Predominance of Multi-Drug Resistant *Klebsiella pneumonia* and Other Gram Negative Bacteria in Neonatal Sepsis in Equatorial Guinea

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Abstract

The study was conducted on new-born babies in whom septicemia was suspected, to determine the prevalence of bacterial strains isolated and their sensitivity to antimicrobial drugs. The study was carried out at La Paz Medical Center, Microbiology section, Malabo, Equatorial Guinea from August 2013 to October 2015. Out of 293 septicemia suspected cases, 29 (10%) blood cultures were positive, 28 with bacterial growth and 1 with growth of *Candida* sp. The mortality rate of neonates caused by Gram negative bacterial sepsis was 34.7%. Among the Gram negative bacteria (24 isolates), the most common types were *Klebsiella pneumoniae* (16 = 69.6%), followed by *Escherichia coli* (4 = 17.4%) and *Acinetobacter* species (4 = 17.4%). Four Gram positive bacteria were also isolated and identified all ascoagulase-negative staphylococci. All the *Klebsiella pneumoniae* isolates and *Acinetobacter* species demonstrated Multi Drug Resistance against different antibiotics with Extended-spectrum *β*-lactamase (ESBL) activity.The most frequent causative agent of bacterial sepsis in new-born children was *Klebsiella pneumoniae*. An alarming level of Multi Drug Resistance (MDR) *Klebsiella pneumoniae* strains to the first choice antibiotic treatment was observed.

Keywords

1. Introduction

At least 1.16 million new-born babies die in sub-Saharan Africa each year. New-born babies in this region are at

the highest risk of death, while progress in reducing mortality is the slowest. The single most common cause of mortality is neonatal infection, particularly sepsis (blood stream infection). In sub Saharan Africa, the sepsis is causing the death about 350,000 newborns a year [1] [2]. The clinical signs of neonatal sepsis are characterized by infection with or without accompanying bacteremia in the first month of life. The infection can be classified in two major categories depending on the onset of symptoms: "Early onset", if the infection occurs within the first 72 hours of life and "Late onset" if it occurs after 72 hours of age [3]. The standard method to determine whether a neonatal sepsis is in progress is a positive blood culture. Both Gram negative organisms such as *Klebsiella* species, *Escherichia coli*, *Pseudomonas* spp., *Salmonella* spp., and Gram positive organisms such as *Staphylococcus aureus*, coagulase negative staphylococci (CONS) and *Streptococcus pneumonia* are the pathogens most commonly known as causing neonatal sepsis [4] [5]. Etiology of community-acquired neonatal sepsis in developing countries may change from region to region [6]. Antibiotic resistance is now a global problem. Reports of multiresistant bacteria causing neonatal sepsis in developing countries are increasing [7]-[9]. However, it is difficult to compare antibiotic resistance between countries because the epidemiology of neonatal sepsis is extremely variable [6]. The prompt administration of the appropriate, efficient antibiotic to treat newborn infections would increase the number of lives saved.

To the best of my knowledge, this is the first study on the microorganisms involved in neonatal sepsis and their antibiotic resistance pattern in Equatorial Guinea.

2. Subjects and Methods

The study was conducted at La Paz Medical Center, Malabo, Equatorial Guinea from 2013 to 2015 on suspected cases of neonatal septicemia. The blood samples were obtained from neonates aged 0 - 28 days. The bacteriological analysis was carried out according to standard protocols for blood culture [10]. The C reactive protein (CRP) level from serum was measured using Cobas Integra 400 plus.

2.1. Samples Processing

The blood samples were inoculated into BD BACTEC Plus Aerobic/Anaerobic F medium and incubated in the BACTEC 9050 (Becton-Dickenson, USA) automat system at 35˚C for 5 days. Positive cultures were inoculated on Triptic Soy Agar with 5% sheep blood (TSAB), Mac Conkey's agar and Chocolate agar (HyLabs Ltd.) by spread plate technique. The isolated bacteria were then identified by using Gram Stain and their biochemical characteristics using Remelrap ID system kits (RapID ONE, RapID Staph Plus).

2.2. Antimicrobial Susceptibility Testing

Antibiotic susceptibility was determined using the disc diffusion method on Mueller Hinton agar according to the Guidelines of the Clinical Laboratory Standards Institute (GCLSI) [11].

