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Evolution of the Level of Antibiotic Resistance of Nosocomial Germs in a Reference Neonatal Unit in Abidjan

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Abstract

Healthcare-associated infections (HAIs) are a major public health problem. The aim of the study was to describe the evolution of the level of resistance of germs to antibiotics. Methods: Prospective and analytical study that took place from January to April 2021 on 67 newborns suspected of bacterial infection and with a positive blood culture. Results: The mean gestational age was 35.7 weeks, 69% of patients were born vaginally, and 48% were in a level 3 maternity hospital. Prematurity (34%) and intrapartum asphyxia (24%) were the most common reasons for admission. The main germs found were *Staphylococcus aureus* (40.2%), *Staphylococcus Coagulase Negative* (28.3%), *Klebsiella pneumoniae* (30%), *Aeromonas salmonicida* (1.5%); 60% of *Staphylococcus Coagulase Negative* (SCNs) and 45% of *S. aureus* were resistant to penicillin; 75% of *K. pneumoniae* produced Extended-spectrum β-lactamase (ESBL). Multi Antibiotic Resistant Bacteria were found in newborns who received care to help them adapt to extrauterine life ($p = 0.0000$) and significantly increased the risk of death ($p < 0.001$). The mean length of hospital stay was 13.2 days, and the mortality rate was 19.4%. Conclusion: Good hand hygiene and optimal disinfection practices are necessary to limit HAI-related morbidity and mortality.

Keywords

IAS, Neonatology, BMR, Antibiotic Therapy

1. Introduction

Sepsis is a preventable and potentially fatal disease. It contributes significantly to

global mortality, especially neonatal mortality, and is recognized as a priority by the World Health Organization [1] [2]. Unfortunately, there is no consensus on the definition that allows for an unequivocal differentiation between neonatal (generalized) and neonatal (localized) infection, which complicates epidemiological evaluations [2] [3]. Despite this lack of consensus, two main categories of neonatal sepsis are widely accepted: early-onset sepsis (EOS) defined as occurring in the first 72 hours of life, thus representing a fetal-maternal infection, and late-onset sepsis (LOS), which occurs between 72 hours and 28 days [3] [4]. LOS can be acquired in the hospital (HALOS) or in the community (CALOS), a key difference when considering etiology, treatment, and outcome [5]. Globally, neonatal sepsis is estimated to affect between 1.3 and 3.9 million newborns and is responsible for 400,000 to 900,000 deaths annually [6] [7], 84% of which are preventable [6]. The highest incidence of neonatal sepsis is in low birth weight and preterm infants [3] in low- and middle-income countries. Low birth weight preterm infants have a 3 to 10 times higher risk of sepsis than term infants [3] [6]. In Côte d'Ivoire, previous studies carried out in reference neonatal units in Abidjan found Klebsiella pneumoniae (47%), Enterobacter cloacae (22%) and Staphylococcus aureus (54.2%) as seeds associated with HAIs [8] [9]. For better care of hospitalized newborns in our resource-limited countries, the ecology of healthcare-associated infections must be constantly studied and disseminated. We therefore carried out this study, the objective of which was to describe the germs encountered during HAIs and their level of resistance.

2. Methods

The study took place in the neonatology unit of the Cocody University Hospital (CHU). This unit is a reference center for peripheral paediatric services in urban or rural areas. This was a descriptive and analytical prospective study that took place from January 1, 2021, to April 30, 2021 (4 months). All neonates aged 0 to 28 days suspected of being infected or showing signs of infection in whom a blood culture was performed were included in the study. The sampling was exhaustive. The samples were taken by staff trained (doctor, midwife, paediatric nurse) in aseptic rules in paediatric blood culture bottle (Bact/ALERT® FP) and then sent for analysis. Blood volumes of 0.5 to 4 mL were inoculated into blood culture bottles (Bact/Alert® FP). These bottles were then incubated in the Bact/Alert® at 37˚C for 5 days according to the manufacturer's instructions. The microbial detection Bact/Alert® detects the presence of microorganisms in a normally sterile physiological fluid. In the event of a positive result, the blood culture bottle is then removed from the machine. GRAM staining is performed, and the contents of the bottle are subcultured on agar. A blood agar is systematically inoculated and incubated in an oven at 37˚C in an aerobic atmosphere enriched with 5% to 10% CO2. Specific agars such as CHAPMAN and Eosine Methylene Blue (EMB) were used based on the results of GRAM staining. The incubation of these agars was carried out in an oven in an aerobic atmosphere at 37˚C. The incubation time was

