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Risk Factors and Prevalence of Mother to New-Born Transmission of Carbapenemase Producing Enterobacteriaceae in Two Hospitals in Yaounde, Cameroon

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Abstract

Background: In African countries, where the burden of neonatal sepsis is the highest, the spread of Carbapenemases Producing Enterobacteriaceae (CPE) in the community, potentially contributing to neonatal mortality, is a public health concern. The transmission routes are not well defined, particularly the possible key role played by pregnant women. The aim of this study was to understand the neonatal acquisition of CPE in Yaounde, Cameroon. **Methods:** A transversal analytical study was conducted in an urban area. Maternal stool samples during delivery and the first stool from their new-born were collected and cultured to isolate Enterobacteria. After isolation, characterization using API20E identification system, and antibiotic susceptibility testing were performed according to the Antibiogram Committee of the French Society of Microbiology. Carbapenemases detection was done on each carbapenem-resistant strain using the Modified Hodge Test (MHT) and their classification using the synergy tests with different inhibitors. **Results:** Out of the 55 CPE isolates identified, *Escherichia coli* was the most encountered bacteria both in mothers (n = 18, 50.00%) and infants (n = 11, 57.89%). Class B and D carbapenemases were found both in mothers and infants. The estimated prevalence of vertical transmission in our study, was 10% (n = 12). Logistic

regression showed that CPE carriage in mothers and CPE acquisition in their new-borns were independently associated with the presence of greenish amniotic fluid (OR = 7.33, p < 0.0001 in mothers and OR = 4.09, p = 0.0086 in new-borns). **Conclusion:** Our results highlight the non-negligeable role played by pregnant women in the neonatal acquisition of CPE.

Keywords

Drug Resistance, Carbapenems, Pregnant Women, Vertical Transmission, Cameroon

1. Introduction

Antimicrobial resistance (AMR) currently represents one of the most important global health challenges [1]. AMR is projected to become the leading cause of death worldwide, claiming an estimated 10 million lives a year by 2050, primarily in low- and middle-income countries (LMIC) [1]. The main drivers of AMR include the misuse and overuse of antimicrobials in human, animal health and agriculture [2]. In LMICs where the burden of neonatal sepsis is the highest [3], the bacteria of the Enterobacteriaceae family represent the major cause of severe bacterial infections [4] with a mortality rate in 2015 amounting 21 per thousand births in Cameroon [5]. The 2014 World Health Organization report on AMR outlines that bacterial resistance has reached alarming levels in most parts of the world, with the highest resistance levels expressed by *Escherichia coli* and *Klebsiella spp* to third generation cephalosporins and carbapenems [6].

Carbapenemase-producing Enterobacteriaceae (CPE) are an important and increasing threat to global health. Both clonal spread and plasmid-mediated transmission contribute to the ongoing rise of the incidence of these bacteria. Among the 4 classes of β-lactamases defined by the Ambler classification system, the carbapenemases that confer carbapenem resistance in Enterobacteriaceae belong to 3 of them: Class A (*K. pneumoniae* carbapenemases, KPC), Class B (metallo-β-lactamases, MBL including New Delhi metallo-β-lactamases, NDM) and Class D (OXA-48-like carbapenemases). KPC-producing CPE are the most commonly occurring CPE in the United States. MBL-producing CPE have been most commonly associated with the Indian Subcontinent as well as with specific countries in Europe, including Romania, Denmark, Spain, and Hungary. The epicentre of OXA-48 production is in Turkey and surrounding countries. Infections caused by CPE are associated with increased mortality rates; prolonged treatment time; higher health care associated costs [5], and therapeutic dead-ends [7]. The acquisition of CPE during the first seven days of life of the new-born principally results from the vertical transmission of bacteria from the mother to her new-born during childbirth [3]. Studies performed on the first stool of the new-born found that bacteria are present in the foetal tract before birth, suggesting possible prenatal colonization [8]. This highlights the mother as an im-

portant potential risk factor for neonatal colonization by CPE [9]. A study carried out in Algeria in 2018, revealed that mothers and their new-borns had a prevalence of 4.6% and 1.6% respectively of OXA-48 CPE carriage [10].

