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# Genotype group B Streptococcus in the newborn in Abidjan (Ivory Coast)

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**Abstract**
Group B Streptococcus is a pathogen commonly responsible for maternal-fetal and neonatal infections. It is the leading cause of death of newborn bacterial infection. The aim of a present study was to identify the capsular types of Group B Streptococcus circulating in Abidjan from newborns gastric fluids. The study included 522 newborns in the delivery room from November 2012 to May 2013 in four general hospitals and a clinic in Abidjan. Only newborns with a maternal infectious context according to National Agency for Accreditation and Health Evaluation were included in study. Capsular types were determined by Polymerase Chain Reaction targeting the types Ia, Ib, II to VIII from gastric fluids. Polymerase Chain Reaction confirmed the presence of Group B Streptococcus in twenty two gastric fluids 4.2% (22/522). Capsular type V was the most common 11/22 (50%). The other capsular types were, in order of frequency the type Ia 13.6% (3/22), type IV with 13.6% (3/22), type II, with 9.1% (2/22) and type III with 9.1% (2/22). One gastric fluid could not be typed. Capsular type V was mostly in the gastric fluids of newborns and the dominant risk factor was stained amniotic fluid.

**Keywords:** Group B Streptococcus, genotype, newborns, Ivory Coast

## 1. Introduction
Group B streptococcus (GBS) is a pathogen commonly responsible for maternal-fetal and neonatal infections[1]. This bacterium is the leading cause of death of newborn bacterial infection such as pneumonia, sepsis and meningitis. Transmission from mother to newborn is aero digestive and occurs after rupture of membranes during passage through the birth canal by inhalation and ingestion of contaminated amniotic fluid. On average, 50% of infants born to carrier mothers are colonized at birth [2, 3, 4, 5, 6]. There are ten distinct polysaccharide capsular types known to date; types Ia, Ib, II to IX. The most dominant are types Ia, III, V and most of cause of neonatal infections [7, 8, 9, 10]. Capsular types circulating in Africa are poorly known. A study in The Gambia reported scarcity of GBS infections in newborn and a low prevalence of serotype III in maternal carriage [11]. However, in developed countries, especially in Europe and the United States, capsular types circulating are well known and make subject of much research, especially in the context of the development of vaccine for prevention of GBS. Internationally, routine screening by maternal vaginal sampling is performed between the 34th and 38th weeks of gestation for prevention of neonatal GBS disease[12]. National Agency for Accreditation and Health Evaluation also recommends pluriorificial and gastric samples in GBS screening in neonates [13, 14, 15, 16]. Many studies are underway for the development of combination vaccines with wall polysaccharides and GBS surface proteins [17, 18, 19]. In Ivory Coast, there is no data on the capsular types that are circulating. However, to benefit from a possible vaccine and optimize preventive strategy in reducing neonatal infection associated with GBS, capsular types circulating in Abidjan should be known. The purpose of this study was to identify the capsular types of GBS circulating from newborns gastric fluids.

## 2. Materials and methods
### 2.1 Materials
Five hundred twenty-two newborns of four hospitals and urban health center in Abidjan were collected over a period of eight months extending from November 2012 to May 2013. Aspiration of gastric fluid was performed immediately after birth and before cleaning the newborn. Gastric fluid was transferred to the Microbiology laboratory on time delivery and packaging. Newborn of all gender were included in our study. They were preterm, term or post-term. Maternal infection risks for inclusion newborn were: urinary tract infection or vaginal vulva during pregnancy, fever a few days before or during birth, premature rupture of membranes (PRM) greater than 12 h, stained amniotic fluid and / or foul, fetid vaginal and/ or purulent. Written consent was given by mothers before any removal. A plug for collected socio demographic, anamnestic and clinical data accompanied all samples. Table (1) summarized the primers sequences (EUROBIO® France) and the expected size of Polymerase Chain Reaction (PCR) products.

