Multi-resistant Bacteria to Antibiotics in Hospitals: The Case of Neonatology Services of the University Hospitals Centers of Abidjan, Côte d'Ivoire

ABSTRACT

Background: Multidrug-resistant bacteria (MDR) represent a global health scourge. Their emergence in hospital services complicates the clinical management of infections caused in particular in immunocompromised persons. The objective of our work was to evaluate the prevalence of MDR in the neonatal services of the University Hospitals Centers of Abidjan.
Material and Methods: The present study took place from September to November 2020 and from January to June 2021. The samples collected consisted of venous blood samples for blood cultures, rectal swabs from newborns, nasal and hand swabs from health care workers, and swabs from inert surfaces and neonatal care equipment. Bacterial identification methods, antibiotic susceptibility testing, and Chi-square testing were performed.
Results: A total of 513 samples were obtained from which 215 organisms were isolated and identified. These bacteria consisted of 52.1% Gram-negative bacilli, of which 77.7% were *Enterobacteriaceae* and 47.9% Gram-positive cocci. *Klebsiella pneumoniae* (*K. pneumoniae*) (25.6%), coagulase-Negative *Staphylococcus* (CoNS) (24.6%), and *Staphylococcus aureus* (*S. aureus*) (23.2%) were the most isolated bacteria. The overall prevalence of MDR was 73.9%. The main antibiotic resistance phenotypes described were the production of Broad Spectrum β-lactamases (ESBL) in 71.9% of *Enterobacteriaceae* and methicillin resistance (Meti-R) in 75.6% of *Staphylococcus*. ESBL-producing *Enterobacteriaceae* (E-ESBL) were mainly observed in rectal carriage and Meti-R strains in blood cultures in newborns in the respective proportions of 45.6% and 56.4%.
Conclusion: In our work, the results obtained showed a high prevalence of MDR in neonatal services and newborns are the most affected subjects. Improving hygiene rules and control and rationalizing the use of antibiotics are highly recommended control strategies to reduce the hospital dissemination of MDR.

Keywords: Bacterial resistance; neonatal infections; healthcare associated infections; Côte d'Ivoire.

1. INTRODUCTION

Neonatology is a branch of pediatrics concerned with the medical management of newborns whose condition requires intensive care and close monitoring. The application of hospital hygiene rules is an underlying factor in the reduction and prevention of pathologies. Because of their immune immaturity, newborns can be prone to infections. This is especially true for low-birth-weight infants.

Generally, neonatal infections result from a series of previous events of maternal carriage (maternal-fetal infections), colonization of the hospital environment, and carriage by health care personnel (nosocomial infections and healthcare associated infections). The bacterial spectrum varies from one hospital to another within the same country or from one region to another. However, the main clinical and environmental pathogens isolated are *Escherichia coli* (*E. coli*), *Klebsiella sp*, *Staphylococcus aureus* (*S. aureus), Pseudomonas aeruginosa* (*P. aeruginosa), Enterococcus sp*, and *Acinetobacter sp* often showing increased antibiotic resistance [1,2].
Multidrug-resistant bacteria (MDR) are defined as bacteria that accumulate one or more resistance mechanisms or are resistant to more than three antibiotic families [3]. The emergence of MDR as a result of antibiotic use significantly reduces treatment options. In Europe, the Center for Disease Control and Prevention (CDCP)

reports 33.000 deaths resulting from MDR infections. An equivalent excess mortality is observed in the United States by the CDC in Atlanta [4]. Like low and middle-income countries, Côte d'Ivoire has few recent epidemiological data on the bacteria circulating in hospital wards.

The objective of the present study is to evaluate the prevalence of MDR infection, carriage and colonization in Neonatology in the University Hospital Centers of Abidjan.

2. MATERIALS AND METHODS

2.1 Period and Setting of the Study

Sample collection took place from September to November 2020 at the University Hospital Center of Treichville and from January to June 2021 at the University Hospital Center of Cocody. Isolation, identification and antibiotic susceptibility testing of bacterial strains were performed at the Antibiotics, Natural Substances and Surveillance of Resistance of Microorganisms to Anti-infectives (ASSURMI) unit of the Institute Pasteur de Côte d'Ivoire (IPCI).

2.2 Material and Bacterial Isolation

The material consisted of venous blood samples in yellow-capped BACTEC/ALERT® PF PLUS pediatric blood culture bottles (bioMérieux, inc, Durham, NC 27712 USA) and rectal swabs from newborns. In addition, this material included nasal and hand swabs for health care workers and its equipment (incubators, bed covers, oxygen masks and probes, door handles, sinks, trays, nursing beds, physicians' offices, baby scales, vacuum cleaners, milk cups).