The data regarding the acquisition of CPE during the neonatal period are scarce. The routes of transmission and particularly the possible key role played by the pregnant women are not well established [3]. This study, therefore, sought to determine the prevalence and the risk factors associated with maternal and neonatal carriage of CPE isolated from pregnant women and their new-borns in two hospitals in Yaounde, Cameroon.

2. Material and Method

2.1. Study Design

From 28th September to 30th October 2020, a cross-sectional analytical study was carried out in two hospitals within the city of Yaounde, including the Yaounde Gynaecology Obstetrics and Paediatrics hospital—a referral hospital with a capacity of 240 beds and the Animation Social and Sanitary centre of Nkoldongo, a medical center with a capacity of 62 beds. Pregnant women in labor were consecutively enrolled in the study after obtaining an informed consent. The neonates born from these women were equally included in the study following their mother's agreement. Were excluded however, all women who did not give their consent or expressed their right of withdrawal. Clinical specimens were collected from all the participants and transported to the Bacteriology Laboratory of the Yaounde Gynaeco-Obstetrics and Paediatrics hospital for analysis. The minimum sample size was calculated using the Lorentz formula. Our minimum sample size was estimated to 68 couples of mothers and their new-borns. The questionnaire was designed following a similar study carried out in Madagascar [3], and included one health aspects, as well as demographic, epidemiological and clinical aspects. The questionnaire was subjected to a pre-test to verify its validity before being used to collect data from the participants. The stool samples were collected from the mothers as soon as they were emitted during labor using a labelled sterile collection cup and a cotton swab. As for the newborns, the heart of the stool was collected using a labelled sterile collection cup and a cotton swab, directly from the newborn carefully avoiding skin contact, or from the diaper if the stool was emitted later on. The sample was then transported to the laboratory immediately after collection using a refrigerated bag containing ice packs which maintained the temperature between 4˚C - 8˚C.

2.2. Sample Processing

Eosine Methylene Blue medium (BIOCHEM Chemopharma, Cosne-cours sur loire, France) supplemented with cefotaxime solution (Bio-Rad Marnes-la-Coquette, France) was used for Enterobacteriaceae selection. Strain identification was performed using Api 20E system (Biomérieux Marcy-l'Etoile France) after an oxidase test (Bio-Rad Marnes-la-Coquette, France). Each identification plate was

inoculated with a bacteria suspension prepared with opacity of 0.5 on the Mc Farland scale. The presence of Extended Spectrum Beta-Lactamase (ESBLs) in isolates was confirmed using the double-disk synergy method, which was performed by placing the disk of cefotaxime (30 µg), (BIOCHEM Chemopharma, Cosne-cours sur loire, France) ceftazidime (30 µg) (Bio-Rad Marnes-la-Coquette, France), and combination of amoxicillin/clavulanic acid (20 µg/10µg) (Bio-Rad Marnes-la-Coquette, France) on a lawn culture of bacteria on Muller-Hinton agar (BIOCHEM Chemopharma, Cosne-cours sur loire, France) plate, with a distance of 20 mm between each disk center to center. The expression of the Extended Spectrum Beta-Lactamase enzyme by an isolate, and a reduced sensitivity (Inhibition diameter < 25 mm) to the antibiotic Ertapenem on an antibiogram were the selection criteria for the detection of carbapenemases. The detection was done using the Modified Hodge Test (MHT) [10] in which the production of the enzyme by the isolate allowed the growth of the carbapenem-sensitive strain (*Escherichia coli* ATCC 25922) around a carbapenem disc, the appearance of a characteristic notched clover leaf was the indication of the presence of a carbapenemase enzyme. Classification tests were done by inhibitory synergy tests, using Boronic acid 30 mg/ml (BIOCHEM Chemopharma, Cosne-cours sur loire, France) and Chelating agent (EDTA) (BIOCHEM Chemopharma, Cosne-cours sur loire, France) tests for class A (KPC) and class B (NDM) respectively. A negative result to the tests using the two inhibitory agents was considered as a class D. The phenotype interpretation was done according to the 2020 guidelines of the Antibiogram Committee of the French Society of Microbiology (AC-FSM) [11].