**Table 1:** PCR primers for amplification of DLTS and capsular types sequences [21].

| | Primer | size (bp) |
|---|---|---|
| specific PCR | dltS-F AGGAATACCAGGCGATGAACCGAT<br>dltS-R TGCTAATTCTCCCCTTATGGC | 952 |
| multiplex PCR 1 | Ia-F GGTCAGATGGATTAATGGTATGC<br>Ia-R GTAGAAATAGCCTATATACGTTGAATGC | 521 et 1826 |
| | Ib-F TAAACGAGAATGGAATATCACAAACC<br>Ib-R GAATTAACTTCAATCCCTAAACAATATCG | 770 |
| | II-F GCTTCAGTAAGTATTGTAAGACGATAG<br>II-R TTCTCTAGGAAATCAAATAATTCTATAGGG | 397 |
| | III-F TCCGTACTACAACAGACTCATCC<br>III-R AGTAACCGTCCATACATTCTATAAGC | 1826 |
| | IV-F GGTGGTAATCCTAAGAGTGAACTGT<br>IV-R CCTCCCCAATTTCGTCCATAATGGT | 578 |
| multiplex PCR 2 | V-F GAGGCCAATCAGTTGCACGTAA<br>V-R AACCTTCTCCTTCACACTAATCCT | 701 |
| | VI-F GGACTTGAGATGGCAGAAGGTGAA<br>VI-R CTGTCGGACTATCCTGATGAATCTC | 487 |
| | VII-F CCTGGAGAGAACAATGTCCAGAT<br>VII-R GCTGGTCGTGATTTCTACACA | 371 |
| | VIII-F AGGTCAACCACTATATAGCGA<br>VIII-R TCTTCAAATTCCGCTGACTT | 282 |

## 2.2 Methods

### DNA extraction

DNA was isolated by the phenol-chloroform method. 500 µl of gastric fluid were precipitated and suspended in 400 µl of lysis buffer (20 mM Tris, EDTA 2 mM, 150 mM NaCl, 10% SDS and Proteinase K 100 µg / ml) and incubated for 1 hour at 60 ℃. 600 µl of phenol-chloroform (24/1, v/v of phenol and chloroform) allowed to precipitate the DNA visible in the form of pellet after adding iced ethanol 70%. The pellet storage was at -20°C in 50 µl of Biomerieux elution buffer.

### Capsular type's genotyping

The first PCR had targeted the DLTS gene in all strains of GBS. The primers used were served for the detection of ST-17 clone which amplify the variant GBS 2018 gene encoding a surface protein [21, 22]. PCR was performed in a final volume of 50 µl containing 5 µl of DNA extract, 1.5 µl of each primer 10 mM, 3µl of MgCl2 25 mM, 1.5 µl of dNTPs 10 mM, and 5 µl of each colored uncolored buffer 5X, 27.3 µl water for preparation and 0.2 µl of Taq DNA polymerase. After heating at 94°C for 2 min, amplification was performed over 30 cycles of : 94°C for 30 s, 57°C for 45 s, 72°C for 1.15 min and 72°C for 5 min. Amplification involved 30 cycles. Each cycle consisting of a denaturation step of 2 min and 30 s at 94 ° C, elongation step of 30 s at 57 ° C and assembly phase of 1.15 min and then 5 min at 72 ° C. Multiplex PCR were conducted to gastric fluids that were identified in GBS-specific PCR as described by Poyart et al. (2007). PCR was performed in a final volume of 50 µl containing 10 µl of DNA extracted, 1µl of each primer 10mM, and 2µl dNTPs 10 mM, 3.5µl MgCl2 25 mM, 5 ml of each colored and uncolored buffer 5X, 14.3 µl of water for injection and 0.2 µl of Taq DNA polymerase. after heating at 94°C for 5 min, amplification was performed over 30 cycles of : 94°C for 30 s, 57°C for 45 s, 72°C for 1.30 min and 72°C for 7 min. The PCR products were resolved in 1.5% agarose gel stained with ethidium bromide and visualized under ultraviolet light [23, 24].

## 3. Results

### Prevalence of risk factors

The factors of neonatal infections most frequently found were stained amniotic fluid, amniotic fluid foul and premature rupture of membranes for more than 12 hours. The stained amniotic fluid was the dominant (Table 2).

**Table 2:** Distribution of infectious risk criteria

| Infectious criteria | Effective N=522 | Percentage (%) |
|---|---|---|
| Bladder or vulvo vaginal infection | 28 | 5.4 |
| Fever before or during childbirth | 49 | 9.4 |
| PRM >12 | 93 | 17.8 |
| Stained amniotic fluid | 522 | 100 |
| Foul amniotic fluid | 209 | 40.1 |
| Fetid and/or purulent vaginal | 123 | 23.6 |

PRM: premature rupture of the membrane

### Identification of GBS capsular types in gastric fluids by PCR

Twenty two strains were identified by the GBS-specific PCR. The DLTS gene encoding a specific surface protein in all GBS strains was amplified and has a size of 952 bp (Fig 1). 1 multiplex PCR showed three different capsular types of Streptococcus group B. These are the types Ia, II, III and IV. Type Ib have not been found (Fig 2). Multiplex PCR 2 revealed a single type capsule V. VI, VII and VIII were not found (Fig 3).

