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Molecular characterization of high-risk infection vaginal bacteria isolated from pregnant women in CHU-MEL of Cotonou (Benin)

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The aim of this study is to determine the distribution of the genotypic pathogenicity traits of vaginal high-risk infectious bacteria (HRIB) collected in the CHU-MEL of Cotonou (Benin). To achieve this, a recto-vaginal swab of 42 pregnant women in the third trimester of pregnancy was collected. Species identification was carried out by specific biochemical tests. Antimicrobial susceptibility was tested according to the microbiology standard recommendation. Macrolide resistance genes in Gram-positive bacteria and virulence genes in *Escherichia coli* were investigated by polymerase chain reaction (PCR). *E. coli* is the most isolated species (14.7%) followed by *Klebsiella pneumoniae* (11.8%). Mono-microbial carriage was 55.9%. Gram-negative antibiotic susceptibility shows strong resistance to beta lactam. While Gram-positive bacteria showed strong resistance to beta-lactamine, tetracycline and macrolides with $cMLS_B$ (70.4%), $iMLS_B$ (3.7%) and M (25.9%) phenotypes. *ErmB* and *ermTR* were not detected in Gram-positive bacteria but *mef(A/E)* was detected at a high. Virulence genes in *E. coli* were detected and *fimA* was the most common (52.2%) followed by *sfa/foc* (30.4%) and *cnf1* (13.0%). *NeuC* and *ibeA* have not been detected. The *hvgA* virulence gene was detected in *S. agalactiae* at a rate of 61.54%. These results demonstrate the importance of introducing antenatal screening for HRIB to improve obstetric and neonatal management in Benin.

Key words: Pregnant woman, vaginal swab, neonatal infection, virulence factor.

INTRODUCTION

Maternal-fetal bacterial infection is an infection of the newborn resulting from vertical mother-to-fetal transmission that occurs during the perinatal period (Arora et al., 2017). Recently, many high-income countries have reported maternal mortality ratios of about 10/100,000 births (Kassebaum et al., 2013; Megli and Coyne, 2021). Vaginal bacterial carriage in the last trimester of pregnancy has been identified as the major

cause of these infections (Akbarian et al., 2016; Madrid et al., 2018). It is mainly due to genital colonization by vaginal bacteria with high risk of infection (Denis et al., 2016). These infectious bacterial can either cause the contamination of the amniotic fluid, or contamination of the newborn during vaginal birth (Rani et al., 2014). The pathogenicity of bacteria involved in maternofetal infections is not only linked to the bacterial species but also to the production of some virulence factors (Six et al., 2014). In developed countries, *Streptococcus agalactiae* and *Escherichia coli* are reported to be responsible for the majority of maternal-fetal infections (Akbarian et al., 2016). However, in developing countries, these infections are caused in major part by Enterobacteriaceae and staphylococci but very rarely to *S. agalactiae* because of the scarcity of vaginal carriage (Iregbu et al., 2013; Ogunlesi et al., 2011).

Indeed, epidemiological and experimental studies have clearly established a link between neonatal infection (Kim et al., 1992) and *E. coli* expressing the fimA, sfa/foc, cnf1 genes (Ott et al., 1986) and the ibeA gene product (Germon et al., 2005). These genes have been shown to promote adhesion and invasion through brain microvascular endothelial cells. *E. coli* with the K1 capsular antigen represents the second leading cause of serious maternal-fetal and neonatal infections after group B streptococci (Simonsen et al., 2014). The pathogenic determinants of *E. coli* meningitis identified are so far mainly surface structures and adhesins. Concerning streptococci, *S. agalactiae* (ST17) have been strongly associated with late neonatal infection (Poyart et al., 2008). These hyper-virulent strains belonging to the clonal complex 17, (CC-17), were defined by the Multi-Locus Sequence Typing method (Poyart et al., 2008; Teatero et al., 2016). *S. agalactiae* (ST17) have specific virulence factors such as adhesins (*Srr*2 and *hvg*A) and a pilus (PI-2b) that may explain their hyper-pathogenicity in newborns and their tropism for the central nervous system (Six et al., 2014).

To address the risk of transmission of maternal-fetal infection, antibiotic therapy for women carrying *S. agalactiae* has been introduced, but its implementation remains a problem (Saizonou et al., 2014). This antibiotic therapy consists of the use of penicillin and aminoglycosides and in case of penicillin allergy, macrolides are indicated (WHO, 2016). However, we are witnessing an emergence of resistance to most of the antimicrobial molecules (Isaacs, 2005; Hays et al., 2016; Srinivasan et al., 2014). The emergence of resistance mechanism to macrolides that involves the *erm* and *mef* genes has been reported (Da Cunha et al., 2014; Hays et al., 2016; Metcalf et al., 2017); although this family constitutes the third critically important in human medicine (WHO, 2019).

Several studies, in Benin, have been carried out on the detection of genes for resistance to antibiotics, in particular to beta-lactams (Anago et al., 2015; Moussé et al., 2019). However, there is few data on the distribution of genes encoding for the resistance to macrolides and its derivatives. In addition, the level of pathogenicity and resistance to antibiotics of highly pathogenic species such as *E. coli* and *S. agalactiae* are not sufficiently documented in the Beninese context. Thus, the objective of the present study was to determine the distribution of genotypic pathogenicity characters of *E. coli* and *S. agalactiae* isolated in Cotonou (Benin).

MATERIALS AND METHODS

Ethics

A cross-sectional study was carried out with a descriptive and analytical aim. Thus, prospective data were collected from July to November, 2020. The protocol was validated by the National Committee of Ethics for Health Research of Benin. All targeted population of this study gave their free and informed consent.

