Analysis of virulence factors and antibiotic resistance genes in Group B Streptococcus from clinical samples

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Abstract

Background: *Streptococcus agalacticae* is one of the most important causative agents of serious infections among neonates. Group B Streptococcus (GBS) virulence factors are important in the development of vaccines, whilst antibiotic resistance genes are necessary in understanding the resistance mechanisms used by these pathogens. This study was carried out to identify the virulence genes and antibiotic resistance genes associated with GBS isolated from pregnant women.

Methods: A total of 43 GBS isolates were obtained from vaginal samples that were collected from all HIV positive and HIV negative women who were 13-35 weeks pregnant attending Antenatal Care at both Chitungwiza and Harare Central Hospitals in Zimbabwe. Identification tests of GBS isolates was done using standard bacteriological methods including molecular tests. Antibiotic susceptibility testing using 3 antibiotics was done using the modified Kirby-Bauer method. The boiling method was used to extract DNA and Polymerase Chain Reaction (PCR) was used to screen for 13 genes in the isolates. Data was fed into SPSS 24.0 and the Spearman rank correlation test used to determine any correlation among genes.

Results: Nine distinct virulence gene profiles were identified. The profiles *hly-scpB-bca-rib* 37.2% (16/43) and *hly-scpB-bca* 18.6% (8/43) were common among GBS isolates. The following virulence gene frequencies were obtained namely *hly* 97.8% (42/43), *scpB* 90.1% (39/43), *bca* 86.0% (37/43), *rib* 69.8% (30/43) and *bac* 11.6% (5/43). Antibiotic resistance genes showed high frequencies for *tetM* 97.6% (41/42) and low frequencies for *ermB* 34.5% (10/29), *ermTR* 10.3% (3/29), *mefA* 3.4% (1/29), *tetO* 2.4% (1/42) and *linB* 0% (0/35). The *atr* housekeeping gene amplification yielded 100% (43/43) positive results, whilst the mobile genetic element IS1548 yielded a low 9.3% (4/43).

Conclusion: The study showed a high prevalence of multiple virulence genes *hly, scpB, bca* and *rib* in *S. agalactiae* strains isolated from pregnant women. Tetracycline resistance was found to be predominantly caused by the *tetM* gene, whilst macrolide resistance was predominantly due to the presence of *erm* methylase, with the *ermB* gene being more prevalent. It was also observed that in vitro phenotypic resistance is not always accurately predicted by resistance genotypes.

Background

Streptococcus agalactiae (Group B Streptococcus; GBS) is the leading cause of neonatal infections and other serious infections in pregnant women in many countries (1). About 15 to 35% of pregnant women have GBS, and approximately 1 to 2% of colonized neonates develop a severe infection (2). It is also known that about 25% of pregnant women have Group B Streptococcus bacteria in their rectum or vagina (1) which can be passed via vertical transmission to the unborn baby leading to early onset disease in the form of sepsis, pneumonia and meningitis (2).

In poverty stricken countries such as Zimbabwe, pregnant women hardly get access to GBS diagnostic tests and antibiotics are expensive for the general public. A study done in three communities in Zimbabwe showed a cumulative 60.3% GBS colonization rate of women who were tested 3 times and that same study showed that GBS colonization is common among pregnant women in the country (3).

Group B streptococcus possesses virulence factors (VFs) encoded by genes which they use to enter, replicate and persist in a host (4). Antimicrobial resistance genes give GBS the ability to resist the effects of medication that would have previously been used to treat them and these antibiotic resistance genes are found everywhere in the environment (5). The increasing emergence of antibiotic resistant Streptococcus agalactiae clearly raises more concerns for sustained measures in treatment of such life-threatening bacterial infection (6).

A number of these virulence factors in GBS include the capsular polysaccharide which is used for strain typing (7,8). There are capsular polysaccharide antigens (CPA) designated Ia, Ib and II–IX in GBS some of which have been studied by serotying and molecular methods in Zimbabwe (9,10). Other genes that have been studied in other countries include those for the alpha/α antigens of the C protein (bca), beta/β antigens of the C protein (bac), surface protein Rib (rib), C5a peptidase (scpB) and hyaluronate lyase (hly) (4,7,11). To our knowledge only one study (9) in this country investigated Rib, Cα and Cβ protein markers in GBS, however data on some of the other known virulence genes is not available.