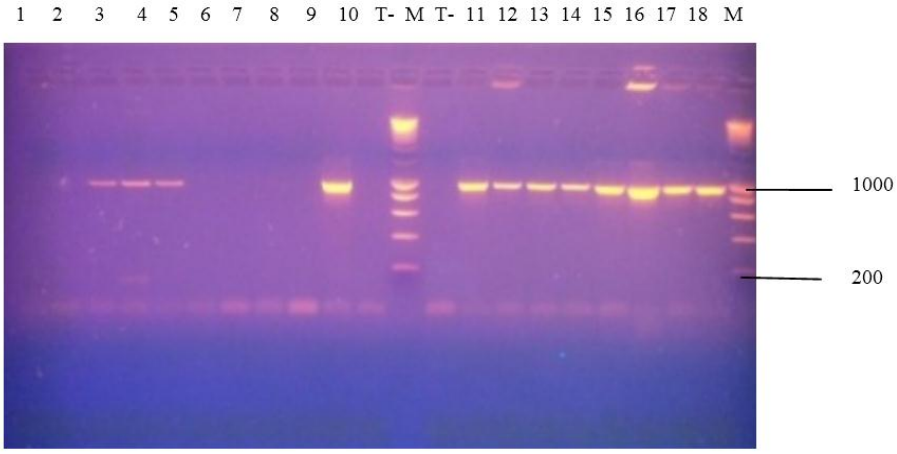


**Fig 1:** GBS-specfic PCR Lane M : 200 bp marker. Lane T- : negative control. Lane 3 to 5, 10, 11 to 18 : fragments DLTS positive (952pb). Lane 2, 6 to 9: fragments DLTS negative.( Numbers to the right are in base pairs)

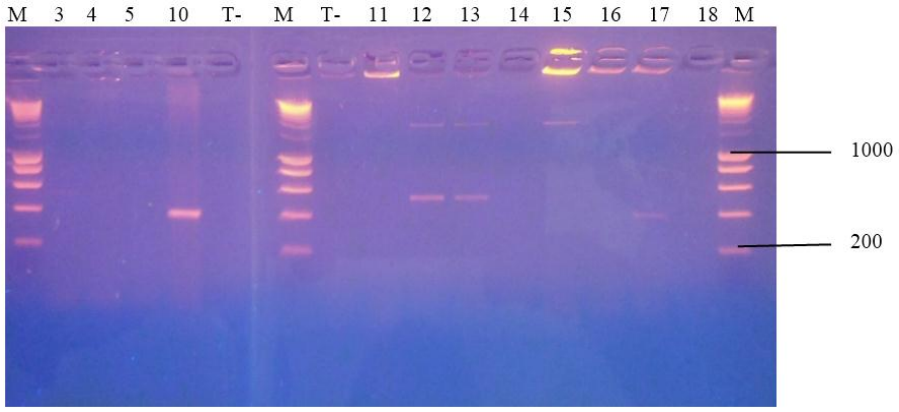


**Fig 2:** Multiplex PCR for detection of capsular types Ia, Ib, II and IV. Lane M : 200 bp marker. Lane T- : negative control. Lane 10 and 17 : Type II positive strains. Lane 12 and 13: Type Ia positive strains. Lane 15: Type III positive strain. Lane 3: Type IV positive strain. Lane 4, 5, 11, 16,18: GBS negative strains.

