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*Article*

# Frequency and Antibiotic Susceptibility Patterns of *Streptococcus Agalactiae* Strains Isolated from Women in Yaounde, Cameroon

**Cécile Ingrid Djuikoue[1], Paule Dana Djouela Djoulako[2], Rodrigue Kamga Wouambo[2], Rosine Yemetio Foutsa[2], Dorine Ekeu[1], Teke Apalata[3,*]**

[1] Microbiology Department of the faculty of Health Sciences of the Université des Montagnes, Bangangté, Cameroon; djuikoe1983@yahoo.fr, dorineekeu812@gmail.com
[2] Department of Microbiology and Parasitology, Faculty of Sciences, University of Buea, Cameroon; danadjoulako02@gmail.com, rodriguekamga89@yahoo.fr, yemet92@gmail.com
[3] Faculty of Health Sciences & National Health Laboratory Services, Walter Sisulu University, South Africa; tapalata@wsu.ac.za
* Corresponding author: Prof. Teke Apalata, MD, PhD; e-mail: tapalata@wsu.ac.za

**Abstract:** Group B *Streptococcus* (GBS), a commensal in the body, causes a wide range of infectious diseases. The colonisation levels of GBS and its resistance profile to antibiotics provide important information useful for orienting prevention strategies. There is little data available on the subject with determination of resistance phenotypes in Cameroon. We therefore aimed to determine the prevalence of colonization, antibiotic resistance, including patterns of inducible resistance to clindamycin of GBS in Yaounde. To achieve this goal, a prospective cross-sectional study with an analytical component was carried out from the 28th June to the 29th August 2020 at the BIOSANTE laboratory and the Yaounde Gynaeco-Obstetrics and Paediatrics hospital. Vaginal swabs and urine were collected on 163 women. This samples were analysed using 5% defibrinated sheep blood agar and chocolate plus polyvitex agar. The isolates were identified using the morphology of the colony, Gram staining, haemolysis, catalase test and latex grouping test. Antibiotic susceptibility testing was done by disk diffusion method following the recommendations of the ACFSM 2019. The double disk diffusion method was used to identify isolates with clindamycin inducible resistance. Our data was analysed by the software SPSS version 2.1. The results obtained showed that the global prevalence of colonization by GBS was 37% (57/163), 40.35% in non-pregnant women and 59.65% in pregnant women. Pregnancy (P-value = 0.019) and gestational age (P-value = 0.025) constituted the risk factors of maternal colonization by GBS. In addition, the strains of GBS were resistant to all antibiotics tested. A D test showcased that 64.7% of GBS were resistant in a constitutive manner to clindamycin. We also note the presence of M phenotypes. As a whole, our results demonstrate that the rate of GBS colonization in this study was similar or higher than those in the previous report in Cameroon. All this indicates that attention should be paid to this bacterium in the monitoring of antimicrobial resistance and in the care of pregnant women and newborns.

**Keywords:** Antibiotic resistance; Colonization; Prevalence; GBS; Resistance phenotype

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## 1. Introduction

*Streptococcus agalactiae*, equally known as Group B Streptococcus (GBS), is a commensal commonly encountered in the human gastro-intestinal and urogenital tract [1]. It provokes a broad range of infectious diseases, in newborns, elderly people, immunocompromised people, in pregnant women and adults (urinary tract infection) [2]. During pregnancy, about 10 to 30% of women are carriers of the bacterium [3] and 60% of them transmit the bacterium to their child during pregnancy or childbirth [4] ; due to this vertical transmission, the mortality rate from GBS infections in infants has been estimated to be between 2% and 4%, but this rate is even higher in premature infants[5]. Widespread use of intrapartum antibiotic prophylaxis to prevent early onset of GBS disease has led to concerns about the development of antibiotic resistance among GBS isolates [6]. In order to prevent multiresistance, universal screening of mothers for vaginal or rectal colonization by GBS between the 35$^{th}$ and 37$^{th}$ gestation week and selective intrapartum antibiotic prophylaxis (IAP) for all women screened positive, is the strategy actually recommended to reduce the incidence of colonization in newborns and to prevent early onset diseases linked to this bacterium [6]. Despite this prevention, a significant amount of evidence suggest that GBS becomes resistant to antibiotics, a multi-analysis done by Mucheye *et al* in Africa in 2019 reported resistance to several families of antibiotics [7] ; moreover according to the statistics published by the Center of Disease Control (CDC), the in vitro level of resistance of GBS to erythromycin and to clindamycin has increased to 25-32% and 13-20% between 2006 and 2009 in the United states [8]. It is then important to determine the prevalence and antimicrobial sensitvity profile of GBS in different regions for the therapeutic strategies. In Cameroon, Nkembe *et al* in 2018 reported a prevalence of 14% with a sensitivity to β-lactamines but reduced to erythromycin [9], Chatte in 2014 had obtained a prevalence of 7.7% with a sensitivity to β-lactamines and macrolids equally [10]. Due to the presence of antibiotic resistance genes such as ermB, ermTR and ermA/E on transposons, which can travel from one organism to the other [11], the antibiotic resistance of GBS should be studied and monitored regularly. Depending on the importance of the problem and the questions mentioned above, the present study was carried out with the aim of estimating the prevalence of GBS colonization in women, antibiotic resistance and characterising the resistance phenotypes.

