of sepsis. The change of antibiotic sensitivity pattern is the major problem to treat septicaemia (Fanaroff, 1998).

Septicaemia can be classified into early onset sepsis (EOS) which is seen within 72 hours of birth and late onset sepsis (LOS) (Jeyamurugan *et al.,* 2012; English and Wilson, 2001) which occurs after 72 hrs after birth. EOS is thought to be primarily due to vertical transmission from mother to child, Early-onset

infections are caused by organisms prevalent in the maternal genital tract or in the delivery area. The associated factors for early-onset sepsis include low birth weight, prolonged rupture of membranes; foul smelling liquor, multiple per vaginal examinations, maternal fever, difficult or prolonged labour and aspiration of meconium. Early onset sepsis manifests frequently as pneumonia and less commonly (Neonatal Sepsis Protocol for Indian Neonatal Intensive Care Unit; Mathur, 2009; Fanaroff, 1998) as septicaemia or meningitis while LOS is more likely to be acquired through horizontal transmission. The associated factors of late-onset sepsis include: low birth weight, lack of breastfeeding, superficial infections (pyoderma, umbilical sepsis), aspiration of feeds, disruption of skin integrity with needle pricks and use of intravenous fluids.

The common presenting features in preterm neonates are nonspecific, they present with convulsions, hypothermia ($<35.5^0$ C) respiratory rate > 60/min, severe chest in drawing, nasal flaring, grunting, bulging fontanelle, hypoglycemia / hyperglycemia, increased pre-fed aspiration and unexplained metabolic acidosis (Mathur, 2009; Sohn, 2001).

Inclusion Criteria

Preterm neonates suffering from septicaemia will be included in this study. Thorough clinical examination will be performed with reference to hypotension, hypothermia, gastrointestinal problems, and metabolic acidosis.

Exclusion Criteria

This study will exclude collection of blood samples from old indwelling vascular catheters.

MATERIALS AND METHODS

Blood culture-A patch of skin approximately 5cm in diameter is thoroughly cleansed with 70% isopropyl alcohol, allow it to evaporate for 1min and then clean with 1% povidone iodine over the proposed vein puncture site. About 0.5ml of blood is drawn into 5ml-10ml of culture media, with help of a sterile needle and inoculated into Brain – heart infusion broth. Blood to broth ratio 1:5 to 1:10 will be maintained, incubated, and observed after 24hrs, 48hrs, 72hrs looked for growth and turbidity.

RESULTS

A total of 116 samples having clinical suspicion of sepsis were sent from pediatric NICU to the laboratory for bacterial culture and only 54 samples revealed growth. The study group included preterm neonates born and admitted at NICU of Dr. B. R. Ambedkar Medical College Hospital.

Table 1: Shows time of isolation of organisms

Time of isolation	Number
EOS	32
LOS	22

Table 1 shows time of isolation of organisms. In the present study early onset sepsis (EOS) constituted 59% (32/54) and late onset sepsis (LOS) showed 41% (22/54). Early onset sepsis was more by 17% in the present study.
The time of isolation of oraganisms is represented in chart 1.

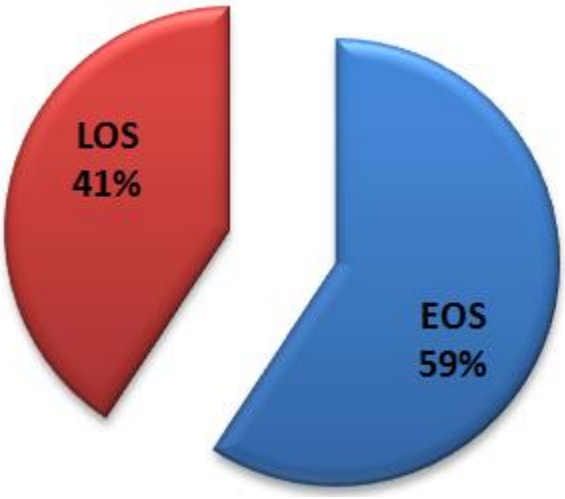

Chart 1: Shows time of isolation of organisms

Table 2: Shows distribution of bacteriological septicaemia with respect to gestational age

Gestational age	Number of preterm neonates	Percentage
<28	11	20.3%
28-29	9	16.6%
29-30	9	16.6%
30-31	8	14.8%
31-32	7	12.9%
32-33	4	7.4%
33-34	2	3.7%
34-35	2	3.7%
35-36	1	1.8%
36-37	1	1.8%