The antibiotics discs obtained from OXOID, UK and their concentration were: Cephems (cefuroxime (30 μg), cefotaxime (30 μg), ceftazidime (30 μg)); Penicillins (Ampicillin (10 μg)); Beta-lactam + inhibitor (Amoxicillin/Clavulanic acid (20/10μg), Piperacilin/Tazobactam (100/10μg)); Aminoglycosides (Gentamicin (10 μg), Amikacin (30 μg)); Carbapenemes (Imipenem (10 μg)).

Multiple Drug-Resistant (MDR) bacteria are defined as resistant to at least three different classes of antibiotics.

Confirmation of Extended-spectrum β-lactamase (ESBL) activity was performed by the standard double-disc synergy testing of Cefotaxime and Ceftazidime in the present and absent of Amoxicillin/Clavulanic acid.

2.3. Statistical Analysis

Statistical analysis was done using Microsoft Office Excel 2003.

3. Results

3.1. Epidemiology of Blood Stream Infection

Out of 293 blood samples suspected to septicaemia, 29 (10%) were positive. 14 (17.3%) positive sample were of male and 15 (23.1%) were of female babies. Out of these positive cultures a total of 28 (96.6%) isolates were

bacteria and 1 (3.4%) were *Candida* sp. Gram negative rods (24 isolates) were more prevalent than Gram positive (cocci, 4 isolates). Among the Gram negative bacteria the most common type was *Klebsiella pneumonia* 16 (69.6%) followed by *Escherichia coli* 4 (13.8%), *Acinetobacter* species 4 (13.8%) (**Table 1**). All of the Gram positive bacteria were coagulase-negative staphylococci.

Early Onset Sepsis of twenty babies was caused by Gram negative strains. *Klebsiella pneumoniae* (n = 13) was the most frequently isolated organism in Early Onset Sepsis, followed by *Escherichia coli* (n = 4) and *Acinetobacter* species (n = 3).

Four babies had Late Onset Sepsis caused by Gram negative bacteria. The causative organism were *Acinetobacter* species (n = 1), *Klebsiella pneumoniae* (n = 3). The C reactive protein (CRP) level in serum of neonates in Early Onset Sepsis was in ranges from 4.0 to 30.6 mg/dL with an average level 11.0 mg/dL.

3.2. Antibiotic Resistance

All the isolates of *Klebsiella pneumoniae*, and *Acinetobacter* sp. were Extended-Spectrum β-Lactamase (ESBL) producing strains and showed resistance to Ampicillin (100%), Amoxicillin/clavulanic acid (100%), 2nd generation cephalosporin Cefuroxime (100%), 3rd generation cephalosporin Cefotaxime (100%), and highest resistance to Gentamycin (81% and 75% respectively). The sensitivity pattern of the Gram negative bacteria is shown in **Table 2**. *Klebsiella pneumoniae* also demonstrated high level of resistance to Piperacilin/Tazobactam (44%). Multidrug resistance (MDR) was observed in all the *Klebsiella* and *Acinetobacter* species.

4. Discussion

The neonatal sepsis is a leading cause of neonatal mortality especially in African countries [1] [2]. Blood culture is still the gold standard for the diagnosis of neonatal sepsis. The rapid and correct choice of the antibiotic enables rapid cure of the patient and the saving of the patient's life.

Results of our study indicate that neonatal sepsis was confirmed in about 10% of the 293 neonates. These data are lower than the figures from previous studies [12]-[15] carried out in Nigeria (33.1%), Kenia (23%), and Uganda (37%) and Sudan (61.3%). The difference in rates of positive samples can be explained by the differences in criteria for determining neonatal sepsis, blood culture system, location and health situation in the region, capacity of hospital, variability between microbiological techniques [1] [4] [5]. Our study showed that out of twenty-nine isolates, Gram negative bacteria (24 = 79.3%) were predominant bacterial pathogens causing the neonatal sepsis.The all coagulase-negative staphylococci were considered as the skin contamination because the

Table 1. Profile of blood culture isolates.