Penicillin and ampicillin are the drugs of choice for treatment of human S. agalactiae infections (12–14) whereas for penicillin-allergic individuals, erythromycin and clindamycin are recommended (1). The prevalence of resistance to erythromycin, tetracycline and clindamycin has recently been increasing in S. agalactiae (12,13,15). Various researchers have linked this resistance to specific genes found in Group B Streptococcus (12,14,16).

Molecular tests are necessary for both therapy and in the monitoring of the spread of resistant organisms and their genes throughout the communities and medical facilities such as hospitals and clinics (17). Such tests are important if we are to control the increase in resistance as well as the emergence of multi-drug resistant bacteria. Monitoring this spread of resistance necessitates the use of such nucleic acid based tests especially in the study of mobile genetic elements (MGEs) which are a type of DNA that can move around and within the genome (18). Strategies to fight off certain bacterial infections by targeting these specific virulence genes and mobile genetic elements have been suggested (19).

The current study focused on screening for 13 genes in GBS including 5 virulence genes hyaluronate lyase, hly; beta/β antigens of the C protein (bac); alpha/α antigens of the C protein (bca); surface protein Rib, rib (7) and C5a peptidase, scpB (20). The 6 antibiotic resistance genes investigated are the tetracycline resistance determinants tetM and tetO (16); erythromycin ribosomal methylase determinants ermB and ermTR; Macrolide efflux mefA and clindamycin resistance determinant linB (12). The Mobile Genetic Element IS1548 (7) and the housekeeping gene atr (21) was also amplified. The atr gene was chosen in this study because it is an excellent target for Streptococcus agalactiae amplification, especially because it has been well studied and it is an essential gene with a very low probability of

mutation. A protein amino acid transporter gs0538, which is extremely specific to S. agalactiae species is encoded by the gene (21).

The aim of this study was to identify virulence factors and antibiotic resistance genes found in GBS isolates from pregnant women clinical samples as well as to study the correlation between the different genes found in GBS isolates.

Methods

Study design and sample collection

The cross-sectional study was done using GBS samples that were collected from pregnant mothers who attended Antenatal Care (ANC) at Harare central hospital and Chitungwiza central hospital in Zimbabwe. The patient's age, gender, HIV status were some of the demographic data collected together with the samples. A consecutive sampling method was used to select the participants then two vaginal swabs were collected from each consenting participant by trained research nurses.

Inclusion and exclusion criteria

Patients who were on treatment for any STI and those who had received antibiotic treatment one month prior to recruitment were excluded from the study. All pregnant women who did not give their consent as well as those who were less than 13 weeks pregnant and less than 18 years of age were also excluded from the study.

Isolation and Identification of Streptococcus agalactiae

A total of 43 suspected GBS isolates were obtained from the vaginal swabs. These isolates were subcultured by streaking onto 5% sheep blood agar plate (Columbia CNA Agar) (Acumedia, USA). The plates were incubated at 37 °C for 18–24 hours in aerobic conditions. The suspected β-hemolytic colonies were identified as GBS using standard microbiological Group B streptococcus identification tests which were confirmed using the Streptex grouping latex reagent (Remel, UK). The isolated identified GBS strains were then stored in Mueller Hinton broth along with 20% glycerol at -80 °C. The negative culture results was only issued after 72hrs.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of GBS was done using the modified Kirby Bauer methods as recommended by the Clinical Laboratory Standard Institute (CLSI, 2017). Incubated, plates were examined to ensure the growth was confluent or near confluent before zone diameters were measured. The susceptibilities of GBS to antibiotics were categorized and determined based on the CLSI guidelines. (CLSI, 2017).

DNA Isolation and Quantification

After subculturing the stored isolates, DNA was isolated using the boiling method. A pipette was used to place 600 µl of sterile Tris-EDTA (TE) buffer pH 8.0 (Amresco, USA) into a 1.5 ml sterile eppendoff. A sterilized loop was used to emulsify the cultured colonies in TE and this mixture was vortexed for 30-45secs before placing the tightly closed eppendoffs into a boiling water bath for 15 minutes. A -20ºC freezer was then used to freeze the boiled mixture for 10 minutes. DNA samples were stored at -20ºC in Tris-EDTA buffer at pH 8.0 to minimize the degradation, before being tested for quality and quantity. The preparation of × 1 Tris-Borate electrophoresis (TBE) buffer, 1% agarose gel and molecular weight markers (1 kb and 50 bp) were all done according to manufacturer's instructions.