2.3. Statistical Data Analysis

The data on socio-demographical and bacteriological characteristics, and potential risk factors were recorded on the software Excel 2016 and analysed using the statview version 4.0. The results were expressed using descriptive statistics and associated to the confidence interval at 95%. The research of potential risk factors associated to the carriage of CPE in mothers and new-borns, and a potential vertical transmission, was done using the logistic regression in univariate and multivariate analysis. P value < 0.05 was considered as significant.

2.4. Ethical Approval

The ethical approval was obtained from the Institutional Ethical Review Board Université des Montagnes and the city local authorities (Authorization N˚2020/164/UDM/PR/DE). Permissions to conduct the study were granted by the Director of the Yaounde Gynaeco-Obstetrics and Paediatrics hospital and at the Social Animation and Sanitary centre of Nkoldongo.

3. Results

3.1. Characteristics of the Study Population

Of the 120 pregnant women enrolled in the study, 120 live newborns were also

included in the study. Table 1 presents the general characteristics of the mothers and newborns. On average, mothers were 28.52 years old with a minimum at 17 years old and a maximum at 42 years old. Majority of the women gave birth in public hospitals and were single with 65.83% and 45.83% respectively. Table 2 shows that our new-born population was dominated by the male sex with a proportion of 54.17% against 45.83% for females. Their mean birth weight was 3211.75 g.

3.2. CPE Carriage in Pregnant Women and in New-Borns

Of the 120 mothers from whom stool samples were collected, 36 (30.0%) were colonized with CPE. The majority were *Escherichia coli* (n = 18, 50.00%) and *Klebsiella pneumoniae* (n = 8, 22.22%). The most identified carbapenemases class was class D (n = 19, 15.83%), followed by class B (n = 17, 14.17%). The class A carbapenemases was not encountered. As for the 120 new-borns, 19 (15.83%) CPE isolates were identified and the most predominant was *Escherichia coli* (n = 11, 57.89%), followed by *Klebsiella pneumoniae* (n = 5, 26.32%).

Table 1. Socio-demographical characteristics of pregnant women.

Socio-demographical characteristics	n (%) or mean
Age (years)	
Mean ± SD	28.51 ± 5.82
Hospital type	
Private	41 (34.17%)
Public	79 (65.83%)
Marital status	
Single	55 (45.83%)
Married	44 (36.67%)
Engaged	8 (6.67%)
Open relationship	13 (10.83%)

Table 2. Socio-demographical characteristics of pregnant women

Socio-demographical characteristics	n (%) or mean
Birth weight (g)	
Mean ± SD	3211.75 ± 471.43
Age (hours)	
Mean ± SD	2.35 ± 8.27
Gender	
Male	65 (54.17%)
Female	55 (45.83%)

The most encountered class was the class D (n = 10, 8.33%) followed by the class B (n = 9, 7.50%) (Table 3).

3.3. Risk Factors of CPE Carriage in Pregnant Women and CPE Acquisition in Their New-Borns

Table 4 and Table 5 show the results obtained from association between risk factors and the maternal and neonatal carriage of CPE respectively. Logistic regression showed that CPE carriage in mothers was significantly associated to the presence of greenish amniotic fluid, and that mothers were seven times more likely to carry a CPE in presence of the latter (OR = 7.33, p < 0.0001). CPE acquisition in their new-borns was independently associated with the presence of greenish amniotic fluid (OR = 4.09, p = 0.0086), and the risk of acquiring a CPE was increased by four times with greenish amniotic fluid).