Table 2 shows distribution of bacteriological septicaemia with respect to gestational age, in the present study septicaemia was high among the neonates with lesser gestational age.
Among 54 preterm neonates who showed positive blood culture growth,
The highest age group showing septicaemia was 20.3%(11 neonates) which was seen in preterm neonates, who were less than 28 weeks of gestation and age group which showed least septicaemia was 1.8%(1 neonate each) , who were from gestational age of 35-36 and 36-37 weeks. The other gestational age groups showing septicaemia are as follows,
16.6% (9 neonates each) were from 28-29 and 29-30 weeks,
14.8% (8 neonates) were from 30-31 weeks,
12.9% (7 neonates) were from 31-32 weeks,
7.4% (4 neonates) were from 32-33 weeks,
3.7% (2 neonates each) were from 33-34 and 34-35 weeks.
The above data is represented in chart 3.

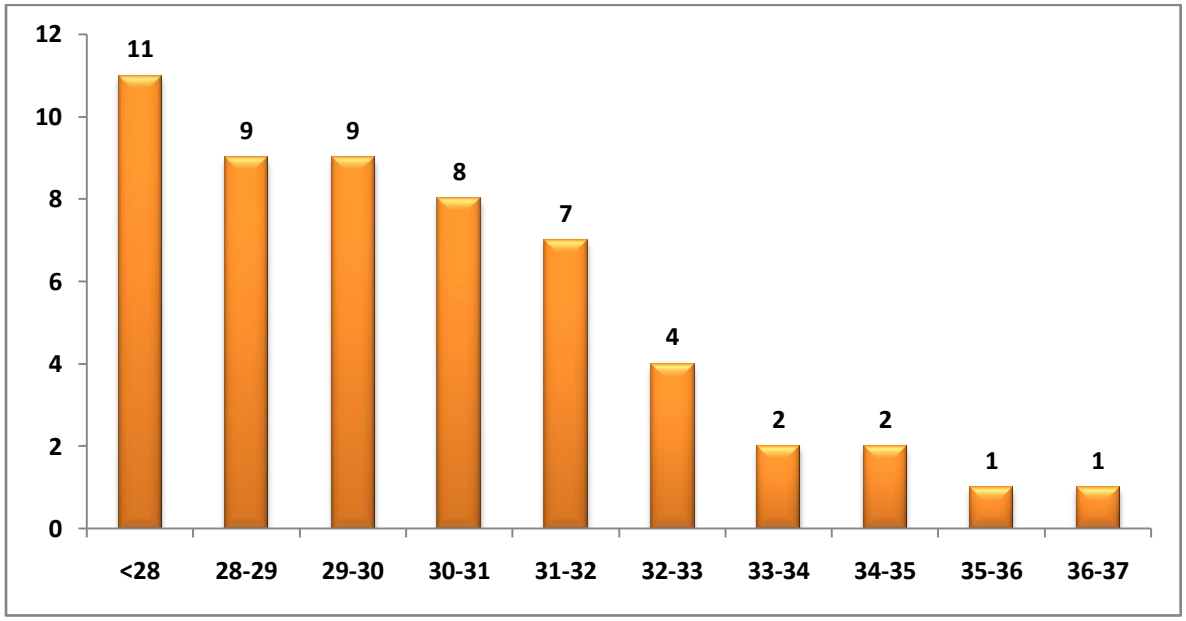

Chart 2: Shows distribution of bacteriological septicaemia with respect to gestational age

Table 3: Shows the distribution of bacteriological septicaemia with respect to birth weight

Birth weight	Number	Percentage
<1005 grams	12	22.2%.
1005-1300 grams	10	18.5%
1300-1500 grams	8	14.8%
1500-1700 grams	7	12.9%
1700-1900 grams	6	11.1%
1900-2100 grams	4	7.4%
2100-2300 grams	2	3.7%
2300-2600 grams	1	1.8%
2600-2800 grams	1	1.8%
2800-<3000 grams	1	1.8%

Table 3 shows the distribution of bacteriological septicaemia with respect to birth weight. 22.2% of preterm neonates had birth weight less 1005gms and mortality too was also very high in these pretem neonates.

Least number of positive bacterial cultures were seen in preterm neonates who weighed 2300-<3000 grams i.e only 1.8% (1/54).