The PCR process was done in four stages, i.e. amplification of the housekeeping gene atr, the mobile genetic element IS1548, the antibiotic resistance genes (ermB, ermTR, mefA, linB, tetM and tetO) and the virulence genes (hly, bca, bac, rib and scpB).

PCR Reactions Mixtures and Conditions

Amplification of the all the genes was done using standard PCR. For each DNA sample a 25 µl reaction mixture was made which consisted of 15.875 µl of nuclease-free water (Amresco, USA), 2.5 µl of × 10 PCR buffer (New England BioLabs, UK), 0.5 µl of dNTPs Solution Mix, 10 mM (New England BioLabs, UK), 0.125 µl Taq DNA polymerase, 5U/µl (New England BioLabs, UK), 5 µl of DNA template and 1 µl of the 10 µM primer (0.5 µl of forward primer and 0.5 µl of reverse primer) (Inqaba biotech, South Africa). The primers used in our study are listed (see Additional file 2: Table S1). Negative controls comprised of a water control. A Labnet Multigene OptiMax (Labnet International Inc, USA) was used for the PCR thermal cycling conditions with an initial denaturation step at 94ºC for 1 min, 35 cycles and a final elongation step at 72ºC for 10 min. Ten microliters of the amplified products were mixed with 5 µl of gel loading dye (6×) then run along a 1% ethidium bromide (Amresco, USA) (EtBr final conc. of 0.5 µl/ml) stained agarose gel with a 1 kb and 50 bp DNA ladder (New England BioLabs, UK) in 1 × TBE buffer (Amresco, USA) for 1hr 30 min at 100V (Biobase, China) and then viewed using a Wealtec KETA UV Transilluminator (Wealtec Corp, USA).

The contents of each 25 µl reaction mixture is summarized in Table 1. Optimization of the PCRs was performed by adjusting the DNA template concentration and the annealing time.

Table 1

Final primer concentrations and volumes used in the PCRs

Components	25 µl Reaction	Final Conc.
10 × PCR Standard Taq reaction buffer	2.5 µl	1×
10 mM dNTPs Solution Mix	0.5 µl	200 µM
10 µM Forward Primer	0.5 µl	0.2 µM
10 µM Reverse Primer	0.5 µl	0.2 µM
Template GBS DNA	5 µl	variable
Taq DNA Polymerase	0.125 µl	1.25U/50 µl PCR
Nuclease Free Water	15.875 µl	-

Results

Statistical analysis

All data entry and analysis was done using IBM Statistical Package for the Social Sciences (SPSS) version 24.0 software. Prevalence figures were calculated for the total sample population. Tests for normality were done such as the Shapiro-Wilks test and Skewness-Kurtosis All Normality Test. P value less than 0.05 was considered statistically significant.

Antibiotic Susceptibility Profiles.

The Group B Streptococcus were assayed against a panel of three antibiotics. The results shown in Table 2 suggest a 97.6% resistance of GBS to tetracyline, 55.8% to clindamycin and 30.2% erythromycin.

Table 2

Antibiotic susceptibility profiles of 43 GBS isolates from pregnant women

Antibiotic (conc.,µg)	Resistant no. (%)	Intermediate no. (%)	Susceptible no. (%)
Tetracycline (30 µg)	42/43 (97.6%)	0 (0%)	1 (2.3%)
Clindamycin (2 µg)	24/43 (55.8%)	11/43 (25.6)	8/43 (18.6%)
Erythromycin (15 µg)	13/43 (30.2%)	16/43 (37.2%)	14/43 (32.6%)

The 43 GBS isolates in Table 2 showed high resistance to tetracycline followed by clindamycin and the least resistance to erythromycin.

Polymerase Chain Reaction

Sample Characteristics (Statistical Analysis)

Tetracyline Resistance Genes

A Shapiro-Wilks test ($p < 0.05$) and a visual inspection of their histograms, normal Q-Q plots and box plots showed that tetracycline resistance was not normally distributed for tetM and tetO with a skewness of 6.481 (S.E = 0.365) and a kurtosis of 42 (S.E = 6.717) for both tetM and tetO.