## 2. Materials and Methods

### Type, location and duration of study

We carried out a cross-sectional descriptive study with an analytical component in a hospital settinG in the centre region of Cameroon. The samples were collected and analysed at the Yaounde Gynaeco-Obstetrics and Paediatrics and the BIOSANTE International laboratory from 29$^{th}$ June to the 28$^{th}$ August 2020.

### Study population, selection criteria and sampling method

We used convenience sampling to recruit women who are pregnant or not who came to consult or carry out exams at the collection sites. Women who were in the first trimester of their pregnancy and women on antibiotics or who had been on antibiotics for less than 14 days had been excluded.

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**Data and sample collection method**

During consultation and exam realisation, we selected elligible patients, the studay was explained to them and those who had accepetd to participate gave their written consent. Relevant information was collected by means of a questionnaire. During our study, we have performed a vaginal swab using cotton swabs and urine sampling with the help of a sterile can previously labeled. The samples after collection were rapidly fowarded to the laboratory.

**Sample analysis**

**Culture :** Once at the laboratory, all the specimens were incoculated on the blood agar supplemented by nalidixic acid-nistatin-colistin (ANC) and cj=hocolate plus polyvitex agar, then incubates at 37°C for 24 hours in a jar with 10% Carbon dioxide. **Identification:** Little greyish colonies, smooth and non-pigmented with a visible beta hemolysis zone appearing after 24 hours of incubation were isolated, and their reactivity to the catalase test evaluated. Colonies having a negative reactivity to the catalase were used for confirmation of the diagnostic with the help of the Pastorex-Strep kit to carry out Lancefield serogrouping. The colonies that only agglutinated with the reagent in B were considered as GBS. **Antibiogram:** The resulting isolates were then used to test antibiotic sensitivity by the Kirby Bauer disk diffusion method on Muller Hinton agar plus sheep blood with the reference strain being *Staphylococcus aureus* ATCC 29213. The antibiotics tested, their disposition and their respective inhibition diameters were those recommended by the Antibiogram Committee of the French Society of Microbiology (ACFSM 2019). **Determination of the Resistance phenotype:** Clindamycin and erythromycin sensitivity and the determination of the different resistance phenotypes to Macrolids-Lincosamide-B Streptogramines (MLBS) were done by the double disk diffusion method on Muller Hinton agar containing 5% of sheep blood. In the case of clindamycin and erythromycin, the regions inferior to 15mm around the two disks indicated a resistance to constitutive MLBS; the appearance of a D-shaped halo on the medium had been considered as indicatif of an inducible clindamycin resistance; Furthermore, the coincidence of resistance to erythromycin and the absence of resistance to clindamycin was indicative of the M phenotype. Finally, the coincidence of resistance to clindamycin and moderate resistance to erythromycin was indicative of the L phenotype [8].

**Data analysis and interpretation**

The different variables and results obtained after verification of their conformity were recordes in Excel 2010 software, then analysed with the statistic software SPSS 21. The principal analysis included the calculation of the frequency and their frequency intervals at 95% (for qualitative variables), and the mean or the median (for quantitative variables). The univariate analysis enabled the determination of the factors influencing the occurrence of a GBS infection (P-value<0,05).