Similarly pretem neonates who weighed 1005-1300 grams showed 18.5% (10/54), 1300-1500grams showed 14.8% (8/54), 1500-1700grams showed 12.9% 9(7/54), 1700-1900grams showed 11.1%, 1900-2100 grams7.4% , 2100-2300 grams3.7%, 2300-2600 grams ,2600-2800 grams and 2800-<3000 grams showed 1.8% each bacteriological septicaemia.

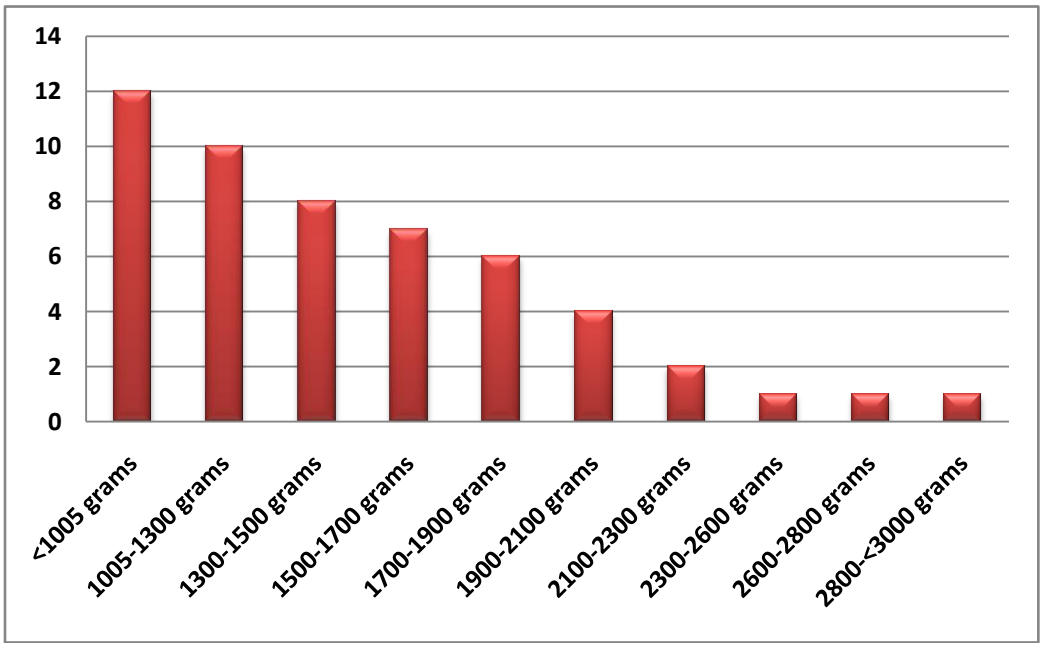

Chart 3: Shows the distribution of bacteriological septicaemia with respect to birth weight

Table 4: Shows various kinds of microorganisms isolated with number and percentage of the distribution of organisms

Name of the isolate	Number	Percentage
Klebseilla spp	13	24%
MSSA	10	18%
CoNS	7	13%
E.coli	7	13%
E .faecalis	5	9%
Citrobacter spp	2	4%
P aeruginosa	4	7%
MRSA	2	4%
A .baumanii	2	4%
E. cloacae	2	4%

Table 4 shows various kinds of microorganisms isolated with number and percentage of the distribution of organisms.
Klebsiella spp is highest with 24% (13/54) isolates.
MSSA is 19% (10/54), CoNS is 13% (7/54), *E.coli* is 11% (7/54),
E.facalis is 9% (5/54/), *P aeruginosa* is 7% (4/54), MRSA is 4% (2/54),
Citrobacter spp is 4% (2/54), *A.baumanii* 4% (2/54)
Enterobacter cloacae are 4% (2/54) and MRSAis 4% (2/54).