Once collected, the samples were sent to the laboratory within two hours of collection. The blood samples were incubated at 37°C in the BAct/Alert 3D® automaton for blood cultures. In case of positivity, a drop of blood was plated on Methyl Blue Eosin, Chapman agar, and regular agar supplemented with cooked or fresh sheep blood. Swab samples were also discharged onto selective and non-selective culture media. The bacterial species were identified by conventional methods. (catalase testing, oxidase testing, Gram staining, Vitek 2 (Biomerieux)).

2.3 Susceptibility Testing

Susceptibility testing was performed using the agar diffusion method. The antibioticsdisks tested (Bio-Rad) as well as their loads were the following: benzylpenicillin (PNG, 1unit), ampicillin (AMP, 10µg), Amoxicillin/clavulanic acid (AMC, 20/10µg), piperacillin (PIP, 30µg), ticarcillin (TIC,75µg), (75/10µg), cefotaxime (CTX,5µg), cefoxitin (FOX, 30µg), ceftazidime (CAZ, 10µg), ceftriaxone (CRO, 30µg), imipenem (IMP,10µg), meropenem (MEM, 10µg), nalidixic acid (NAL,), ciprofloxacin (CIP, 5µg), levofloxacin (LVX, 5µg), amikacin (AKN, 30µg), kanamycin (KAN, 30µg), gentamycin, (GMI, 10µg), erythromycin (ERY, 15µg), clindamycin (CMN, 2µg), tetracycline (TET, 30 µg), chloramphenicol (CHL, 30µg), fusidic acid (FAD, 10µg) and trimethoprim /sulfamethoxazole (SXT, 1.25/23.75µg). The categorization of strains into susceptible (S), intermediate (I) and resistant (R) was done in accordance with the recommendations of CASFM/EUCAST (versions 2020 and 2021).. (It is the reference)

2.4 Testing for ESBL Detection

The method consisted in the use of the synergy between two discs on the standard antibiogram, i.e. a disc of cefotaxime, ceftazidime and a disc containing clavulanic acid (e.g. amoxillin-clavulanic acid: AMC) 30 mm apart from the cephalosporin discs on Mueller-Hinton agar. The presence of an ESBL is expressed by the appearance of a "champagne cork" synergy between the disc containing clavulanic acid and the cephalosporin (CASFM/EUCAST).

2.5 Identification of Methicillin Resistance

Methicillin resistance was confirmed by the cefoxitin disk diffusion method according to CASFM/EUCAST. A strain of *S. aureus* or CoNS is resistant to methicillin if it is resistant to cefoxitin.

2.6 Statistical Analyses

The KHI 2 or PEARSON test was used using XLSTAT version 2018 software to analyze a correlation between random variables. The results were interpreted according to the *p. value*:

- *P. value* ≤0.05 (confidence level): non-independent variables; existence of a relationship or no statistically significant differences between the variables.

- P. value >0.05: independent variables; no relationship or existence of statistically significant differences between the variables tested.

3. RESULTS

3.1 Samples and Bacteria Isolated

During our study period, a total of 513 samples consisting of 150 blood samples, 125 rectal swabs, 26 hand swabs, 26 nasal swabs and 186 environmental samples were taken with a positivity rate of 39.6%, i.e. 203 pure cultures obtained. From these cultures, 215 bacteria were isolated and identified, of which 52.1% (112/215) were gram-negative bacilli and 47.9% (103/215) were gram-positive cocci. Gram-negative bacilli were mainly isolated from rectal carriage with a rate of 42.9% (48/112) in newborns and 24.1% (27/112) in the environment. The highest proportion of gram-positive cocci, 47.6% (49/103), was observed in blood cultures.

Klebsiella pneumoniae (*K.pneumoniae*) (55/215, 25.6%), coagulase-Negative *Staphylococcus* (CoNS) (53/215,24.6%) and *S. aureus* (50/215, 23.2%) were the most isolated germs. Although these bacteria were present in all ecosystems at different rates, their distribution was related to the type of sample taken (p<0.0001).

