Susceptibility of Zimbabwean *Streptococcus agalactiae* (group B *streptococcus*; GBS) isolates to four different antibiotics

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Abstract

Objective: To establish the susceptibility of Zimbabwean GBS strains isolated from hospitalised patients to four antibiotics.
Design: Cross sectional survey.
Settings: Four regions of Zimbabwe (Bindura, Bulawayo, Harare, and Masvingo).
Subjects: 113 GBS isolates from hospitalised patients in Bindura, Bulawayo, Harare and Masvingo, of whom most were suffering from infectious diseases.
Main Outcome Measures: All isolates were tested for their susceptibility to clindamycin, erythromycin, penicillin and tetracycline.
Results: All isolates were 100 % sensitive to clindamycin, 98 % to penicillin, 86 % to erythromycin; 2 % of the isolates showed intermediate susceptibility to penicillin and 100% showed resistance to tetracycline.
Conclusion: Penicillin is still the antibiotic of choice for treatment of GBS infections and for *intrapartum* chemoprophylaxis in Zimbabwe. For patients who are allergic to penicillin, clindamycin will be the drug of choice for both treatment and/or chemoprophylactic use in Zimbabwe.

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Introduction

Streptococcus agalactiae (group B *Streptococci*; GBS) is an important cause of infections in humans, in particular in immunocompromised patients and in newborns. In the infant, GBS disease may occur as an early-onset disease with serious infection occurring before the seventh day of life or as a late-onset disease beginning between one to 12 weeks of life.[1] In intra-uterine or early onset disease, GBS is usually acquired from the mother who is a GBS carrier.[1] In the USA *intrapartum* prophylaxis with penicillin has been advocated to prevent neonatal GBS disease, according to defined guidelines.[1,2] This regimen has proved effective.[3,4] In the case of maternal allergy to penicillin, erythromycin has been advocated for the prophylaxis.[2] However, over the last few years, the emergence of resistance of GBS to macrolides and also to lincosamides has been reported from a variety of areas which makes erythromycin a less reliable alternative to penicillin.[5,9] Considerable regional differences in resistance rates have been noted.[5,10] For this reason it is important to monitor the sensitivity of GBS strains isolated in different countries and regions to various antibiotics.

In this study we have tested GBS of the three most prevalent GBS serotypes based on capsular antigen typing, occurring in Zimbabwe, for susceptibility to erythromycin, clindamycin, penicillin and tetracycline. The results confirm that these GBS isolates have sensitivity patterns essentially similar to those found in other parts of the world.

Materials and Methods

Bacterial Isolates.

A total of 113 GBS isolates were recovered from a variety of specimens and sites from patients hospitalised for various diseases, mostly infectious diseases. The strain collection includes a few isolates cultured from cerebrospinal fluid (CSF) and blood cultures from neonates and adult patients. Strict categorisation of strains in relation to patient and possible invasive disease could not be carried out due to inadequate data.

The patients were from four regions of Zimbabwe (Harare, Bulawayo, Masvingo and Mashonaland Central [Bindura]).

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The specimens were collected, transported and cultured and strains presumptively identified according to procedures recommended by the Public Health Laboratories of Zimbabwe. Putative GBS strains were forwarded to the Department of Medical Microbiology, University of Zimbabwe (UZ), Harare for further identification by one of us (SRM) as described previously.[11] Briefly, further identification of CAMP positive strains, was done by using the Strep Check test (Lorne Laboratories, United Kingdom). Strains were stored at -80°C in Greave's medium. Frozen bacteria were sub-cultured on blood agar plates before being transported to the Department of Microbiology, Norwegian University of Science and Technology (NTNU), Trondheim, Norway, for further analysis.

Capsular Antigen Typing.

Capsular antigen typing (CAT) was performed by using the indirect fluorescent antibody test (FAT) as previously described.[12] Briefly, the antisera used were raised against whole cells of reference or prototype strains for the various CATs, and made CAT specific by appropriate cross-absorption.[12] Smears were prepared from GBS cultured on blood agar plates and suspended in 0.15M phosphate-buffered saline pH 7.2 (PBS), air-dried, heat-fixed, and incubated with rabbit antisera appropriately diluted in PBS. After incubation, the smears were washed with PBS and further incubated with FITC- conjugated anti-rabbit immunoglobulin (Dakopatts). The slides were washed, mounted, and read with a Nikon epi-fluorescent microscope. The signalling was graded from 0 to 3+ with 2+ and 3+ recordings considered FAT positive.