Study population

The study took place in southern Benin and took into account samples collected from 42 pregnant women at the Lagune University Hospital of Mother and Child (CHU-MEL) in Cotonou, Benin. The samples were collected in the delivery room of the CHU-MEL. The present study took into account all pregnant women whose gestational age ≥29 weeks, presenting for prenatal or gynecological consultation and having given their free consent. Forty-two women (42) pregnant women were selected based on the following criteria:

Inclusion criteria: All pregnant women in good mental and physical health, in their third trimester of amenorrhea, hospitalized in the delivery room of the center during the study period were included.

Non-inclusion criteria: All the women who did not give their consent to participate in the study.

Sampling and samples collection

The samples used in this study were collected from 42 pregnant women in the CHU-MEL, Cotonou (Benin) from July to October 2020. For each sampled pregnant woman, rectovaginal swabs samples were performed by a nurse or midwife as described by Mengist et al. (2016). After passing the sterile swabs over defined areas they were returned to their protective cases. The collected samples are transported using an icebox containing coolers (~ 8°C) to laboratory for microbiological analysis in the parasitology

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laboratory of the CHU-MEL.

Microbiological analyses

Identification of S. agalactiae

The identification of enterococci and streptococci was performed according to the method described by Mohammed et al. (2012). Briefly, the Todd-Hewitt broth was incubated for 24 h at 35 to 37°C then streaked on 5% sheep blood agar and incubated anaerobically for 24 h. All small greyish colonies suspected to be enterococci (with narrow beta-hemolysis) were sub cultured on blood agar and subjected to a Gram control and catalase test. All Gram positive and catalase negative bacteria were subjected to the CAMP test (Guo et al., 2019). Identification of enterococci was confirmed molecularly by detection of the species-specific *dltR* gene.

Identification of other vaginal bacteria with high infectious risk

The MH broth (oxoid) in which the vaginal swab is immersed was incubated for 24 h at 35-37°C. After incubation period, selective media such as Eosin Methylene Blue (to select Gram negative bacteria), Mannitol Salt agar (for staphylococci) and fresh blood agar (for bacteria such as enterococci) were used. Mannitol Salt agar (MSA) and Eosin Methylene Blue (EMB) cultures were incubated at 37°C for 24 h, the fresh blood agar plates were incubated anaerobically under a bell in a hermetically sealed jar to facilitate the growth of enterococci such as previously described by Mugalu et al. (2006).

Identification of Gram-negative bacteria

From the EMB agar, selected colony was deposited on a humidified oxidase disc placed on a slide for 20 to 60 s. The presence of a cytochrome oxidase is manifested by the appearance of a red color turning to dark purple (Shields and Cathcart, 2010). This test was used to differentiate Enterobacteriaceae from *Pseudomonas* species. The LIMINOR gallery and API 20 E gallery were used for the biochemical identification. All positive cultures on EMB, with negative oxidase test were subjected to a Gram control and to the LIMINOR gallery (Kiwanuka et al., 2013). The strains with unclear result on classical gallery and those with negative glucose fermentation were re-isolated on EMB and indication was performed by API 20E gallery (Okinda et al., 2014).

Identification of Staphylococcus aureus

The staphylocoagulase was performed on golden yellow colonies and positive mannitol (Sperber and Tatini, 1975). Briefly, 2-3 identical colonies were inoculated in 1 ml of brain-heart infusion and then incubated for 24 h at 37°C. The tube coagulase test was carried out by adding 0.5 ml of the 24 h preculture in brain-heart infusion to 1.5 ml of rabbit plasma (BioMérieux) in a hemolysis tube. After gentle mixing, the tubes were incubated at 37°C and examined after 2, 4 and 24 h.

Identification of enterococci

All colony types present on fresh blood agar, Gram-positive bacteria, with a diameter of less than 2 µm grouped were suspect genera such as streptococcus and enterococcus. A catalase negative test was used to classify colonies in enterococci. The resistance to bile test and hydrolysis of esculin was used to dissociate the streptococci, the complex *Streptococcus bovis*/ *Streptococcus equinus* and enterococci.

Antibiotic susceptibility of isolates

Disc diffusion method was used to evaluate the antibiotic susceptibility of the isolated strains according to the standards and recommendations of the Antibiogram Committee of the French Society of Microbiology (CASFM, 2020). For the Gram-negative bacteria, the used antibiotics agents (Oxoid®) were amoxicillin + clavulanic acid (AMC 20/10 µg), ceftriaxone (CRO 30 µg), cefoxitin (FOX 30 µg), cefotaxime (CTX 30 µg), piperacillin (PRL 30 µg), ampicillin (AMP 10 µg), gentamicin (CN 10 µg) and imipenem (IPM 10 µg). The antibiotics agents (Oxoid®) used for streptococci and enterococci were cefotaxime (CTX 30 µg), ampicillin (AMP 10 µg), gentamicin (CN 10 µg), tetracycline (TE 30 µg), vancomycin (VA 5 µg), clindamycin (MY 10 µg), erythromycin (E 15 µg) and penicillin G (1 µg).

Molecular characterization of *S. agalactiae* and *E. coli* strains

The molecular cauterization was performed in the Laboratory of Biology and Molecular Typing in Microbiology of the University of Abomey-Calavi. The following steps were used to reach our goal: DNA extraction and classic PCR targeting specific genes.

DNA extraction

DNA extraction was performed according to an adaption of the previously described method by Aranishi et al. (2006). Thus, from fresh bacterial culture (about 18-h old), 3 to 4 colonies were used for a preculture in 1 ml of brain-heart infusion before incubation (37°C for 18 h). The tubes were then centrifuged at 12,000 g for 5 min. The supernatant is discarded and 500 µl of Tris Borane EDTA (TBE 1x) were added to the bacterial pellet then mixed and heated in a dry bath at 95°C for 15 min. After heating, the mixture was centrifuged again at 12000 g for 5 min. The supernatant was recovered into sterile tube and 500 µl of ethanol before another centrifugation at 12,000 g for 5 min. The DNA pellets were suspended in 50 µl of sterile distilled water and stored at 4°C for imminent use or at -20°C for long-term storage.