24 to 48 hours. Germ identification was carried out using standard bacteriological methods. The Vitek MD 2 Compact® system, which used the microdilution method in broth, was used to determine the antibiotic susceptibility of the identified germs. When no microbial growth is detected after 5 days, the blood culture vial is marked as negative and removed from the automaton. An absence of bacteria at the staining of GRAM and a negative subculture of the said vial confirm its negativity. Quality control strains were used: Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 700603 and Staphylococcus aureus ATCC 29213. An antibiogram was carried out on all the strains considered by the microbiologist to be likely to be involved in an infectious process. Two categories have been selected for the interpretation of the antibiogram:

- Strains categorized S (susceptible) are those for which the probability of therapeutic success is high in the case of systemic treatment with the recommended dosage.
- Strains categorized R (resistant) are those for which there is a high probability of treatment failure regardless of the type of treatment and the dose of antibiotic used.

Partial results of the fresh state (presence or absence of leukocytes and bacteria) were communicated to the clinician to enable him to orient his therapeutic approach after 24 to 48 hours. Data were collected using a survey form containing epidemiological (age at admission, sex, place of birth, mode of delivery), clinical (gestational age, APGAR, temperature, physical examination), biological (blood count, blood glucose, serum calcium, thick gout and CRP), bacteriological (blood cultures and antibiotic susceptibility testing), therapeutic (antibiotic therapy instituted, duration of treatment) and evolutionary parameters (cure or death). The data was entered into the Excel software for WINDOWS OFFICE 16 and analyzed with the SPSS 18 software. The statistical tests used were Pearson's Chi-2 and Fisher's Exact test with a significance threshold of 5%.

3. Results

3.1. Prevalence of HAIs

Four hundred and forty-three newborns were admitted to the neonatal unit during our study. 114 blood cultures were performed: 28 came back negative, 19 soiled and 67 positive. The prevalence of HAIs was 58.77%.

3.2. Characteristics of Newborns

Among the sixty-seven (67) newborns with a positive blood, culture included, 35 (52%) were referred from a peripheral maternity ward (Out born) and 48% to the delivery room of the Cocody University Hospital (in born). There was a predominance of men (sex ratio = 1.54). More than half of the newborns were preterm infants (51%) and very preterm infants (gestational age (GA) between 28 and 32 amenorrhea weeks) accounted for 25.4%. The mean gestational age was 35.7 amenorrhea week, and the average weight was 2393 g with extremes ranging from

950 to 3900 g. Most of them were born vaginally (69%) and hospitalized before 48 hours of life (71.6%). The main reasons for admission were prematurity (34,3%), fever (24%), jaundice (16,5%), and seizures (15%). The mean length of hospital stay was 13.2 days with extremes of 2 to 46 days. The evolution was favorable in 80.6% of cases. The mortality rate was 19.4%.

3.3. Bacteriological Study and Antibiotic Resistance Profile

All newborns received a double antibiotic therapy with beta-lactam and an aminoglycoside (88%) on admission. Nearly half of the blood cultures (49.3%) were performed before the first 48 hours of hospitalization. On direct examination, 68.5% of the bacteria found were gram-positive Cocci and 31.5% were gram-negative bacilli. *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Staphylococcus coagulase negative* and *Aeromonas salmonicida* were the isolated germs, with 27 cases, 20 cases, 19 cases and 1 case, respectively. *S. aureus* was the most isolated bacterium (55.6%) in newborns referred from the CHU maternity ward (in born). Among those born in structures other than the CHU (out born), *K. pneumoniae* was found mainly (60%). The antibiogram performed showed that 60% of *S. coagulase negative* and 45% of S. aureus were resistant to penicillin and 75% of *K. pneumoniae* produced ESBL. (40% resistant to ceftriaxone and 35% resistant to cefotaxime). The antibiotic resistance profile of germs is presented in Table 1.

Table 1. Distribution of germs according to antibiotic susceptibility.