**Ethical considerations**

An ethical clearance was obtained on the basis of the evaluation and validation of the research protocol by the Ethics Committee of the University of Douala.Collection

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authorisations from the BIOSANTE International Laboratory and the Yaounde Gynaeco-Obstetrics and Paediatrics Hospital were also obtained after validation of the collection request coupled with the research protocol.

## 3. Results

### 3.1. *General characteristics of the population*

**Table 1.** General characteristics of the population

| Characteristics | Effectif (n) | Percentage (%) |
|---|---|---|
| Pregnant women | 90 | 55.21 |
| Non-pregnant women | 73 | 44.79 |
| **Age group (in years)** | | |
| [17-25] | 41 | 25.15 |
| [26-34] | 57 | 34.97 |
| [35-43] | 32 | 19.63 |
| [44-52] | 20 | 12.27 |
| 53 and ABOVE | 13 | 7.98 |
| **Mean age : 32.34 years±10.48** | | |
| **Gestational age** (in weeks) | | |
| [27-31] | 41 | 45.56 |
| [32-36] | 22 | 24.44 |
| [37-41] | 27 | 30 |
| **Sample type** | | |
| Vaginal secretions | 138 | 84.66 |
| Urine | 25 | 15.34 |
| **Number of GBS strains isolated** | | |
| Pregnant women | 34 | 59.65 |
| Non-pregnant women | 23 | 40.35 |

A total of 163 patients participated, of whom 90 were pregnant (55.21%) compared to 73 who were not (44.79%). The mean age of the population was 32.34years ±10.48 with a minimum of 17 years and a maximum of 73 years. The GBS was isolated only in the vagina from vaginal secretions representing 88.66% of the patient samples giving us a prevalence of **37%**. Out of the 57 strains of GBS isolated, 34 were from pregnant women (59.65%) against 23 only in non pregnant women (40.35%).

### 3.2. Frequency of GBS strains

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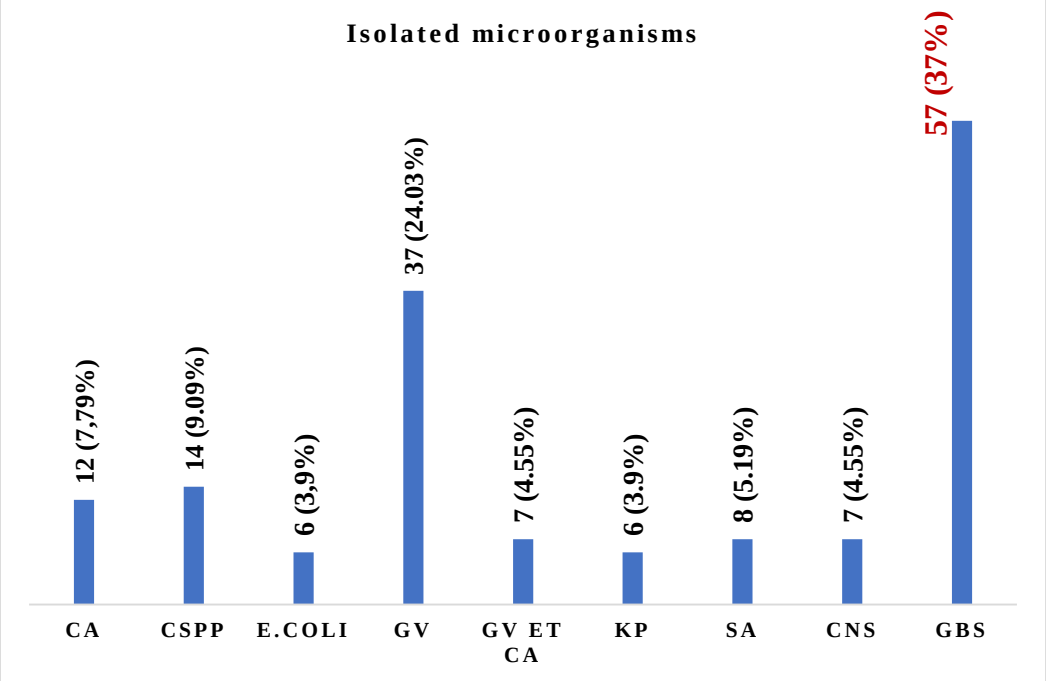