Virulence and antibiotic resistance genes of S. agalactiae and E. coli

Investigated genetic *E. coli* virulence determinants include *ibeA*, *sfa*/*foc*, *cnf1*, *fimA* genes (Huang and Jong, 2001; Wang and Kim, 2002). The presence of the K1 capsular antigen was sought by targeting the *neuC* gene (Moulin-Schouleur et al., 2006). The species-specific (*dltR*) of *S. agalactiae* and its hyper virulent clones (*hvgA*) were targeted for the species and sequence type 17 (ST-17) identification. Three erythromycin resistance genes (ermB, ermTR and mef A/E) were targeted using a set of specific primers (Bolukaoto et al., 2015; Gygax et al., 2016; Seppälä et al., 1998). The sequences and the expected fragment length were compiled in Table 1. All strains of Gram-positive bacteria phenotypically resistant to erythromycin and/or clindamycin were tested for resistance genes.

For each gene, the reaction was performed in a 20 µl reaction mixture containing 10x buffer (2 µl), dNTP (0.2 µl), 10 µM of each primer (1 µl), $Mgcl_2$ (2 µl), Taq DNA polymerase (0.3 µl) and DNA (3 µl). The amplification program for each targeted gene is presented in the Table 2. The amplification products were migrated on a 1.5% agarose gel containing ethidium bromide at 110 V for 30 min.

Table 1. Characteristics of the primers used for molecular characterization *S. agalactiae* and *E. coli* isolates.

Target gene	Primer	Sequences (5' →3')	Size of amplicon (pb)
ermB	*ermB1* *ermB2*	F: FGAA AAG GTA CTC AAC CAA ATA R: AGT AAC GGT ACT TAA ATT GTT TAC	639
ermTR	*ermTR1* *ermTR2*	F: GAA GTT TAG CTT TCC TAA R: GCT TCA GCA CCT GTC TTA ATT GAT	395
mef(A/E)	*mef(A/E)1* *mef(A/E)2*	F: AGT ATC ATT AAT CAC TAG TGC R: TTC TTC TGG TAC TAA AAG TGG	346
dltR	*dltRS* *dltRAS*	F: TTGACAGGTCTCTATGATTTAGTC R: GTCTGGTTCTCAGCCTAATTC	234
hvgA	*ST-17S* *ST-17AS*	F: ATACAAATTCTGCTGACTACCG R: TTAAATCCTTCCTGACCATTCC	210
neuC	*neuCF* *neuCR*	F: AGGTGAAAAGCCTGGTAGTGTG R: GGTGGTACATCCCGGGATGTC	676
FimA	*Fim AF* *FimAR*	F: CGGCTCTGTCCCTSAGT R: GTCGCATCCGCATTAGC	500
ibeA	*Ibe AF* *ibeAR*	F: TGAACGTTTCGGTTGTTTTG R: TGTTCAAATCCTGGCTGGAA	814
cnf1	*cnf1F* *cnf1R*	F: GGGGGAAGTACAGAAGAATTA R: TTGCCGTCCACTCTCACCAGT	1111
sfa/foc	*sfa/focF* *sfa/focR*	F: 5'-CTCCGGAGAACTGGGTGCATCTTAC R: CGGAGGAGTAATTACAAACCTGGCA	401

ermB: Gene encoding for macrolide-lincosamide-streptogramin B resistance protein, *ermTR*: gene encoding for erythromycin resistance methylase, *mef(A/E)*: gene encoding for macrolide resistance A and E, *dltR*: gene encoding for transcriptional regulatory protein, *hvgA*: gene encoding for hypervirulent GBS adhesin , *neuC: UDP-N-acetylglucosamine 2-epimerase*, *FimA: gene* encoding *for Type-1 fimbrial protein, A chain*, *ibeA*: gene encoding for Invasion protein , *cnf1*: gene encoding for Cytotoxic necrotizing factor 1, *sfa/foc*: Gene clusters for S fimbrial adhesin (sfa) and F1C fimbriae (foc).

Data analysis and processing

The data were entered into the Excel 2013 spreadsheet and analyzed by SPSS version 22 software. The chi-square test was used to assess relationships between species, antibiotics resistance and between genes. The difference was considered statistically significant for $p < 0.05$. GraphPad Prim 8 software was used graphing.

RESULTS

Distribution of pregnant women by age

The distribution of pregnant sampled women according to their age is shown in Figure 1. Thus, the average age of targeted women is 28 years. However, the women are between 18 and 45 years old and the most represented ages are between 20 and 35 years old.

Frequency of mono-microbial and poly-microbial carriage

The rate of mono-microbial carriage of bacteria is presented in Table 3. The overall frequency of mono-microbial vaginal carriage of species isolated from vaginal samples (*E. coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Klebsiella rhinoscleromatis*, *Pseudomonas* spp., Coagulase Negative Staphylococci (CNS), *S. aureus*, *S. agalactiae*, *Streptococcus* species

Table 2. PCR condition for each of the amplified genes.