Antibiotics		*S. aureus* N = 27	SCN* N = 19	*K pneumoniea* N = 20	*S salmonicida* N = 1
Céfotaxim	S (%)	41	37	30	
	R (%)	-	5	35	
Ceftriaxon	S (%)	4	-	-	
	R (%)	-	-	40	
Pénicillin	S (%)	15	5	-	-
	R (%)	45	60	-	
Fusidic acid	S (%)	63	53	-	
	R (%)	22	37	-	
Bactrim	S (%)	19	21	20	
	R (%)	22	32	-	100
Lévofloxacin	S (%)	-	5%	5	
	R (%)	19	-	15	
Ciprofloxacin	S (%)	-	-	25	-
	R (%)	4	-	15	100
Gentamicin	S (%)	44	37	-	100
	R (%)	30	58	50	-

Continued

Méropénèm	S (%)	30
	R (%)	-
Imipenèm	S (%)	65
	R (%)	-
Amikacin	S (%)	35
	R (%)	5

*SCN = S Coagulase negative.

In the case of place of birth, multidrug-resistant bacteria (MDR) were mainly gram-positive Cocci in the delivery room (17 cases) and in peripheral maternity wards, gram-negative bacilli (12 cases); 80% of the strains of S. aureus found in newborns were multidrug-resistant and 73.3% of the strains of *K. pneumoniae* found in out born newborns were multidrug-resistant. The presence of these bacteria was significantly associated with death ($p < 0.0001$), the presence of respiratory disorders ($p < 0.0001$) and the performance of resuscitation procedures at birth ($p = 0.04$). After the results of blood cultures and antibiotic susceptibility tests, 31% (25 cases) of patients benefited from antibiotic readjustment.

4. Discussion

HAIs are part of late-onset neonatal sepsis contracted in hospitals. Globally, neonatal sepsis is estimated to affect between 1.3 and 3.9 million newborns and is responsible for 400,000 to 900,000 deaths annually [6] [7] which are preventable [6] in 84%. In this study, the prevalence of HAIs was 58.77%. It was higher than the prevalence found by Folquet and Col in a study of the same structure in 2010 (22.7%) [9]. A quarter of the affected newborns were very premature babies (25.4%) and the average weight was 2392 grams. Indeed, the highest incidence of neonatal sepsis is in low-birth-weight newborns and preterm newborns [3] in low- and middle-income countries. Low birth weight preterm infants have a 3 to 10 times higher risk of sepsis than term infants [3] [6] due to immature immunity, potential intrauterine exposure to infection, and, in preterm infants, alteration of skin and mucosal barriers [10]. The diagnosis of neonatal sepsis is difficult because the clinical presentation is non-specific, the disease progresses very rapidly, and the warning signs are similar to those of hypoglycemia, hypothermia, or respiratory distress syndrome [11]-[13]. Leukocyte counts and differential counts have low sensitivity and are of little use due to wide physiological variation in the first few days of life [13] [14]. Creactive protein (CRP) is the most studied acute phase reagent in neonatal infection. It has been shown to be useful in high-income settings [15]-[17], where a single CRP value between 8 h and 36 h post-admission had a negative predictive value of sepsis of >99% [17]. The use of serial CRP measures to guide antibiotic therapy in newborns has been shown to be safe and