Erythromycin Resistance Genes

A Shapiro-Wilks test ($p < 0.05$) and a visual inspection of their histograms, normal Q-Q plots and box plots showed that erythromycin resistance was not normally distributed for ermTR, mefA and ermB, with a skewness of 2 (S.E = 1.014) and a kurtosis of 4 (S.E = 2.679) for ermTR, a skewness of 0.816 (S.E = 0.369) and a kurtosis of -1.405 (S.E = 0.724) for mefA and a skewness of 0.321 (S.E = 0.409) and a kurtosis of -2.023 (S.E = 0.793) for ermB.

Clindamycin resistance and the linB gene could not be tested for normality because linB's absence was constant and that showed a complete absence of correlation which is represented by 0. The VFs could not also be tested for normality, because they did not have an established dependent and independent variable, however a correlation did exist.

Since the data that was tested is not normal, we used non-parametric test, such as the Spearman rank correlation test and Odds ratios. A p-value of 0.05 was used.

Odds Ratio Report

Table 3 shows that the odds ratio of being resistant to tetracycline is higher for GBS with the tetM gene (ExpB > 1) and the odds of being resistant to tetracycline is lower for GBS with the tetO gene (ExpB < 1). It also shows that the odds of being resistant to erythromycin is higher for GBS with the ermB and ermTR (ExpB > 1) and the odds of being resistant to erythromycin is lower for GBS with the mefA (ExpB < 1).

Table 3
Odds ratios for Tetracycline and Erythromycin Resistance and their determinants.

Variables in the Equation	B	Exp(B)
Step 1[a] tetM tetO Constant	17.514 -17.514 -21.203	40386871.982 0.000 0.000
Step 1[a] ermB ermTR mefA Constant	37.135 0.811 -18.855 -38.469	13414314835479176.000 2.250 0.000 0.000
a. Variable(s) entered on step 1: tetM, tetO.		
b. Variable(s) entered on step 1: ermB, ermTR, mefA.		

Spearman Rank Correlations

Table 4 shows that there was a negative correlation between tetO and all the macrolide resistant determinants; between ermB and ermTR and also between ermTR and mefA. A positive correlation was observed between the tetM and all the macrolide resistant determinants (such as ermB); between tetM and tetO and also between ermB and mefA. However, the P values for all these correlations are greater than the level of significance implying that correlations are not statistically significant, meaning they have occurred by chance. Hence, they cannot be generalized into the general GBS population. The linB gene could not be computed because it was a constant. Table 4 also shows that there is a negative correlation between hly and IS1548 as well as between bac and rib and this is statistically significant at ($p < 0.01$). These relationships can be generalized into the pregnant women GBS population. However, some non-statistically significant positive correlations were also observed between IS1548 and (rib/scpB/bca) as well as bac and (hly/scpB/bca). The remainder of the genes had a negative correlation which was also not statistically significant.

Table 4
GBS Virulence and Antibiotic Resistance Genes Spearman Rank Correlations

Virulence Genes	**IS1548**	**rib**	**hly**	**scpB**	**bca**	**bac**
IS1548	1	0.18	− .563**	0.088	0.11	-0.099
rib	0.18	1	-0.102	-0.036	-0.265	− .393**
hly	− .563**	-0.102	1	-0.049	-0.062	0.056
scpB	0.088	-0.036	-0.049	1	-0.129	0.116
bca	0.11	-0.265	-0.062	-0.129	1	0.146
bac	-0.099	− .393**	0.056	0.116	0.146	1
Antibiotic Resistance Genes	tetM	tetO	ermB	ermTR	mefA	linB
tetM	1	0.024	0.085	0.049	0.034	.a
tetO	0.024	1	-0.085	-0.049	-0.034	.a
ermB	0.085	-0.085	1	-0.176	0.14	.a
ermTR	0.049	-0.049	-0.176	1	-0.071	.a
mefA	0.034	-0.034	0.14	-0.071	1	.a
linB	.a	.a	.a	.a	.a	.a
* Correlation is significant at the 0.05 level (2-tailed).						
** Correlation is significant at the 0.01 level (2-tailed).						
a Cannot be computed because at least one of the variables is constant.						