Subtyping of these strains on the basis of surface-localised proteins was not taken into consideration in the present study.

Antibiotic Susceptibility Testing.

The susceptibility testing with determination of minimal inhibitory concentration (MIC) was done by the Etest method (AB BIODISK, Solna, Sweden). MICs of clindamycin, erythromycin, penicillin and tetracycline for 113 GBS strains were determined. For a few strains, MICs of quinupristin/dalfopristin were also tested by the Etest method. The tests were performed and the results recorded according to Etest technical guidelines. MIC breakpoints were determined for sensitivity (S), intermediate (I), and resistance (R) as recommended by the National Clinical Committee for Laboratory Standards (NCCLS; 13). They were as follows (in mg/l): clindamycin ≤ 0,25 (S), ≥ 1.0 (R); erythromycin ≤ 0,25 (S), ≥ 1,0 (R); penicillin ≤ 0.12 (S), ≥ 4.0 (R); tetracycline ≤ 2.0 (S), ≥ 8.0 (R).

Results

A total of 113 GBS strains from Zimbabwean patients hospitalised for various diseases, mostly infectious diseases, were tested against clindamycin, erythromycin, penicillin and tetracycline, respectively. The isolates included CAT Ia strains (n=25), CAT III strains (n=62) and CAT V strains (n=26).

When applying the breakpoints as defined in this report, clindamycin was the only antibiotic for which the isolates were uniformly susceptible (Table I). Erythromycin resistance was demonstrated by 16 (14%) of the strains, 15 (24 %) of the 62 serotype III strains and one (4 %) of the 26 type V strains. None of the type Ia strains tested showed resistance to erythromycin and non of the erythromycin resistant isolates demonstrated combined clindamycin/ erythromycin resistance. The erythromycin resistant isolates were all sensitive to quinupristin/dalfopristin, a streptogramin combination of two weakly bacteriostatic pristinamycin derivatives.

Table I: Susceptiility to four antibiotics of Zimbabwean GBS strains of the capsular antigen types Ia (n=25). III (n=62), V (n=26), with strains categorised as sensitive (s), intermediate (I), and resistant (R).

		All strains			Ia straints			III straints			V straints		
Antibiotic		S	I	R	S	I	R	S	I	R	S	I	R
Clindamycin	n	113	0	0	25	0	0	62	0	0	26	0	0
	%	100	0	0	100	0	0	100	0	0	100	0	0
Erythromycin	n	97	0	16	25	0	0	47	0	15	25	0	1
	%	86	0	14	100	0	0	76	0	24	96	0	4
Penicillin	n	111	2	0	25	0	0	60	2	0	26	0	0
	%	98	2	0	100	0	0	97	3	0	100	0	0
Tetracycline	n	0	0	113	0	0	25	0	0	62	0	0	26
	%	0	0	100	0	0	100	0	0	100	0	0	100

Sensitivity to penicillin was demonstrated by 98.2% of the isolates. Two type III strains showed intermediate resistance to penicillin with MIC of 0,25mg/l.

All of the isolates tested showed resistance to tetracycline. Distribution of the MICs of the antibiotics for which resistant GBS strains were detected, tetracycline and erythromycin are shown in Figure 1A and Figure 1B, respectively. For erythromycin, a bimodel distribution of MICs was found as is common when a proportion of the strains show resistance to an antibiotic. For tetracycline, a close to normal distribution of MICs at levels above the breakpoint for resistance is evident (Figure 1A).

Figure IA and IB: Distribution of MICs of tetracycline (A) and erythromycin (B) for 113 GBS stains isolated from patients in Zimbabwe. Break points for susceptibility (S), intermediate susceptibility (I) and resistance (R) are shown.