practical in developing countries [18]. As sepsis in newborns progresses rapidly, CRP-guided antibiotic therapy can be used to reduce the duration of antibiotic exposure by stopping antibiotics early [15] [19] [20]. Blood cultures remain the gold standard for confirming neonatal sepsis. However, the sensitivity of cultures decreases with blood samples smaller than 1 mL, which are very difficult to collect from newborns due to anatomical and technical difficulties [21]. Sensitivity may also be negatively influenced by previous maternal or neonatal exposure to antibiotics or poor laboratory skills [12]. Preventive initiation of antibiotic therapy in cases of suspected neonatal sepsis is a generally accepted good clinical practice. Laboratory results help to limit antibiotic choice and duration of treatment that otherwise remain entirely empirical. WHO still recommends initiation of ampicillin and gentamicin therapy as a first-line choice in neonatal sepsis [22], and if meningitis is suspected, when available, with a third-generation cephalosporin (ceftriaxone or cefotaxime). Data on local patterns of antimicrobial resistance are often lacking. The bacteriological profile of HAIs varies from one department to another, from one hospital to another and from one country to another, in relation to technical habits and methods of prescription. The bacterial spectrum of nosocomial infections in intensive care is dominated by gram-positive Cocci in developed countries [23], while the predominance of gram-negative bacilli, among which *K. pneumoniae* occupies a predominant place, has been found in most studies from developing countries [24]. In this series, the most common germs were gram-positive Cocci (68.5%) with a predominance of *S. aureus* (40.2%); SCoN accounted for 28.3% of germs. Gram-negative bacilli accounted for 31.5% of germs and were composed of *K. pneumoniae* (30%) and *Aeromonas salmonicida* (1.5%). Folquet *et al.* found the same distribution in 2010 in the same structure for neonatal bacteremia with 54.2% of *S. aureus* and 16.9% of *Klebsiella* [9]. A meta-analysis shows alarming results in newborns in sub-Saharan Africa. In this literature review, 89% of all *E. coli* were resistant to ampicillin. Gentamicin resistance for *E. coli* and *Klebsiella spp.* was 47% and 66%, respectively. The authors also described a high proportion of *E. coli coli* and *Klebsiella spp.* resistant to ceftriaxon (38% and 49%, respectively). In addition, 50% of *Staphylococcus aureus* were resistant to methicillin [25]. More than half (61%) of the bacteria found in these patients were multidrug-resistant bacteria (MDR). Indeed, 75% of the isolated strains of *Klebsiella pneumoniae* produced extended-spectrum β-lactamases (ESBLs). Their resistance was 75% to C3G, 50% to gentamicin and relatively low to amikacin (5%) and ciprofloxacin (15%). They remained sensitive to imipenem in 65% of cases. As for SCoN strains, they were resistant to penicillin in 60% of cases and to gentamicin in 58% of cases. *S. aureus* was resistant to penicillin in 45% of cases, with gentamicin in 30% of cases and fusidic acid in 22% of cases. In Folquet's 2010 study [9], all strains of *K. pneumoniae* were also resistant to β-lactams; sensitivity was maintained for amikacin (92.3%) and imipenem 88.2% and the vast majority of strains of methicillin-susceptible Staphylococci were found, which reflects a worrying change in bacterial ecology in our structure. Most

of the *K. pneumoniae* MDR (73.3%) were isolated from the newborns from the peripheral maternity wards and 80% of the *S. aureus* MDR were found in the newborns referred from the maternity ward of the University Hospital. The multiresistance of bacteria in nosocomial infections makes the therapeutic component difficult. MDR significantly increased the risk of death ($p < 0.001$), which was 19.4% in this study, and was mainly found in newborns who received care to help them adapt to extrauterine life ($p = 0.0000$) and presented with respiratory distress ($p < 0.0001$). Antibiotic therapy must be adapted to the antibiogram. Imipenems are a therapeutic choice in nosocomial gram-negative bacilli infections, given their resistance profile [26]. Fusidic acid is used in cases of staphylococci that are resistant to aminoglycosides and rifampicin in combination with vancomycin. In our study, 63% of *S. aureus* and 53% of SCNs were sensitive to fusidic acid, making it a good therapeutic alternative for gram-positive Cocci. The combination of cefotaxim and fusidic acid had given good results in gram-positive Cocci bacteremia. The combination of imipenem and amikacin appeared to be the most appropriate treatment for nosocomial gram-negative bacilli bloodstream infections. Carbapenems are the beta-lactams with the broadest antibacterial spectrum, those for which the percentage of resistant strains is currently the lowest. Their indications should be limited to proven or strongly suspected infections with gram-negative bacteria resistant to other beta-lactams [27]. However, it is still not very accessible in our practice, due to their high cost. In France, the combination of vancomycin, amikacin, and cefotaxime is currently recommended for the management of neonatal bacterial infections associated with resistant germ care (CoNS meti R; Enterobacteriaceae; *S. aureus*) [27].

5. Conclusion

The prevention and control of HAIs has become an urgent issue, particularly due to the increase in multidrug-resistant pathogenic microorganisms. Hand hygiene, a centuries-old concept, remains the main strategy used worldwide to prevent HAIs. It helps maintain a safe hospital environment and stop the transmission of contagious and infectious microorganisms, including multidrug-resistant microbes. Finally, antibiotic stewardship also plays a crucial role in reducing the impact of HAIs by keeping antimicrobials currently available.

Authors' Contributions

Kouakou Cyprien: conception, data collection, data entry, data analysis, and drafting of the manuscript.

Djivohessoun Augustine: participation in study design and manuscript writing.

Djoman Isabelle, Gro Bi Andre, Mansou Komenan: participation in drafting the manuscript.

Folquet A. Designing and carrying out the work of collecting the results, Reading and revising the manuscript. All authors have read and approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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