4. Discussion

The neonatal acquisition of CPE and the role played by the mothers where the

Table 3. CPE carriage in pregnant women and in new-borns.

Carbapenemase enzyme	Pregnant women (n = 120)	New-borns (n = 120)
Overall	36 (30.0%)	19 (15.83%)
Class A	0	0
Class B	17 (14.17%)	9 (7.50%)
Class D	19 (15.83%)	10 (8.33%)

Table 4. Association between risk factors and maternal carriage of CPE.

Parameters	n	OR	95%CI	P value
Age				
Mean	28.51	1.05	[0.98 - 1.12]	0.18
Hospital type				
Private	13 (31.71%)	1		Ref
Public	22 (27.85%)	0.83	[0.37 - 1.89]	0.6
Amniotic fluid				
Clear	6 (8.33%)	1		Ref
Greenish	13 (27.08%)	7.33	[3.05 - 17.61]	<0.0001

Table 5. Association between the maternal risk factors and neonatal carriage of CPE.

Parameters	OR	95%CI	P value
Age	1.01	[0.92 - 1.11]	0.78
Amniotic fluid: Greenish	4.09	[1.43 - 11.68]	0.0086
Hospital type: Public	1.23	[0.36 - 4.25]	0.73

subject of this study. In this study, 120 couples (mothers and newborns) were enrolled for an overall prevalence of 22.92%. The class D carbapenemases enzymes were more frequently encountered and the class A completely absent which can be explained by the fact that class A carbapenemases are actually endemic mostly in the United States, in Colombia, in Greece and in Italy and the class D on the African continent [12]. Asymptomatic carriage of carbapenemase producing *Escherichia coli* and *Klebsiella pneumoniae* was reported in this study in mothers and newborns. The presence of greenish amniotic fluid was statistically significant and associated to CPE carriage in mothers, and the acquisition of CPE in newborns, therefore represented a risk factor. When we considered the acquisition of CPE during the first week of life, 12 pairs of mother/infant carried the same pathogen. Although other studies found that maternal CPE was associated with CPE colonization in newborns [13], this finding suggests that mother to child transmission during delivery might play a significant role in the acquisition of colonization in the first week of life.

However, we cannot exclude that the none statistically significant associations between hospitalization, use of antibiotics during the first month of life, maternal ESBL-PE carriage and CPE acquisition in neonates, which are contrary to the findings of Mairi *et al.,* [3] where hospitalization is considered as a risk factor. Upon completion of our study, no correlation was highlighted between the risk factors; birth weight, sex, maturity, hospital facility, and age at sample collection ($p = 0.19$, $OR = 1.04$) and the acquisition of CPE. These results were calculated by univariate analysis and can be explained by the low population size. Statistical crossovers were done by crossing the maternal risk factors with the neonatal carriage of CPE*,* and the neonatal risk factors with the maternal carriage of CPE*.* As a result of the crossing, greenish amniotic fluid was independently associated to CPE acquisition in new-borns.

The limitation of this study was the inability to carry out molecular characterisation of carbapenemase producing enterobacteriaceae due to lack of funding.

5. Conclusion

This study showed that the MHT technique is highly sensitive for detecting class A, B, and D carbapenemases. However, the limitations of the MHT in terms of clinical performance remain its lack of specificity and the delay in obtaining the results (24 to 48 h) after isolation of a bacterial colony.

Consent for Publication

All authors consented for publication.

Availability of Data and Material

All data generated or analysed in the course of this study are included in this manuscript.

Authors' Contributions

CID and conceived the project and designed the study. CID and NTC searched relevant literature, scrutinized all relevant information and draft the manuscript. CID, NTC and FW conducted and coordinated the field study. NTC, FW, JDN, PDDD, CSN, MF, EK; VMN, JMT collected and processed the samples and data.

CID and PDDD analysed the data. CID, NTC, and BDTP interpreted the results. CID and BDTP critically revised the manuscript. All authors read and approved the final manuscript.

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Conflicts of Interest

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

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