Virulence Gene Profiles

Nine distinct virulence gene profiles were identified and the virulence gene profiles hly-scpB-bca-rib 37.2% (16/43) and hly-scpB-bca 18.6% (8/43) were common among GBS isolates (Table 5).

Table 5
Virulence gene profiles of the 43 Streptococcus agalactiae isolates

Virulence Gene Profile	Number of Isolates	Frequency %
hly, bca	1	2.3
hly, bca, rib	3	7.0
hly, scpB, rib	6	14.0
hly, scpB, bca	8	18.6
hly, scpB, bca, rib	16	37.2
hly, scpB, bca, rib, bac	1	2.3
hly, scpB, bca, bac	4	9.3
scpB, bca, rib, IS1548	1	2.3
hly, scpB, bca, rib, IS1548	3	7.0

Prevalence of Virulence Genes and Antibiotic Resistance Genes

Table 6 shows the percentages of all the genes being investigated (see Additional file 1: Table S2). The housekeeping gene atr was present in all 43 isolates, whilst the Mobile Genetic Element IS1548 was present in only 4 of the isolates. The virulence gene hly had the highest frequency followed by scpB, bca, rib and then bac which was found in a few isolates. The VFs frequencies were calculated for the whole GBS population (43 isolates). The antibiotic resistance gene tetM had a high percentage when compared with tetO. The ermB determinant was moderately present followed by ermTR then lastly mefA which was rarely found. The linB gene was not detected in any of the isolates. The percentage of the resistant determinants was obtained by combining only the intermediate and resistant populations (I + R) (see Additional file 2: Table S3).

Table 6

Frequency of all the 13 genes in the 43 GBS isolates.

Name of Gene	Primer Name	Frequency (%)
Housekeeping Gene		
	atr	100
Mobile Genetic Element		
	IS1548	9.3
Virulence Genes		
Hyaluronate lyase	hly	97.8
C5a peptidase	scpB	90.1
alpha/α antigens of the C protein	bca	86.0
Surface protein Rib	rib	69.8
beta/β antigens of the C protein	bac	11.6
Antibiotic Resistance Genes		
Tetracycline Resistance	tetM	97.6
Tetracycline Resistance	tetO	2.4
Erythromycin Ribosomal Methylase	ermB	34.5
Erythromycin Ribosomal Methylase	ermTR	10.3
Macrolide efflux	mefA	3.4
Clindamycin Resistance	linB	0
Additional file 1		
Gel Electrophoresis Pictograms		
Amplification of the atr housekeeping gene.		
PCR was done to detect the presence of housekeeping genes. Forty-three genomic DNA samples were amplified using primers targeting for presence of the gene: atr (780 bp) in GBS. Figure 1 shows the results for the housekeeping gene, atr.		

Discussion

Our study is the first prevalence report of direct detection of some of the GBS virulence genes and antibiotic resistance genes in pregnant women in Zimbabwe. Odds ratios showed (Table 3) that the antibiotic resistance genes are associated with drug resistance, whilst Spearman rank correlations showed (Table 4) the different associations between the investigated genes.

The atr gene and the Mobile Genetic Element

The sensitivity of the atr gene amplification, in this study was 100% (43/43) (Table 6) which is similar to what is reported in other studies done by Gavino and Wang (22), de-Paris et al., (21) and Alfa et al. (23), where sensitivity was 95.8%, 100% and 90.5% respectively. This high sensitivity may be attributed to the use of selective and enriched media prior to performing PCR. Since this PCR test has shown to be reliable and robust, future studies can focus on rapidly testing pregnant women who present in labor with unknown GBS status. This test was used as a GBS confirmatory identification test. The Mobile Genetic Element IS1548 was present in only 9.3% (4/43) (Table 6). This is contrary to an Iranian study showed that the gene is more prevalent in human than in bovine (77% vs 5%) GBS isolates (7). The origin of the isolates could be a possible explanation for such a difference.