3.2 Bacterial Resistance Profiles to Antimicrobials

- ***K. pneumonia***

High rates of resistance were described towards penicillins (100% to ampicillin, piperacillin and ticarcillin) and third generation cephalosporins (from 91.7 to 100% to cefotaxime, from 25 to 100% to ceftazidime). Other less important resistances were observed with carbapenems (from 8.3 to 23.1% with imipenem). Amikacin also showed good anti-bacterial activity with rates ranging from 20 to 36.3% (Table 1).

- ***S. aureus***

S. aureus strains showed resistance rates from 90.9 to 100% to benzylpenicillin and from 20 to 100% to cefoxitin. Concerning the aminoglycosides represented by gentamycin and kanamycin, important resistances have been described with values ranging from 33.3 to 100%. In the case of macrolides, lincosamides and streptogramins, although high rates of resistance have been reported, the molecules (erythromycin and clindamycin) tested were effective in the presence of *S. aureus* strains isolated from healthcare workers hands (Table 2).

- **CoNS**

CoNS strains that were subjected to benzylpenicillin showed resistance rates of 100%. With cefoxitin, the highest resistance was 66.7%. As for the aminoglycosides, the lowest resistance rate was 28.6% while the highest value was 87.5%. Levofloxacin was the least effective molecule regardless of the origin of the strains. In the presence of erythromycin and clindamycin, resistance rates ranged from 50 to 71.4% and 44.4 to 62.5%, respectively (Table 2).

3.3 Prevalence of MDR According to Bacterial Genera and Species

Out of a total of 215 strains identified, an overall prevalence of 73.9% (159/215) of MDR was obtained. These bacteria included 51.6% (82/159) of *staphylococcus* strains, 40.2% (64/159) of *enterobacteria* and 8.2% (13/159) of non-fermenting bacteria. The distribution of MDR according to bacterial families is detailed below:

Among the *Enterobacteriaceae*, a rate of 73.6% (64/87) of MDR was noted, distributed as follows: 65.6% (42/64) of *K. pneumoniae*, 7.8% (5/64) of *Klebsiella sp*, 6.2% (4/64) of *E. coli*, 4.7% (3/64) of *Klebsiella oxytoca*, 4.7% (3/64) of *Morganella morganii* and 2.1% (1/64) of *Proteus vulgaris*. No MDRs were observed among *Providencia rettgeri*, *Citrobacter freundii*, and *Citrobacter koseri* strains.

In the non-fermentative group, the prevalence was 52% (13/25). Multidrug resistance was obtained only with *P. aeruginosa* strains (81.2%; 13/16).

Among *Staphylococcus*, the prevalence of MDR observed was 80.4% (82/103) including 51.2% (42/82) of *S. aureus* and 48.8% (40/82) of CoNS.

3.4 Prevalence of MDR According to the Origin of the Sample

The MDRs were mainly isolated from blood cultures (37.7%; 60/159) and from rectal carriage (35.2%; 56/159) in neonates.

3.5 Phenotypic Characteristics of MDR

Among the 64 multi-resistant *enterobacteriaceae*, 71.9% (46/64) were producers of Extended Spectrum Beta- Lactamases (E-ESBL) and 28.1% (15/64) were resistant to third generation cephalosporins (3GCR-E). *K. pneumoniae* accounted for 69.6% (32/46) of the E-BLSE. More than 10% (5/46) of these E-ESBL developed resistance to carbapenems and 17.4% (8/46) to ciprofloxacin (Fluoroquinolone's resistance). Among *Pseudomonas aeruginosa*, ESBL production (7.7%; 1/13) and resistance to 3GCR (7.7%; 1/13) have also been described. Resistance to ceftazidime was observed with a rate of 15.4% (2/13). E-ESBL was observed in particular in rectal carriage in newborns (45.6%; 21/46).

Table 1. Antimicrobial resistance rates of *K. pneumoniae* strains by origin of samples

Antibiotics	Blood cultures(%)	Rectal swabs(%)	Hand swabs(%)	Nasal swabs(%)	Environment(%)	*P-value*
Amikacin (AKN)	36.3	20	0	-	0	
Amoxicillin/ clavulanic acide (AMC)	86.67	87.5	25	87.5	71.43	
Ampicillin (AMP)	93.75	100	50	100	100	
Cefotaxime (CTX)	91.67	100	100	-	100	
Cefoxitin (FOX)	58.33	100	100	62.5	50	
Ceftazidim (CAZ)	66.67	100	-	25	-	
Ceftriaxone (CRO)	100	100	75	-	40	
Chloramphenicol (CHL)	66.67	55.55	75	0	25	
Ciprofloxacin (CIP)	50	66.67	-	25	-	*P*<0.05
Gentamycin (GMI)	100	63.63	-	25	50	
Imipenem (IMP)	23.08	8.33	0	0	0	
Levofloxacin(LVX)	50	60	75	-	25	
Meropenem (MEM)	0	0	-	0	0	
Nalidixic acid (NAL)	50	75	50	25	50	
Pipéracillin (PIP)	100	100	100	100	100	
Ticarcillin (TIC)	100	100	100	100	100	
Trimethroprim/Sulfamethoxazole (SXT)	60	100	-	37.5	50	