Amplification steps	Temperature/Time				
	fia/ibeA	***cnf1***	***neuC/ sfa-foc***	***ermB/erm TR/ mef(A/E)***	***dltRS/hvgA***
Initial denaturation	94°C/3 min	94°C/5 min	94°C/3 min	95°C/3 min	95°C/10 min
Denaturation	94°C/1 min	94°C /1 min	94°C/1 min	95°C/1 min	95°C/10 s
Annealing	52°C/1 min	55 °C/1 min	56°C/1 min	57°C/1 min	55°C/5 s
Elongation	72°C/1 min	72°C /30 s	72°C/1 min	72°C/1 min	72°C/10 s
Final elongation	72°C/10 min	72°C/10 min	72°C/10 min	72°C/10 min	72°C/10 min
Number of cycles	30	30	30	35	40

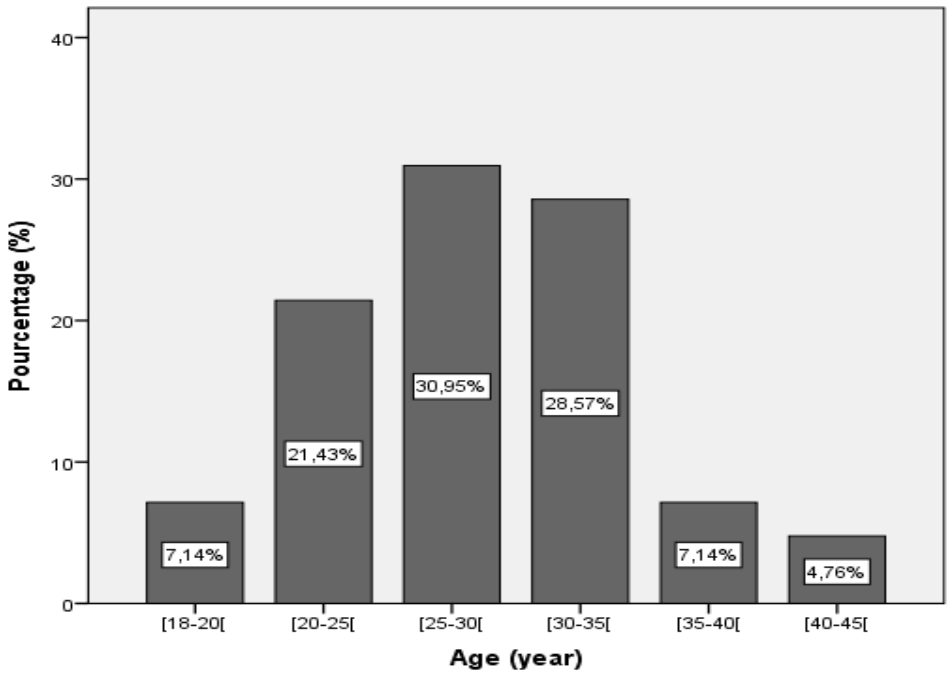

Figure 1. Pregnant women by age group.

and *Enterococci*) is 55.9% while the recorded frequency is 44.1% in poly-microbial. *E. coli* is found as a mono-microbial (Table 3) in 14.7% of cases, followed by *K. pneumoniae* (11.8%), *S. agalactiae*, *Streptococcus* spp., and enterococci (5.9%), Coagulase Negative Staphylococci and *S. aureus* (2.9%).

The poly-microbial carriage rate is presented in Table 4. *E. coli* is found in association with other bacteria in 32.34% of cases against 11.76% for *S. agalactiae*, 8.82% for *K. rhinocleromatis* and 5.88% for *S. aureus*.

Antibiotic resistance profile of Gram-negative bacteria

Figure 2 shows the antibiotic resistance profile of Gram-negative bacteria. Globally, there is a high rate of resistance of Gram-negative bacteria to ampicillin. *E. coli* showed a low level of resistance to cephalosporins with the exception of cephotaxime (91.3%). There is also a low resistance rate (30.4%) of *E. coli* to imipenem. *K. oxytoca* did not show any resistance to the tested antibiotics apart from ampicillin. On the other hand, *K. pneumoniae* (9.1%) and *K. rhinoscleromatis* (25%) showed variable resistance rates to cephalosporins and imipenem. *Pseudomonas* spp. shows resistant to all cephalosporins but sensitive to penicillin, in particular to piperacillin.

Resistance profile of Gram-positive bacteria to antibiotics

Figure 3 shows the resistance profile of the antibiotics

Table 3. Frequency of monomicrobial carriage of Gram-positive and negative bacteria.

Bateria	Bacterial species	Proportion (%)
Gram positive	*S. agalactiae*	5.9
	S. aureus	2.9
	CNS	2.9
	Streptococcus spp.	5.9
	Enterococci	5.9
Gram negative	*E. coli*	14.7
	Klebsiella pneumoniae	11.8
	Klebsiella rhinocleromatis	2.94
	Pseudomonas spp.	2.94
	Total	**55.9**

Table 4. Frequency of polymicrobial vaginal carriage.

Bacterial species	Proportions (%)
E. coli + *K. oxytoca*	2.94
E. coli + *K. pneumoniae*	5.88
E. coli + *S. agalactiae*	5.88
E. coli + *S. aureus*	2.94
E. coli + *Streptococcus* spp.	2.94
E. coli + *Enterococcus*	2.94
E. coli + *K. oxytoca* + *K. pneumoniae*	2.94
K. pneumoniae + *K. rhinocleromatis*	2.94
K. pneumoniae + *S. aureus*	2.94
E. coli + *K. rhinocleromatis*	2.94
E. coli + *K. rhinocleromatis* + *K. pneumoniae*	2.94
CNS + *S. agalactiae*	2.94
CNS + *S. agalactiae* + *Streptococcus* spp.	2.94
Total	44.10

tested on Gram positive bacteria. A high level of resistance is observed for betalactamines (cefotaxime, penicillin and ampicillin) and for Macrolides and derivatives (Macrolide-Lincosamide-Streptogamine). For erythromycin, 100% resistance was observed in enterococci and CNS, while high resistance rates were observed for *S. aureus* (75%) and *S. agalactiae* (76.9%). Among the isolated bacteria, only one strain of *S. agalactiae* (7.7%) was resistant to gentamycin. Vancomycin shows high activity against all *Staphylococcus* whereas enterococci (60%) and *S. agalactiae* (15.4%) show variable a resistance rate. High resistance rate was recorded with tetracycline on all isolated species.