Bacteria isolated	Total number	(%)
Klebsiella pneumonia	16	55.1
Escherichia coli	4	13.8
Acinetobacter sp.	4	13.8
Coagulate Negative staphylococci	4	13.8
Candida sp.	1	3.5

Table 2. Bacterial isolates and antibiotic sensitivity pattern (%).

Drugs name and concentration	*K. pneumoniae* (n = 16)	*E. coli* (n = 4)	*Acinetobacter* sp. (n = 4)
Ampicillin (10 μg)	0	0	0
Amoxicillin + clavulanic acid (20/10μg)	0	50	0
Piperacillin + tazobactam (100/10μg)	56	75	75
Cefuroxime (30 μg)	0	50	0
Cefotaxime (30 μg)	0	75	0
Gentamicin (10 μg)	19	100	25
Amikacin (30 μg)	81	100	75
Imipenem (10 μg)	87	100	75

neonate was clinically well, the CRP level was normal and after receiving the negative results of repeated blood culture. This is consistent with studies in Nigeria [9] [16], South Africa [17], Sudan [15] and differs from other studies in Kenia [12], Nigeria [9], Uganda [14] where Gram positive cocci were the most commonly isolated bacteria from blood culture (78%, 54.1%, 62.7% respectively). Early reports indicated that the C-reactive protein (CRP) levels may be useful for early diagnosis of Blood stream infection especially if the infants have clinically unclear infectious status [18]. In our study, all neonates with Early Onset Sepsis had the C reactive protein (CRP) level in serum of was >4 mg/dL that coincides with previous studies where described that CRP levels > 2 mg/dL were associated with a risk of probable neonatal sepsis [19]. In the present study, *Klebsiella pneumoniae* (69.6%) was the most prevalent Gram negative bacteria and was implicated in almost 2/3 (65%) cases of Early Onset Sepsis. Similarly, prevalence of *Klebsiella pneumoniae* among Gram negative organisms associated to neonatal Early Onset Sepsis has been recorded by several authors [9] [12] [15]. On the other hand, some authors reported that *Escherichia coli* [20] and Gram positive cocci [14] [21] were the predominant isolates in Early Onset Sepsis.

Out of the 25 babies who had true positive blood culture, six neonates died in Early Onset Sepsis and two neonates died in Late Onset Sepsis. Neonatal mortality rate was 34.7%. This rate was similar to that found in Nigeria (32.2%) [14] but was higher than in Uganda (18.7%) [14], Sudan (14.5%) [15] and in South Africa (20.8%) [17].

Antibiotic resistance is now a global problem. It is difficult to compare antibiotic resistance between African countries because the epidemiology situation of neonatal sepsis is very variable [4]. Previous studies [7] [12] [20] reported on the efficiency of Gentamicin, second and third generation cephalosporin against gram negative bacteria that caused neonatal sepsis. However our data seem to show a lower effectiveness of these antibiotics for treatment of *Klebsiella pneumoniae* and *Acinetobacter* sp. with the 100% resistance to Cefuroxime and Cefotaxime. The resistance level to Gentamicin was 87% and 75% respectively. Moreover, an alarming level of *Klebsiella pneumoniae* (44%) resistance to Piperacilin/Tazobactam and *Acinetobacter* sp. (25%) resistance to Imipenem was found. All the *Klebsiella pneumoniae* and *Acinetobacter* sp. Strains were multidrug resistant (MDR) organisms and possessed Extended-Spectrum β-Lactamase (ESBL) activity. This is the highest rate as compared with other studies conducted in Africa [9] [17] [20]-[22].

5. Conclusion

All the *Klebsiella pneumoniae* and *Acinetobacter* sp. strains in this study were multidrug resistant (MDR) organisms and possessed Extended-Spectrum β-Lactamase (ESBL) activity. Our results showed inefficiency to the first choice line drugs such as Ampicillin, Gentamicin, Amoxicillin/Clavulanic acid, second and third generation of cephalosporins for the treatment of blood stream infection due *Klebsiella pneumoniae* and *Acinetobacter* sp. isolates. Just Imipenem was broadly the most sensitive drug, followed by Piperacilin/Tazobactam and Amikacin. Considering the increasing frequency with which resistance to antibiotics is detected in African hospitals, our results are alarming.

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Conflict of Interest

The author declares that he has no competing interests.

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