Table 2. Antimicrobial resistance rates of *S. aureus* (in blue) and CoNS (in bold) strains by origin of the samples

Antibiotics	Blood cultures (%)	Rectal swabs (%)	Hand swabs (%)	Nasal swabs(%)	Environment (%)	*P-value*
Benzypénicillin	90.9	100	100	100	100	
	100	50	-	100	-	
Cefoxitine	92	91.6	100	20	20	
	57.1	66.7	0	50	20	
Clindamycin	36	61.5	0	0	20	
	50	44.4	0	62.5	62.5	*P*<0.05
Erythromycin	72	57.1	0	25	66.7	
	50	57.1	-	62.5	60	
Fusidic acid	32	46.1	100	40	60	
	62.5	44.4	50	62,5	60	
Gentamycin	100	90.9	100	0	50	
	765	50	-	60	0	

Antibiotics	**Blood cultures (%)**	**Rectal swabs (%)**	**Hand swabs (%)**	**Nasal swabs(%)**	**Environment (%)**	***P-value***
Kanamycin	88.2	84.6	100	33.33	50	
	73.7	42,8	-	87.5	28.6	
Levofloxacin	100	80	100	100	33.3	
	94.4	83.3	-	100	100	
Tétracyclin	55	42.8	-	25	0	
	68.4	57.1	0	66.7	37.5	
Trimethroprim/Sulfamethoxazole	76.9	81.8	-	0	0	
	58.3	50	50	33.3	16.7	

(-) : not tested

Table 3. Distribution of antibiotic resistance phenotypes by sample type

Resistance phenotypes Samples types	ESBL n (%)	Meti-R n (%)	*P-value*
Blood cultures	11 (23.9%)	35 (56.4%)	
Rectal swabs	21 (45.6%)	17 (27.4%)	
Hand swabs	2 (43%)	1 (1.6%)	0.003
Nasal swabs	2 (4.3%)	5 (8.1%)	
Environment	10 (21.7%)	4 (6.4%)	
Total	46 (100%)	62(100%)	

Methicillin resistance was described in 75.6% (62/82) of *staphylococcus* strains. The prevalence of methicillin-resistant *S. aureus* (MRSA) was 61.3% (38/62) and that of coNS (MRcoNS) 37.7% (24/62).

Among the *Staphylococcus* Meti-R strains, 79% (49/62), 62.9% (39/62) and 29% (18/62) developed resistance to fluoroquinolones (Fq-r), gentamycin (KTG phenotype) and macrolides, lincosamides and streptogramins (MLSb) respectively. A combination of at least three antibiotic resistance phenotypes was noted in 56.4% (35/62) of these strains. Methicillin resistance mainly concerned strains of *Staphylococcus* isolated from blood cultures (56.4%; 35/62). The results obtained showed a significant relationship between the distribution of these different resistance phenotypes and the different samples taken (p=0.003) (Table 3).

4. DISCUSSION

In our study, the bacterial ecology of the samples taken consisted of *K. pneumoniae* (55/215; 25.6%), CoNS (53/215; 24.6%) and *S. aureus* (50/215; 23.2%). The predominance of these three bacterial species could be explained by their ubiquitous nature and their ability to adapt to any host. Described as etiological agents of infections and carriage in immunocompromised patients, especially in newborns, in low-income countries [5-8], but also organisms colonizing the hospital environment and healthcare personal [9-10,1], these bacteria often present an increased resistance to one or more families of antibiotics, hence the notion of multi-resistant bacteria. The emergence of MDR is a health concern because it is the cause of high morbidity and mortality rates, as well as long periods of hospitalization.

The objective of the present study is to evaluate the proportion of MDR in neonatal services, showed a high prevalence of 73.9% (159/215). This result highlights the alert situation that prevails in our hospital services, particularly in neonatology.