Table 5 shows the distribution of cMLSB, iMLSB and M phenotypes within the species isolated. All isolated species were resistant to erythromycin and/or clindamycin. Inaddition, 100% of the enterococcus were of cMLSB phenotypes; 70% of GBS were of cMLSB phenotype and 30% of phenotype M. A single strain (CN).

Distribution of virulence genes of *E. coli* involved in maternofetal pathology

Figure 4 shows the frequency of *E. coli* virulence genes isolated from pregnant women. Thus, 52.2% of isolates harbor *fim*A, whereas *sfa*/*foc* (30.4%) and *cnf1* (13.0%) were detect at different rate. The *neu*C and *ibe*A genes were not detected.

Distribution of MLSB resistance genes

In the Table 6, none of the *erm* genes (*erm*B and *erm*TR) were detected among species showing resistance to erythromycin and/or clindamycin. However, a high level of *mef* (A/E) is detected in all Gram-positive bacteria.

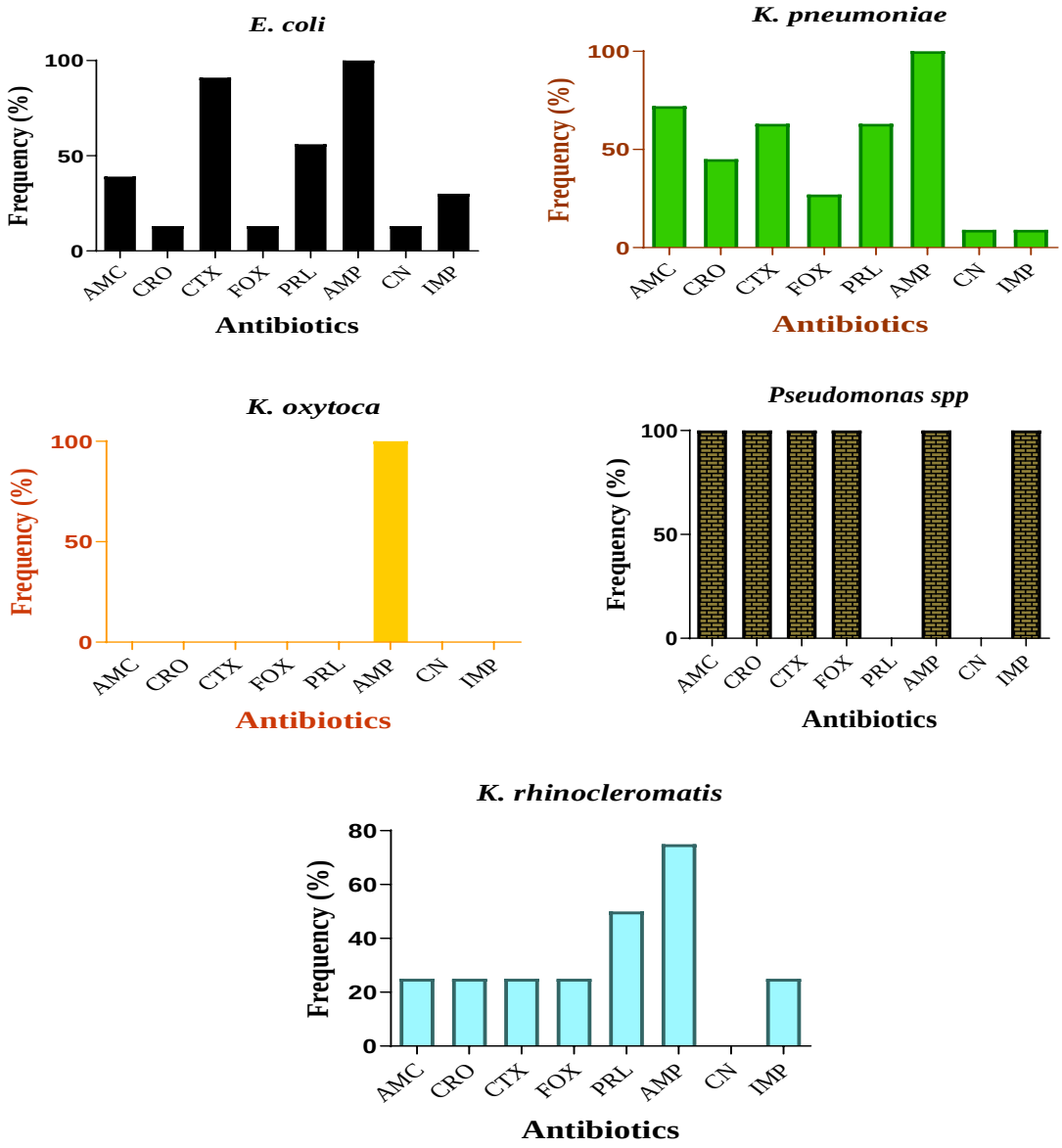

Figure 2. Profile of Gram-negatives bacteria susceptibility to antibiotics. AMC: Amoxicillin + Acide cluvulanique; CRO: Ceftriaxone; CTX: Cefotaxime; FOX: Cefoxitin; PRL: Piperacillin; AMP: Ampicillin; CN: Gentamicin; IMP: imipenem.

Frequency of hyper virulent strains of *S. agalactiae*

Among the isolated *S. agalactia* strains, 61.5% were hyper virulent and harbor *hvg*A genes.