CA : *Candida albicans* CNS : Coagulase negative Staphylococcus

CSPP : *Candida species* E. coli : *Escherichia coli* GBS : Group B *Streptococcus*

GV : *Gardnerella vaginalis* KP : *Klebsiella pneumoniae* SA : *Staphylococcus aureus*

**Figure 1.** Frequency of the different microorganisms isolated

It emerges from the figure 1 that we had eight isolated germs among which the most presented was *Streptococcus agalactiae* (37%), followed by *Gardnerella vaginalis* with a percentage of 24.03%, then we had 9.09% Candida species, and 7.79% *Candida albicans* and *Staphylococcus aureus* (5.19%), *Escherichia coli*, *Klebsiella pneumoniae* 3.9% each... We had six (3.9%) co-infections between *Gardnerella vaginalis* and *Candida albicans*.

### 3.3. Antibiotic resistance profile of *Streptococcus agalactiae*

**Table 2.** Antibiotic resistance profile of the isolates of Group B *Streptococcus*

| Antibiotics | Effectifs (n)/57 | Percentages (%) |
|---|---|---|
| **Penicillin G** | 34 | 58.8% |
| **Oxacilline** | 34 | 58.8% |
| **Amoxicillin** | 40 | 70.6% |
| **Ceftazidime** | 27 | 47.1% |
| **Vancomycin** | 34 | 58.8% |
| **Gentamycin** | 27 | 47.1% |
| **Streptomycin** | 50 | 88.2% |
| **Erythromycin** | 27 | 47.1% |
| **Clindamycin** | 37 | 64.7% |
| **Tetracyclin** | 50 | 88.2% |
| **Doxycyclin** | 57 | 100% |
| **Chloramphenicol** | 50 | 88.2% |
| **Norfloxacin** | 17 | 29.4% |

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| | | |
|---|---|---|
| **Levofloxacin** | 10 | 17.6% |
| **Cotrimoxazol** | 57 | 100% |
| **Bacitracin** | 57 | 100% |

Of the 57 strains of GBS isolated, all were antibiotic resistant (Table 2). 34 (58.8%) were resistant to Penicillin G, and 40 (70.6%) to amoxicillin, 50 (88.2%) to streptomycin and chloramphenicol. The cyclin family was the most resistant with 100%, and 88.2% resistant to doxycycline and tetracycline. The least resistant family, the fluoroquinolones (levofloxacin: 17.6% and 29.4% to norfloxacin) was also observed.