The bacteria which were mostly affected by multidrug resistance were *K. pneumoniae* (65.5%), *S. aureus* (51.2%), CoNS (48.8%), and *P. aeruginosa* (100%). Most of these germs have been listed by the World Health Organization as pathogens for which the development of new antibiotics is an absolute emergency [11].

The distribution of MDRs by origin of samples showed relatively high rates in blood cultures (37.7%) and rectal carriage (35.2%) in neonates. These results are in conformity with that of Zou et al, 2021 in China and Arhoune et al, 2021in Morocco [12,13]. Several works have, in addition, shown that the main correlative risk factors for the emergence of MDRs in newborns have been low birth weight, gestational age, long hospital stay associated with prematurity and cumulative antibiotic exposure, among others [14-16]. At the phenotypic level, the most described MDRs, remain multidrug-resistant *Pseudomonas*, carbapenem-producing *Enterobacteriaceae* (CPE), E-EBLS, MRSA, vancomycin-resistant *Enterococcus*, and multidrug-resistant *Acinetobacter baumanii* [17-20]. In our study, 71.9% of the isolated *enterobacteriaceae* were ESBL producers. This rate is significantly higher than 57.7% and 58% reported by Teklu et al., 2019 in Ethiopia and Ouedraogo et al., 2016 in Burkina-Faso, respectively [21-22]. This high prevalence could be due to the fact that almost all of these bacteria were of clinical origin and therefore subject to high selective pressure generated by the excessive use of antibiotics. Among the E-ESBL, 10.9% were carbapenemase producers and 17.4% were resistant to Fluoroquinolones. According to some authors, ESBL production is often associated with resistance to several families of antibiotics; the genes encoding these enzymes are carried by large plasmids on which other resistance genes would be localized [23-25]. *K. pneumoniae* was the bacterial species most involved in ESBL production with a rate of 69.6%. Our results agree with those of Teklu et al, 2019 and Kagia et al, 2019 who also noted a high prevalence of the same germ [21,26]. Indeed, *K. pneumoniae* and *E. coli* are the *Enterobacteriaceae* frequently

associated with the production of Beta-Lactamases although this resistance mechanism is also described in the genera *Pseudomonas*, *Proteus*, *Citrobacter*, *Morganella*, *Salmonella* [27]. In our study, more than 45% of E-ESBL were isolated from neonatal rectal carriage. Studies conducted in Morocco have shown prevalence of 22.4% at the time of admission of newborns to the neonatal unit and 92.4% during their stay in the unit [13]. Other authors have reported a rate of 77% of rectal carriage E- ESBL in this age group [27]. In addition to the production of ESBLs in *Enterobacteriaceae*, the predominance of methicillin resistance was noted in 75.6% of *Staphylococcus* strains, including 61.3% of *S. aureus*. The prevalence of MRSA shows inter and intra country disparities. The most recent data for 85 WHO member countries reported rates exceeding 20% in all regions and even 80% in some countries [28]. MRSA has caused several hospital outbreaks in neonatal care units [29-31]. Reports from various studies have discussed the important risk factor of colonized caregivers in the occurrence of these outbreaks [32-33]. *Staphylococcus* Méti-R strains showed high rates of resistance to Fluoroquinolones (79%), Aminosides (62.9%) and relatively low to Macrolides (29%). This resistance towards other families of antibiotics, which mainly concerned *S.aureus*, has been described in several studies [34-36]. The majority of our Meti-R strains (56.4%) were isolated from blood cultures in neonates. A study conducted by Almutairi et al., 2021 in Kuwait obtained a rate of 15, 15% [37]. while other work conducted in Ethiopia, South Africa and China reported 69%, 70% and 79% respectively [38-40]. MRSA is one of the leading causes of late neonatal sepsis in low-income countries. The variability of prevalence observed from one region to another would be due to the policies of antibiotic use and the implementation of preventive measures of hygiene and control.

5. CONCLUSION

The present study revealed a high prevalence of MDR in our neonatal services. The most concerned bacteria were *P. aeruginosa*, *K.pneumoniae*, *S.aureus* and CoNS with the main antibiotic resistance phenotypes being ESBL production and methicillin resistance. MDR were mainly isolated from rectal carriage and sepsis in neonates. The vulgarization of these results could encourage health authorities to improve hospital hygiene measures in order to limit the dissemination of MDR and to rationalize the use of antibiotics.

CONSENT AND ETHICAL APPROVAL

This study was approved by the National Ethics Committee for Life Sciences and Health (CNESVS) under the number IRB000111917. All participants gave their consents.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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