DISCUSSION

Among the strains isolated, in monomicrobial, *E. coli* was the most isolated species (14.7%), followed by *K. pneumoniae* (11.8%) then *S. agalactiae* (5.9%). *Pseudomonas* spp. was the least represented species (2.9%). These results are different to those reported by Salou et al. (2015) on pregnant women in the last trimester of pregnancy with premature rupture of the membrane. In their study, they isolated *E. coli* (23%), *K. pneumoniae* (17.1%), *S. agalactiae* (8.5%) and *S. aureus*/*K. oxytoca* (2.9%). The difference may be due to several parameters, including the vaginal sampling method (the sample is taken without a speculum in our study). Nevertheless, these results are close to those found by Rani et al. (2014) in India who showed a high prevalence of Gram-negative bacteria with the most frequently isolated *E. coli*. Genital carriage of *S. agalactiae* and *E. coli* has been studied in several countries because of the incidence of infection caused by

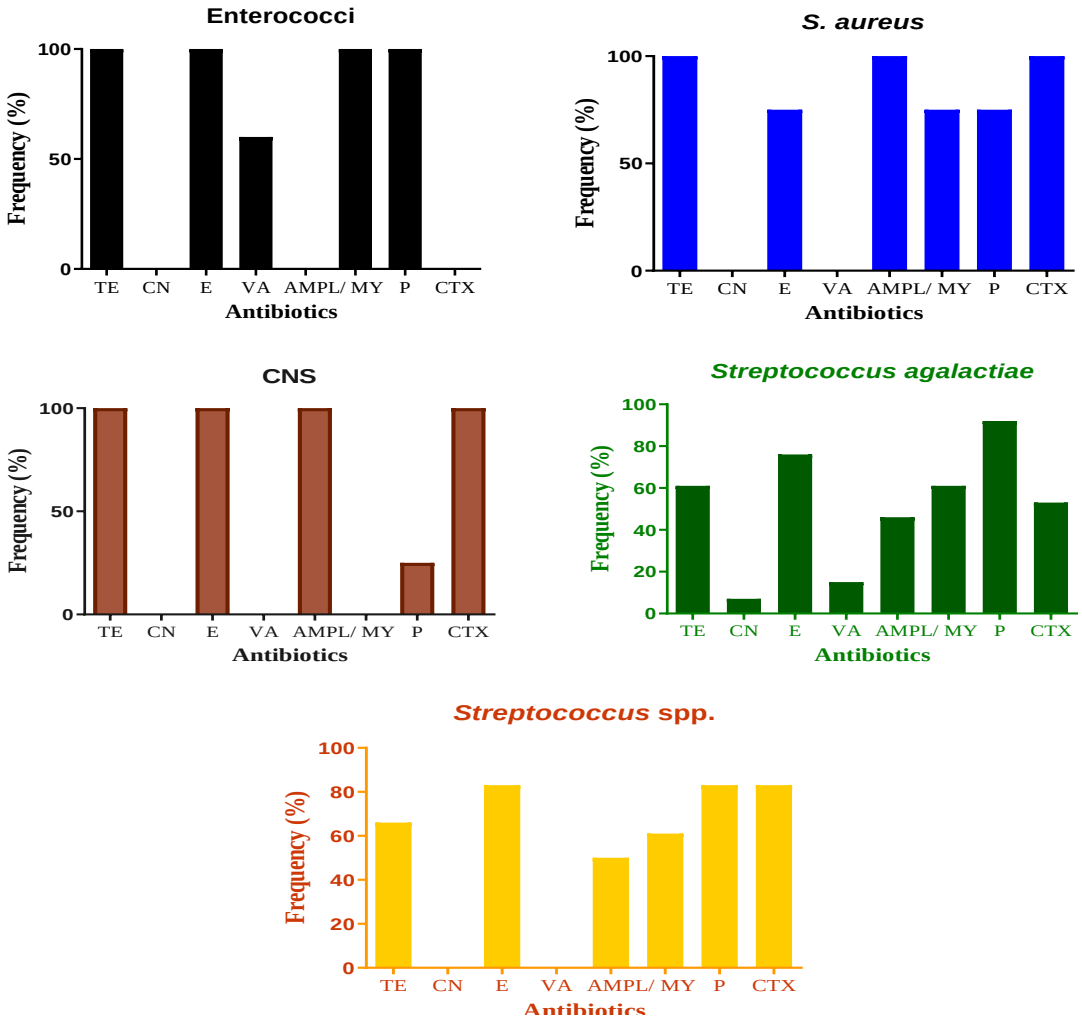

Figure 3. Profile of Gram-positives bacteria susceptibility to antibiotics. TE: Tetracycline; GN: gentamicin; E: erythromycin; VA: vancomycin; AMP: ampicillin; L/MY: lincomycin/clindamycin; P: penicillin G; CTX: cefotaxime.

Table 5. Phenotypical clustering of resistance to erythromycin and clindamycin of isolated bacterial strains.

Bacteria	Phenotype		
	$cMLS_B$ (%)	$iMLS_B$ (%)	M (%)
Enterococci	100	0.0	0.0
CNS	0.0	33.3	66.7
S. aureus	75.0	0.0	25.0
S. agalactiae	70.0	0.0	30.0
Streptococcus spp.	80.0	0.0	20.0
Total	70.4	3.7	25.9

these bacterial species and the carriage rate varies according to geographic area and region (Brochet et al., 2009; Gizachew et al., 2019; Lekala et al., 2015; Madrid et al., 2017). In Benin, Edmond et al. (2017) reported a 19.76% enterococci carriage rate of 11.86% for *S. aureus* and 6.62% for *E. coli* in pregnant women with a vaginal

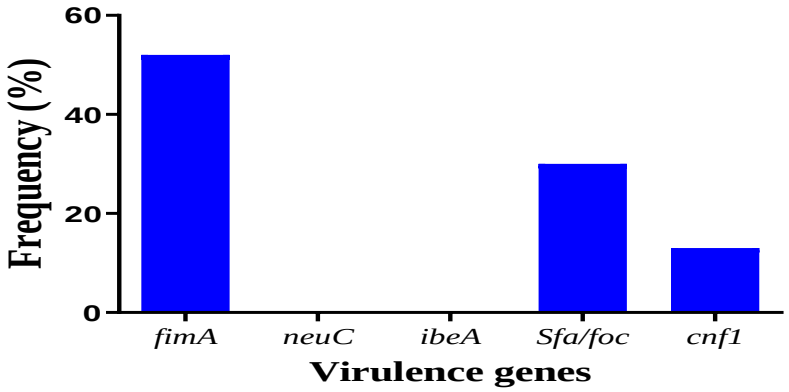

Figure 4. Profile of virulence genes involve in physiopathology of infection caused by *E. coli*.