### 3.4. Resistance phenotypes

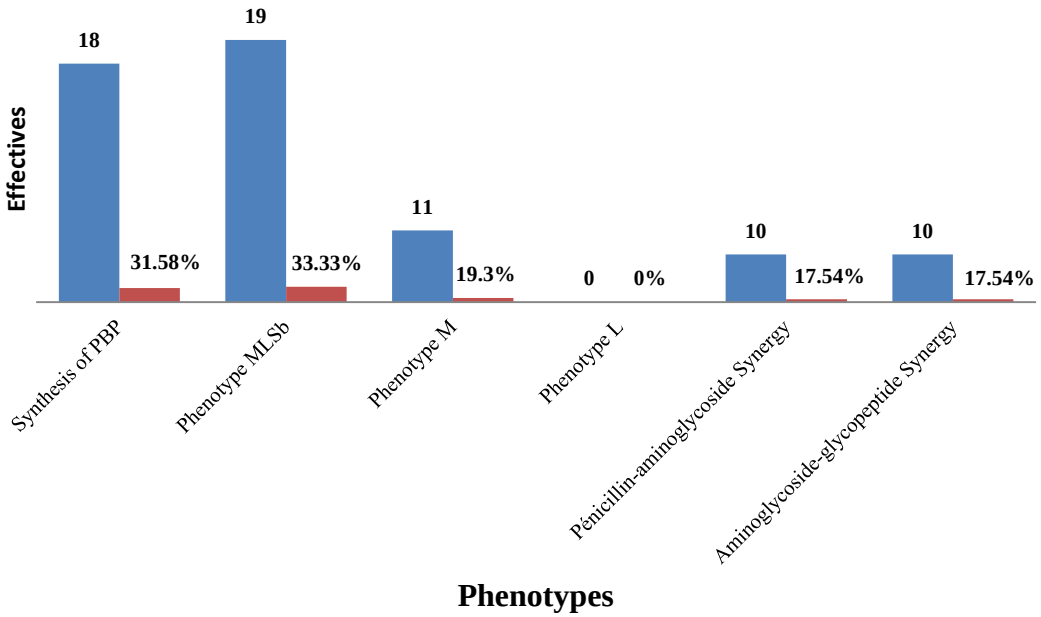


**Figure 2.** Resistance phenotypes and Synergies

We note that the resistance phenotypes of the Macrolide-Lincosamide-Streptogramine family were represented by the cMLSB type with 33.33%, and M (19.3%); the synthesis of Penicillin Binding Protein (31.58%) and synergies were observed from the antibiogram (Figure 2).

### 3.5. Risk factors associated to GBS infection in women

Our analyses showed that 39.02% of pregnant women with a gestational age between 27-31 weeks were GBS carriers compared to only 31.82% for those between 32-36 weeks and 40.74% for those between 37-41 weeks of pregnancy. This difference was statistically significant (P-value=0.025). The difference in prevalence of GBS in pregnant and non-pregnant women was also significant (P-value=0.019) (Table 3).

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**Table 3**. Risk factors of GBS infection in women

| VARIABLE | | GBS | | TOTAL | P-value |
|---|---|---|---|---|---|
| | | NO | YES | n (%) | |
| **Pregnancy** | NO | 50 (94.33%) | 23 (40.35%) | 73 (44.79%) | **0.019** |
| | YES | 56 (80%) | 34 (59.65%) | 90 (55.21%) | |
| **Gestational age** (in weeks) | [27-31] | 25 (73.5%) | 16 (39.02%) | 41 (45.56%) | **0.025** |
| | [32-36] | 15 (93.8%) | 7 (31.82%) | 22 (24.44%) | |
| | [37-41] | 16 (80.0%) | 11 (40.74%) | 27 (30%) | |
| **Age** (in years) | [17-25] | 27 (81.81%) | 17 (41.46%) | 41 (25.15%) | 0.89 |
| | [26-34] | 43 (87.75%) | 14 (24.56%) | 57 (34.97%) | |
| | [35-43] | 21 (87.5%) | 11 (34.38%) | 32 (19.63%) | |
| | [44-52] | 11 (91.66%) | 9 (45%) | 20 (12.27%) | |
| | 53 AND ABOVE | 4 (80%) | 9 (69.23%) | 13 (7.98%) | |

*P-value is significant if < 0.05*

## 4. Discussion

The objective of our study was to determine the frequency and sensitivity of GBS strains. A total of 163 patients were recruited and all samples collected were inoculated on specific media and identified by the latex agglutination test.

The most represented microorganism in our study was GBS (37%) followed by *Gardnerella vaginalis* (24.03%), with *Candida albicans* (7.79%) coming only fourth after *Candida spp*. (9.09%). These results differ from those reported by Chatté *et al*, in 2014 where *Candida albicans* (45.16%) was the most represented germ [10] and those of Nkembe *et al*, in 2018 where *Candida albicans, Gardnerella vaginalis* and *Candida spp*. with frequencies of 22%, 18% and 16% respectively [9]. This can be explained by an improved intimate hygiene but with an increase in sexual partners resulting in an increase in the prevalence of certain germs.

The prevalence of GBS was 37% which is consistent with that reported by Nkembe *et al*, 2018 in Cameroon, and Ali in Ethiopia who had 14% and 13.2% respectively [9, 12] and slightly lower than that of vinnemier in Ghana at 19.1% [13]. In our study, the carriage rate was 40.35% among non-pregnant women and 59.65% among pregnant women. This rate among pregnant women is higher than those reported in Cameroon and Ethiopia 21.2% and 25.5% [14, 15]. This difference can be explained by the fact that the prevalence of GBS infection varies according to the region in the world and testifies to the evolution of GBS infection in pregnant women in Cameroon.