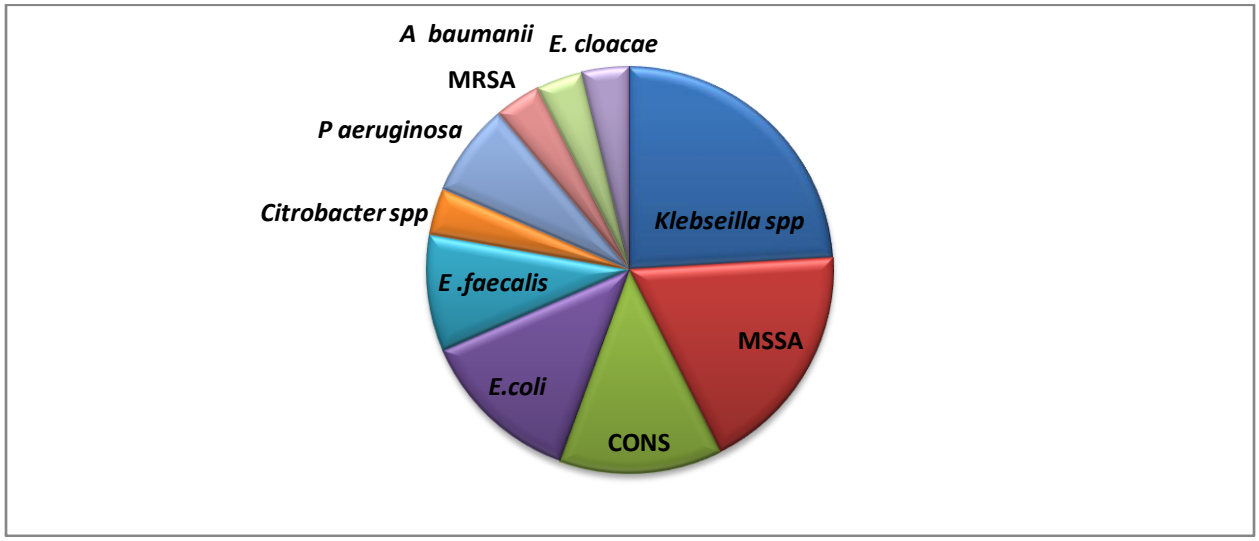

Chart 4: Shows various kinds of microorganisms isolated with number and percentage of the distribution of organisms

Two MRSA organisms showed 100% resistance to Pencillin, Ampicillin, Ampicillin - sulbactum, Cefoxitin and Ciprofloxacin, 50% of isolates showed resistance to Cotrimoxazole, Levofloxacin , Linezolid, Gentamicin, Amikacin and both the isolates were sensitive to Vancomycin.
In Seven CONS isolates, 71.4% (5/7) were sensitive to Pencillin, Ampicillin, Ciprofloxacin and Cotrimoxazole. 85.7% (6/7) were sensitive to Ampicillin- sulbactum, Levofloxacin; these were 100% sensitive to Gentamicin, Amikacin, Linezolid, Cefoxitin and Vancomycin.
In Ten MSSA isolates 30% (3/10) isolates were sensitive to Pencillin, Ampicillin. 80% (8/10) isolates were sensitive to Ampicillin- sulbactum, 90% (9/10) sensitive to Ciprofloxacin, Levofloxacin, Linezolid, Gentamicin, all isolates were 100% (10/10) sensitive to Cefoxitin, Amikacin and Vancomycin. In Five *Enteroccocus spp* 20% (1/5) were sensitive to Pencillin and Ampicillin. 60% (3/5) were sensitive to Ciprofloxacin and Levofloxacin 80% (4/5) were sensitive to high level Gentamicin and Linezolid and all the isolates were sensitive to Vancomycin.
The predominant organisms to be isolated were *Klebseilla spp* which were 13 in number.
They showed 100% resistance to Pencillin, only 50% (6/6) of isolates were sensitive to Cotrimoxazole and Piperacillin, 58.3% (7/12) were sensitive to ciprofloxacin. 66.6% (8/12) sensitive to Cefuroxime, 83% (10/12) were sensitive to Piperacillin- tazobactum, Ceftazidime, Gentamicin and Amikacin and 100% (13/13) were sensitive to Meropenem two of the isolates produced ESBLs.
In Two *Entrobacter cloacae* species there was 100% resistant to Pencillin G and Ceftazidime, only 50% (1/2) isolates were sensitive to pipercacillin, 100% sensitive to Piperacillin-tazobactum, Gentamicin, Amikacin and Meropenem.
In Seven *Eshcherichia .coli isolates* there was 100% resistance is seen to Pencillin and Ampicillin. 57.1% (4/7) isolates were sensitive to Ampicillin sulbactum, Ceftriaxone, Cefuroxime and Levofloxacin. 71.4% (5/2) isolates were sensitive Ceftazidime, Gentamicin and Amikacin. 100% (7/7) were sensitive to Meropenem. This data is represented in chart no 10. Table 11 shows the number and antibiogram of *Acinetobacter* and *Pseudomonas species.* Two species of *Acinetobacter* were isolated one *Acinetobacter baumani* and *Acinetobacter lowfii.* There was 100% resistance to Piperacillin, Cotrimoxazole and Gentamicin. 50% (1/2) isolates were sensitive to Piperacillin-tazobactum and Amikacin. Both the isolates were 100% sensitive to Meropenem and Azetreonem. In Four *Pseudomonas aeruginosa* species. 100% resistance was seen to Piperacillin. 25% (1/4) isolates were sensitive to Cotrimoxazole. 50% (2/4) isolates were sensitive to Piperacillin-tazobactum, Levofloxacin, Ceftazidime and Gentamicin. 75% (3/4) isolates were sensitive to Amikacin. All isolates were sensitive to Meropenem and Azetreonem.