Table 6. Distribution of ermB, ermTR and mef(A/E) genes among Gram- negative bacteria isolate with MLS_B resistance.

Bacteria	Genes of resistance to MLS_B			*P-value*
	***ermB* (%)**	***ermTR (%)***	***mef(A/E) (%)***	
Enterococci	0.0	0.0	80.0	
CNS	0.0	0.0	66.7	
S. aureus	0.0	0.0	75.0	0.691
S. agalactiae	0.0	0.0	50.0	
Streptococcus spp.	0.0	0.0	60.0	

infection (vaginosis or vaginitis). Vaginal carriage in mono-microbial is 76.2% against 35.7% in poly-microbial. Balaka et al. (2005) also found similar results with an overall rate of 79.2% in mono-microbial and 20.8% in poly-microbial.

Among the beta-lactams tested, ampicillin shows high resistance in almost all species. Gentamicin was found to be the most active molecule with a higher resistance rate obtained in *E. coli* (13%). This result is similar to that reported by Rani et al. (2014) who reported 10% resistance to gentamycin. A meta-analysis carried out in Africa by Okomo et al. (2019) on bacteria isolated from neonatal infectious showed that in West Africa, 47% resistance to gentamycin for *K. pneumoniae* was recorded against 52% for *E. coli*. In the present study, the low rate of resistance obtained for this molecule (13% for *E. coli* and 9.1% for *K. pneumoniae*) can be explained by the asymptomatic characteristics of vaginal carriage and therefore an absence of contact between strains with gentamycin. Resistance to imipenem has been observed for some bacterial species such as *E. coli* (7/23), *K. pneumoniae* (1/11), *K. rhinoscleromatis* and *Pseudomonas* spp. (1/1).

The search for carbapenem resistance genes for these species is therefore necessary to assess the distribution of these genes in Benin. However, the analysis by Okomo et al. (2019) also shows rates of phenotypic resistance to imipenem (3% in South Africa and 17% in West Africa for strains of *Klebsiella* spp.).

The results of the present study show that the enterococcus strains exhibit 100% resistance to clindamycin, and to penicillin G. But no resistance was observed for ampicillin; that seems to contradict the fact that enterococci are naturally resistant to penicillin. This can be due either to a synergy between gentamycin and ampicillin, or that it is streptococci. But the insufficiency of identification up to the species for this group, was one of the weaknesses for a good interpretation of this result. The high resistance profile to penicillin's (Penicillin G and Ampicillin) for Gram-positive bacteria, especially *S. agalactiae*, calls into question the efficacy of antibiotic prophylaxis and of first-line treatment of maternal-fetal and neonatal infections.

The results of the present study on the resistance of *S. agalactiae* to macrolides are different from those reported by a study carried out in some Asian countries by on strains of *S. agalactiae* (Zeng et al., 2006). They reported 13% resistance to erythromycin in *S. agalactiae* of which 58.2% were cMLSB phenotype, 26.9% M phenotype and 14% iMLSB phenotype. Nevertheless, we found that the most common phenotype is cMLSB. Higher resistance rates to erythromycin and clindamycin were reported in a

study carried out in Congo by Ngoulou et al. (2019), while a lower resistance rate was obtained for these two antibiotics in Iran (Ghanbari et al., 2016). For strains resistant to clindamycin or erythromycin, we obtained 75% cMLSB phenotype and 25% M phenotype. Our results differ from those reported by Ngoulou et al. (2019) who reported a predominance of the iMLSB phenotype in *S. aureus*. The most sensitive molecule was gentamycin with 0% resistance to Gram positive bacteria except *S. agalactiae* (1/13 resistance). According to the study conducted in Benin on strains isolated from vaginal samples in women presenting symptoms, 66.7% of *S. agalactiae* were resistant to ampicillin, 61.5% resistant to gentamycin, 50.5% to erythromycin, and 91.7% with tetracycline (Edmond et al., 2017). Compared to the results obtained for the sensitivity of *S. agalatiae* to these antibiotics, the resistance rates reported by Edmond et al. (2017) were globally higher. This difference can be explained by the asymptomatic nature of the patients recruited and therefore less contact with antibiotics. In general, a high rate of resistance has been observed for tetracycline, erythromycin, clindamycin, and also for beta-lactams.

All species except *S. agalactiae* showed high sensitivity to gentamycin and vancomycin. These results confirm the observation made by several authors who have reported for *S. agalactiae*, a high rate of resistance, not only for tetracycline, but also for fluoroquinolones and aminoglycosides. Finally, there is resistance emergence to vancomycin and macrolides (Da Cunha et al., 2014; Hays et al., 2016; Metcalf et al., 2017; Srinivasan et al., 2014). The resistance profile to penicillins and aminoglycosides constitutes a real public health problem, since these two families of antibiotics are recommended in the treatment of infections. Monitoring with this antibiotic is therefore necessary in order to assess the effectiveness of the management of maternal-fetal and neonatal infections. Third generation cephalosporins and macrolides are an alternative for people who are allergic to penicillins. Few studies have been carried out on the susceptibility of germs to macrolides in Benin. For this, we targeted, by PCR, the genes encoding for macrolides and derivatives resistance in the genome of strains displaying iMLSB, cMLSB or M phenotypes. Results showed that none of the strains carried the *erm*B or *erm*TR genes but high prevalence of the mef (A/E) gene. The *erm* genes is reported to confer a cMLSB and iMLSB phenotype, inducing resistance to macrolides, lincosamides and streptogramins whereas *mef* (A/E) gene confers the M phenotype inducing resistance to only macrolides. Our results showed a high prevalence of the cMLSB phenotype (70.4%). Despite this high rate, no *erm* gene was detected. This leads us to hypothesize that strains with a cMLSB phenotype, and which have the mef (A/E) gene, also have the lin (A/B) gene which confers resistance to only lincosamides. Several studies carried out in the same direction have shown an increase in the prevalence of these genes over time (Gygax et al., 2006; Kataja et al., 2000; Leclercq, 2002; Saderi et al., 2011). The work carried out by several authors has revealed the involvement of *erm*A, erm B, ermC gene and an *msr*A efflux pump in resistance to MLS in *S. aureus* and clinical coagulase negative staphylococci (Moosavian et al., 2014; Zmantar et al., 2011). These genes have also been detected in other Gram-positive bacteria such as enterococci and have been mainly associated with strains exhibiting an MLSB phenotype (Zeng et al., 2008; Quincampoix and Mainardi, 2001). It seems surprising that neither *erm*B nor *erm*TR was detected in the present study. A study, focused on the detection of all genes for resistance to macrolides and derivatives specific to bacterial species will help to better understand this paradox.