Resistance of *Streptococcus agalactiae* to penicillin, amoxicillin, vancomycin, clindamycin and other tested antibiotics is observed in the current study. This observation may help to alert the relevant organisms to minimise empirical therapy and establish

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antimicrobial management in the study area. Similarly, the reported penicillin resistance patterns are different between studies. Some studies in Cameroon found 100% resistance to penicillin [15] while others in the same country showed no resistance to penicillin, ampicillin and/or vancomycin [9, 10]. This can be explained by the fact that the phenomenon of antibiotic resistance is expanding because antibiotics are nowadays used in all sectors of activity (livestock, agriculture, etc.).

GBS isolates showed resistance to erythromycin and clindamycin in our study. This could reduce the possibilities of prophylaxis in pregnant women allergic to penicillin. Studies in Cameroon showed that the strains were sensitive to these molecules, but one was intermediate at 6% to erythromycin [9]. A study in China reported 69.4% and 47.2% resistance to erythromycin and clindamycin [16]; another in Ethiopia reported 26.5% and 21.4% [14] These reports are in accordance with the results of our study.

The highest rate of antibiotic resistance was observed for the cyclin family in our study (88.2% and 100% resistance for tetracycline and doxycycline respectively). Similar resistance rates for tetracycline were reported in Ethiopia (73.4%) [14]; Tunisia (97.3%) [17]. The resistance rates to tetracycline, ceftriaxone, erythromycin and clindamycin observed in our study could be due to the widespread use of these antibiotics for different clinical cases, which could lead to the emergence of antibiotic-resistant GBS.

A phenotypic analysis of the 57 GBS resistant/intermediate to erythromycin and/or clindamycin in our study revealed that 33.33% of them contained cMLSB phenotypes and 19.3% M phenotypes. Among the erythromycin and/or clindamycin-resistant isolates analysed in Ethiopia 30.6% had an L phenotype, 28.3% of them M phenotypes, 26.1% of the cMLSB and 15.2% of the iMLSB [14]. A Tunisian report also showed that among erythromycin-resistant isolates, 78.7%, 10% and 2.2% had cMLSB, iMLSB and M-phenotypes respectively [17]. A study in the United States found that 8% of patients had a positive D-test, indicating inducible resistance to clindamycin [18] and another study in Germany demonstrated 100% resistance to the M-phenotype [19]. It is believed that differences in antibiotic use, the practice of prophylaxis, the widespread and indiscriminate use of antibiotics in various clinical cases, variation in susceptibility testing methods and/or disparities in the distribution of serotypes may lead to regional differences in GBS resistance rates to different antibiotics.

Knowledge of the risk factors associated with maternal colonization is useful in reducing morbidity and mortality from GBS diseases. This study has shown that pregnancy (P-value= 0.019) and gestational age (P-value= 0.025) are associated with maternal colonisation as reported in other studies, for example in Tunisia only [20], the latter also showed an association with age, which was not the case in our study.

## 5. Conclusions

This section is not mandatory but can be added to the manuscript if the discussion is unusually long or complex. The present study showed a higher prevalence of maternal GBS colonisation compared to previous Cameroonian studies. Pregnancy and gestational age were found to be the risk factors for maternal colonization. GBS was found to be resistant to antimicrobials, penicillin, amoxicillin, vancomycin, ceftazidime and other

antimicrobials tested. GBS with constitutive resistance to clindamycin have been identified. In addition, GBS-containing M-phenotypes were detected. Based on these results, GBS screening of pregnant women at the end of the third trimester of pregnancy, pre-prescription antibiotic susceptibility testing, intrapartum antibiotic prophylaxis and large-scale epidemiological studies should be implemented in the study area.

**Author Contributions:** ICD conceived the project and designed the study. ICD and searched relevant literature, scrutinized all relevant information and draft the manuscript. ICD and conducted and coordinated the field study. PDDD, RY, DE, collected and processed the samples and data. PDDD, RKW and RY analyzed the data and wrote the article. All authors provided additional information. RKW and DDPD further analyzed the data. DIC, RKW and TA revised the manuscript. All authors read and approved the final manuscript.

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**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Human Health Research ethics committee of the Yaoundé Gynaeco-Obstetric and Paediatric Hospital (Authorization N°1124/CIERSH/DM/2020).

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** All data generated or analysed in the course of this study are included in this manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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