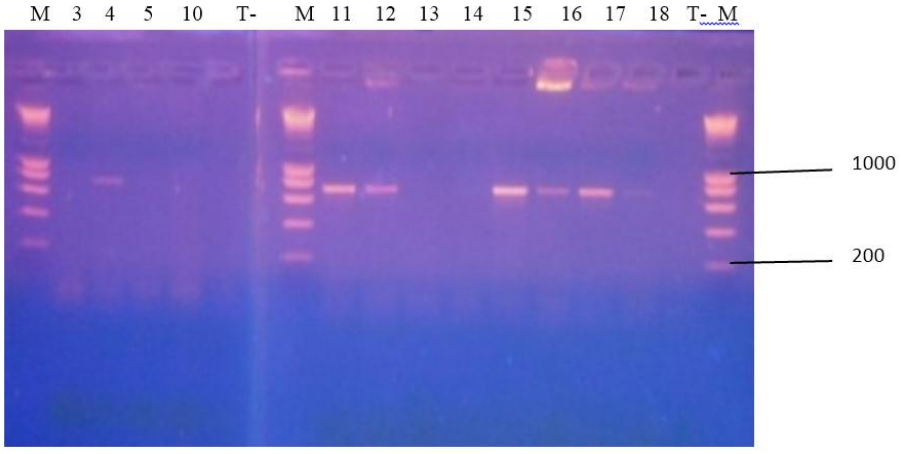


**Fig 3:** Multiplex PCR for detection of capsular types V, VI, VII and VIII. Lane M : 200 bp marker. Lane T- : negative control. Lane 4, 11, 12, 15 to 18 : Type V positive strains. Lane 3, 5, 10, 13, 14: GBS negative strains.

**Determination of capsular types**

The rate obtained on the basis of the positive GBS gastric fluids collected from newborns globally was 4.2% (22/522). Types Ia, IV and V were the most frequent. Type V was predominant with 50%, the types Ia and IV with 13.6% each. Types II and III were rare with 9.1% each. Types Ib, VI, VII, VIII were absent (Table 3).

**Table 3:** Different types of polysaccharide capsules

| Capsular types | Number of GBS | Percentage (%) |
|---|---|---|
| Ia | 3 | 13.6 |
| Ib | — | — |
| II | 2 | 9.1 |
| III | 2 | 9.1 |
| IV | 3 | 13.6 |
| V | 11 | 50 |
| VI | — | — |
| VII | — | — |
| VIII | — | — |
| UT | 1 | 4.5 |
| TOTAL | 22 | 100 |

UT: untyped

## 4. Discussion

The maternal-fetal infections have become a major public health problem with a prevalence of infections with GBS[21]. The overall attack rate of GBS sepsis in studies conducted in the United States is 1.7 per 1,000, only country for which epidemiological data are regularly established. Published studies show that 46% of invasive infections due to GBS occur during the first month of life[25,26]. This study targeted newborns in the delivery room for the development of symptomatic infection (2% of cases) occurs most often (80% of cases) in the first 24 hours of life (early syndrome). In indeed, the contaminated amniotic fluid ingested or inhaled at birth is found in the child's stomach and hence interest to collect gastric fluid.

GBS identification rate in gastric fluid of infants in our study was 4.2%. The important contamination of the gastric fluid is compared with a higher likelihood of infections. The isolation of GBS strains in gastric fluid of the newborn at birth has a high predictive value of infection in newborns according to ANAES 2002[12]. It's why all newborns in whom gastric fluid showed the presence of GBS will require a special medical supervision because of the risk of the occurrence of diseases called early or late onset. Note also that ANAES recommends analysis of gastric fluid and two peripheral samples (ear and another site choice) for better diagnostic performance.

Infectious risk factors in newborns were dominated by stained amniotic fluid. It's a major risk factor especially as expressed fetal distress whether mechanical or infectious. When amniotic fluid is infected, the risk of neonatal infection is 1 to 5%. Indicating a high risk for children included in the study since all had this risk factor. This risk will be increased for children who SGB will be identified in their gastric fluid. And our results are similar to those of Gérardin et al, 2008 were as dominant risk factor associated with the stained amniotic fluid and / or foul[27].

This study also shows that different capsular types circulating in Ivory Coast are the types Ia, II, III, IV and V with a preponderance of the type V. The primers used have not identified a capsular type that is probably the type IX recently discovered or a untyped that is not yet discovered[28]. These results are congruent with those of Jordan et al, 2008 in the United States and Fluegge et al, 2005 in Europe which have shown that the capsular types III, V and Ia are most commonly found in invasive disease of the newborn[29,30]. Furthermore, the publication of a meta-analysis of the global geographic distribution of GBS serotypes showing that serotype III was predominant in Europe, Africa, Australia and Asia, suggests a mismatch in results[31]. Diversification of samples could have according our results to others. As recommended ANAES, two others devices levies of ear and anus could have been incurred in addition to gastric fluid. This would permit to have a variety of samples and a significant number of capsular types to identify.

## 5. Conclusion

Five capsular types have been identified, these are the types Ia, II, III, IV and V the predominant type is the type V (50%). It appears that after this study, genotypic mapping was carried out and that database would be used for other studies on GBS therapy or prevention, which would significantly reduce the incidence of early-onset disease or late in Ivory Coast.

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