Among the five investigated *E. coli* virulence genes, the most common identified is *fim*A (52.2%) followed by sfa/foc (30.4%) then cnf1 (13.0%). *neu*C and *ibe*A genes were not detected in our isolated strains. Not all strains therefore seem to carry the K1 antigen (*E. coli* K1-). This could explain why neonatal *E. coli* meningitis was less compared to other types of infections due to this species, in a study carried out in Benin (Agossou et al., 2016). However, this result differs from that reported in the study on *E. coli* responsible for neonatal meningitis and which reported the presence of neuC (83%), sfa/foc (41%) and ibeA (32%) (Bingen et al., 1998). The IbeA gene (ibe10) is an invasion determinant contributing to the invasion by *E. coli* K1 of the blood-brain barrier in the pathophysiology of neonatal meningitis (Huang et al., 2001). The characterization of 30 virulence genes of *E. coli* strains has shown that the presence of these genes differs according to the type sequence (ST) to which the strain belongs. Thus, the genes involved in adhesion such as fimH, papACEFG1, the siderophores fyuA, traT were more associated with *E. coli* K1 (ST95) while the sfaS, hlyA, and cnf genes are found most often in *E. coli* K5 (ST127) (Alkeskas et al., 2015). In addition, a comparison of the carriage of virulence genes between *E. coli* K1+ and *E. coli* K1- showed the presence of the fimA both in *E. coli* K1+ (83.6%) and in *E. coli* K1– (86.3%). *E. coli* K1+ is reported to have significantly more virulence genes than *E. coli* K1 (Kaczmarek et al., 2012). These observations led to hypothesize that there is a low prevalence of *E. coli* K1 strains, particularly in vaginal samples from asymptomatic pregnant women. A study based on housekeeping gene sequencing (MLST) will provide a better understanding of this distribution of virulence genes.

The hvgA virulence gene specific to hyper virulent strains (ST-17) was targeted for the identification of ST-17 clones. High prevalence (61.54%) of these strains was isolated in rectal and vaginal samples from pregnant women approaching childbirth. This result is higher than those reported by Kardos et al. (2019) on strains isolated from non-pregnant women. The high prevalence of this

strain could be linked to the dissemination of the same clone in southern Benin and a characterization based on the MLST method of the strains could help to better understand this high rate in the population of pregnant women. However, the presence of these strains at a high carrier rate, especially in pregnant women in the last trimester of pregnancy, should alert the health authorities of Benin in order to improve the control of maternal-fetal infection.

Conclusion

Maternal-fetal and neonatal infection is one of the black beasts against which any health authority is fighting. The last trimester of pregnancy is a critical period with a high rate of genital bacterial carriage with a high risk of maternal-fetal and neonatal infection. Prophylactic measures against *S. agalactiae* infection should be applied in southern Benin in view of the alarming results of this study. The antibiotic resistance profile revealed a high rate of resistance to the majority of tested antibiotic families prescribed in a maternal-fetal infectious. Molecular characterization of highly pathogenic species such as *E. coli* and *S. agalactiae* reveals a high level of virulence genes including fimA, sfa/foc and cnf1 in *E. coli* and hvgA responsible for hyper pathogenicity in *S. agalactiae*. There is circulation of highly virulent strains with macrolide resistance genes (mefA/E) in southern Benin. These results show the importance of initiating antenatal screening for genital bacteria at high risk of infection in order to improve obstetric and neonatal care. Larger studies are needed to better orient these measures.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ABBREVIATIONS

CHU-MEL, Lagune University Hospital of Mother and Child; **HRIB,** high risk of infection bacteria; **CNS,** coagulase negative staphylococci; ***ermB*,** gene encoding for macrolide-lincosamide-streptogramin B resistance protein; ***ermTR*,** gene encoding for erythromycin resistance methylase; ***mef(A/E)*,** gene encoding for macrolide resistance A and E; ***dltR*,** gene encoding for transcriptional regulatory protein; ***hvgA*,** gene encoding for hypervirulent GBS adhesion; ***neuC,*** *UDP-N-acetylglucosamine 2-epimerase*; ***FimA,*** *gene* encoding *for Type-1 fimbrial protein A chain*; ***ibeA*,** gene encoding for Invasion protein; ***cnf1*,** gene encoding for cytotoxic necrotizing factor 1; ***sfa/foc*,** gene clusters for S fimbrial adhesin (sfa) and F1C fimbriae (foc); **TE,** tetracycline; **GN,** gentamicin; **E,** erythromycin; **VA,** vancomycin; **AMP,** ampicillin; **L/MY,** lincomycin/ clindamycin; **P,** penicillin G; **CTX,** cefotaxime.

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