Virulence Genes

Nine distinct virulence gene profiles were observed but 30/43 strains (69.8%) belonged to hly-scpB-bca-rib 37.2% (16/43), hly-scpB-bca 18.6% (8/43) or hly-scpB-rib 14.0% (6/43) profiles (Table 5). The PCR assay for detection of VFs revealed that high percentage of the GBS isolates were positive for hly 97.8% (42/43); scpB 90.1% (39/43) and bca 86.0% (37/43) (Table 6). The high prevalence of these genes in GBS has been previously reported (7,24–26). Comparisons with a 2008 study in country shows the importance of investigating the CPA of GBS isolates (9). A comparison with other studies done in countries such as the USA (27), Sweden (28), Argentina (29) and Egypt (20), shows that any discrepancies are possibly a result of the geographical locations among other factors.

The results also showed that the majority of the isolates had more than three virulence genes. This high prevalence of various VFs in S. agalactiae isolates from the vaginal canal of pregnant women could lead to the development of serious maternal and neonatal infections (30). The high incidences of VFs except for bac, also suggest that GBS vaccines containing the proteins ScpB, α protein (31), Rib (9) and hyaluronate lyase (32) could potentially be effective against our population of pregnant women in Zimbabwe.

The present study observed that all the isolates 100% (n = 5) carrying the bac gene also carry the unrelated bca gene, whilst 86.5% (n = 32) of the bca carrying isolates did not also have the bac gene (see Additional file 2: Table S2). This finding agrees with the report that, isolates that express β tend to also express the unrelated α protein, while the α protein is often expressed on its own isolates (31). The majority of the Spearman rank analysis were not statistically significant and with some showing weak associations. However, a significant negative correlation (Table 4) was detected between hly and IS1548 as well as between bac and rib genes ($P < 0.01$). This negative association are most likely because some of these genes are not genetically linked.

Tetracyline Resistance

The high resistance for GBS to tetracycline 97.7% (42/43) (Table 2) can be attributed to a high presence of the tetM gene 97.6% (41/42) (see Additional file 1: Fig. 13). The high tetM gene presence is as a result of the ubiquitous presence of tet genes in pathogens, opportunistic pathogens and members of the normal flora (33). The high resistance to tetracycline is also because the antibiotic a relatively cheap, extensively used prophylaxis in the therapy of animal and human infections and it is known that bacterial strains tend to be resistant to frequently used antibiotics (34). A low presence rate of the tetO 2.4% (1/42) (Table 6) gene indicates that it is not common in GBS isolates that are resistant to tetracycline. These results agree with work done in Canada (35) and Nigeria (36). In Kuwait, they also reported that 89.5% tetracycline-resistant isolates contained 94.5% tetM and 3.9% tetO (37).

Erythromycin and Clindamycin Resistance

The results showed a 30.2% (13/43) and 55.8% (24/43) resistance to erythromycin and clindamycin respectively (Table 2). Such resistance decreases the options of prophylaxis in penicillin allergic women (38). We recommend that antibiotic susceptibility testing should be performed if clindamycin or erythromycin therapy is needed in the prevention of neonatal GBS infection. PCR detected 34.5% (10/29) ermB, 10.3% (3/29) ermTR and 3.4% (1/29) mefA from the intermediate and resistant erythromycin GBS (Table 6). The prevalence of the ermB determinant shows that GBS commonly use target methylation as the mechanism of macrolide resistance.

Studies done in Italy (39), South Africa (12), USA (14), Iran (13) and France (16) reported similar findings, with most of the studies also observing that the ermB gene is more prevalent in distribution than the ermTR gene among GBS strains. This study also confirmed findings by Poyart et al., (2003) that the mefA gene is rare among GBS isolates, thus efflux pumps mediated by this gene are not a common mechanism of macrolide resistance (16). Contrary to Bolukaoto et al., (2015) this current study did not find any linB genes 0% (0/35) in any of the clindamycin resistant and intermediate strains (12). In such cases, where multiple independent resistance genes can cause resistance, the observed phenotypic resistance can be attributed to any of the other known genes or genes that are yet to be discovered. This limits the usefulness of such diagnostic tests.

It was interesting to note that 63.6% (7/11) GBS which were resistant to both erythromycin and clindamycin had the ermB gene (see Additional file 2: Table S2 and S3) and that one GBS strain which was resistant to erythromycin and susceptible to clindamycin carried the ermTR gene. Such observations are similar to other studies (14,40,41).