DISSCUSSION

One of the commonest clinical entity confronting neonatologists in our country is neonatal septicaemia. Birth of premature babies has been escalating steadily and alarmingly over the past two decades. The incidence and aetiology of the disease differs in various part of our country. Hospital acquired infection also plays a key role in spread of septicaemia.

In our study, the incidence of bacteriological septicaemia in preterm neonates is 46.5% (54/116). Similar results of culture positivity rate were seen in preterm 31%, (15/48) (Nawshad Uddin Ahmed *et al.*, 2010; Dhaka, 1993).

In the present study, early onset sepsis (EOS) constituted 59% (32/54) and late onset sepsis (LOS) showed 41% (22/54). Early onset sepsis was more by 17% in the present study. The majority of preterm infants showed early onset sepsis i.e 70.7% in study conducted by choudhury Habibur Rasul *et al.*, (2009).

Nawshad Uddin Ahmed *et al.*, (2010) and Dhaka (1993) studied that case fatality was 10.71% and incidence of septicaemia was probably high with birth weight of 1.5 kg. In the study of Ramesh *et al.*, (2011) and Mathur (2009), the mortaity rate was 47.52% and mortality was significantly higher in lower birth weight and lower gestational age. Vergnano *et al.*, (2005) in their prospective study had mortaliy of 44% during EOS and LOS had 46%. In our present study the rate of preterm neonate mortality is 35.1% (19/54). Among 54 preterm neonates who showed positive growth maximum number of septicaemic cases were 20.3% (11 neonates) which were in the age group less than 28 weeks of gestation, preterm neonates of gestational age group 37 weeks showed least septicaemia of 1.8% (1 neonate each. Similar results were shown in studies by David Kaufman *et al.*, (2004). According to the National Neonatal Perinatal Database of India, *Klebsiella pneumonia*, *Staphylococcus aureus*, and *E. coli* are the three most common organisms causing neonatal sepsis both in hospital and community. Moreover, the causative organisms of EOS and LOS sepsis are similar especially in hospital setting in developing country.

In the present study, MSSA is 19% (10/54), CONS is 13% (7/54) and MRSA was 4% the total staphylococcus isolates showed 35.1%. Similar results were seen in study conducted by Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network.

Shane *et al.*, and Stoll *et al.*, (2002) they were resistant to pencillins but all were sensitive to Vancomycin. Similar results were seen studies conducted by D'Angio, *et al.*, (2009) and Roy *et al.*, (2008) in the present study, *E.facalis* is 9% (5/54/). VRE was not isolated. McNeely *et al.*, (2006) also isolated 19% of *Enterococcus species*.

Klebsiella spp is highest with 24% (13/54) isolates**.** Most of the isolates showed resistance to number of the antibiotics. *Klebsiella pneumoniae* was (25%) similar resistance pattern was seen in studies conducted by other worker. Thaver *et al.*, (2009) reported the resistance of all the *Klebsiella species* to Ampicillin in their study. Ganatra *et al.*, (2010) discussed a similar growing antibiotic resistance of neonatal pathogens in developing countries in their review on an international perspective on early onset neonatal sepsis.

In present study *E.coli* is 11% (7/54), these isolates were completely resistant to Ampicillin as in study conducted by Alos *et al.*, (2004) and an increasing Ampicillin resistance among *E.coli* of EOS has been reported by Hide *et al.*, especially in prematurely born infants.

Citrobacter spp is 4% (2/54), *Enterobacter cloacae* is 4% (2/54) David Kaufman *et al.*, (2004) also isolated similar organisms.

NFGNB are all so increasing in incidence, *Pseudomonas aeruginosa* is an opportunist pathogen s which causes severe infections in patients with immune deficiency and newborns. *P aeruginosa* is 7% (4/54) and *A baumanii* 4% (2/54) similar results were seen in a retrospective study conducted by Marioara *et al.*, (2004).

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