Genetic Linkage in Macrolide/Tetracycline Resistance Determinant

It was also observed that 100% (n = 10) isolates that carried the ermB gene also carried the tetM gene. The association of erythromycin and tetracycline resistance may be due to the conjugative transposon

Tn1545, which encodes erythromycin resistance via the ermB gene and tetracycline resistance via the tetM gene (42–44). This association was supported by our analysis which showed a positive correlation (Table 4) between the two genes, but this lacked statistical significance according to the Spearman Rank test. Contrary to an Egyptian study (45), we report that there is no evidence suggesting genetic linkage of tetO with ermB, ermTR or mefA in our GBS population.

Unexpressed Resistant Genotypes

An unexpected observation in our study was found in the following three (7.0%) isolates. Isolate 127 which was tetracycline sensitive, and yet the tetM gene was detected. Isolates 243 and 322, were both erythromycin sensitive however, at least one gene ermTR or mefA was found, respectively (see Additional file 2: Table S2 and S3). Similar findings were reported in GBS and other streptococcal species (44,46–48). A confirmatory test to check Minimum Inhibitory Concentration (MIC) levels of these three isolates is recommended. However, based on the disk diffusion test, this observation suggests that the Kirby Bauer disk diffusion method is inadequate for detection of resistance. Although the reason(s) behind this lack of gene expression still has to be determined, some possible explanations include gene mutations, low expression levels of the gene and the possibility of a weak, distant or absent promoter (44). This study therefore supports the idea that the resistance genotype does not always accurately predict phenotypic resistance.

Conclusion

The tetracycline resistance encoded by ribosome protection gene tetM and the ribosomal methylation encoded by erythromycin ribosomal methylase gene, ermB were the two most common mechanisms of resistance in Group B Streptococcus from pregnant women. Current study results show that resistance genotypes do not always accurately predict phenotypic resistance.

A high frequency of the virulence genes: hly, scpB, bca and rib was detected in Group B Streptococcus and the majority of the isolates carried multiple virulence genes which showed that GBS's ability to adhere, colonize, destroy and invade tissues is important in the organism's pathogenesis. Hence the present study recommends these VFs as vaccine components. The bac and the MGE IS1548 were rarely found among the GBS isolates.

Limitations Of The Study

It would have also been necessary to test each isolate using an in vivo animal model, such as mice to demonstrate actual virulence and investigate the expression of resistant genes. In future studies it may be useful to get more information on the condition and status of the patients (i.e. whether they are suffering from cystitis or pyelonephritis) we may be able to tell which VFs are associated with a particular condition.

Additional Files

Additional file 1: Gel Electrophoresis Pictograms (Fig. 1 to Fig. 9).
Additional file 2: Three supplementary tables; Table S1: The oligonucleotide primers used for amplifying various genes in GBS. Table S2: Presence or absence of the expected amplicons in the 43 GBS isolates. Table S3: Presence or absence of antibiotic resistance genes in the resistant and intermediate isolates.

Abbreviations

GBS: Group B Streptococcus; VF: virulence factors; CDC: Centers for Disease Control; WHO: World Health Organization; CPA: capsular polysaccharide antigens; MGE: mobile genetic elements; MLSB: Macrolides, Lincosamides, and Streptogramin B; ANC: Antenatal Care; PCR: Polymerase Chain Reaction

Declarations

Ethics approval and consent to participate

This study obtained approvals from the institutions involved namely Harare Central Hospital (HCHEC 080416/21); Chitungwiza Central Hospital ethics committees (dated 28/04/16) which approved sample collection from their hospital antenatal clinics and the Joint Research Ethics Committee (JREC/341/17) for the College of Health Sciences and Parirenyatwa Group of Hospitals. The study was also approved by Medical Research Council of Zimbabwe (MRCZ A/2054) and (MRCZ/B/1403). All information of participants was kept confidential. Written informed consent from the study participants was sought and granted by the participants. All information and data regarding of the participants was kept confidential and it was only accessible to personnel involved in the study. All electronic data was password protected.

Consent for publication

Not Applicable.

Availability of data and material

The datasets generated during and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

RM, **RTM**, **MGM**: conceived the idea and contributed to the study design. **MGM**: Cohort recruitment, sample collection and completed the antibiotic resistance testing. **RM**: designed and performed the molecular testing. **RTM**: designed and supervised the project. **RM**, **RTM**: analyzed data and interpreted the results. **RM**, **RTM**, **MGM**: wrote manuscript. All authors read and approved the final paper for publication.

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