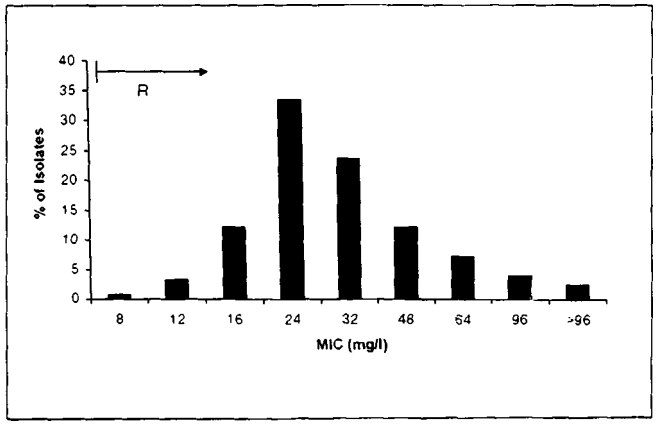
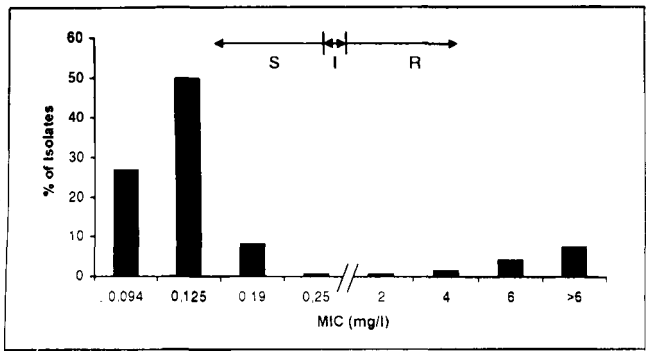


Figure 1.



Discussion

In this study, 113 GBS strains isolated from different body sites from hospitalised patients in Zimbabwe were tested for susceptibility to clindamycin, erythromycin, penicillin, and tetracycline. Studies along these lines are important as a basis for selection of antibiotics for *intrapartum* chemoprophylaxis and for the treatment of GBS infections both in neonates and in adults.

Although two of the 113 GBS strains tested showed intermediate sensitivity to penicillin, this may not mean resistance of these isolates in clinical settings. With this reservation in mind, the results of the present study are consistent with studies from various areas of the world, which have shown that resistance to penicillin (or ampicillin) has not as yet emerged in GBS.[5,10] Thus penicillins can still be considered effective in prophylaxis and treatment of GBS infections in Zimbabwe.

Erythromycin on the other hand cannot be relied upon to the same extent, since 16 (14%) of the isolates showed resistance to this antibiotic. Resistance of GBS to erythromycin has emerged in many countries over the last few years and its prevalence in other studies compares favourably with that found in the present study.[5,8]

Combined erythromycin/clindamycin/streptogramin resistance, which has been demonstrated in several studies,[6,8] is encoded by the *erm* family of resistance genes.[14] Combined resistance did not occur among the Zimbabwean GBS strains. Thus, erythromycin resistance in these isolates, was probably determined by the *mef* or *mre* family of resistance genes, both encoding protein which results in increased efflux of erythromycin but not of lincosamides or streptogramins.[14]

Our results obtained by testing Zimbabwean GBS from heterogeneous human sources compare quite favourably with results from 1996 in which vaginal GBS isolates from Zimbabwean women were examined with some discrepancies.[15] These authors detected resistance to erythromycin in 4% of the strains versus 14% in the present study, clindmycin resistance in 5% versus none in our study and no resistance to penicillin as in the present study. Thus both reports confirm the susceptibility of Zimbabwean GBS strains to penicillin (or ampicillin).

Interestingly, the erythromycin resistance was almost exclusively linked to serotype III GBS. Linkage of resistance to both types III and V strains have been described previously.[6,9] Type III strains were responsible for 91% of neonatal GBS infection in Cameroon[16] and also played a major role (41.8%) in colonised pregnant women in an area of Zimbabwe.[11] The results of the present study emphasise that erythromycin should be critically viewed in relation to prophylaxis and treatment of GBS infection in Zimbabwe, whereas clindamycin can be considered a safer alternative to penicillin for usage according to our results.

All of the 113 GBS strains showed resistance to tetracycline. Although this resistance rate is unusually high, resistance rates of more than 50% are common around the world.[6,9] Tetracycline resistance can be determined by one or more of a number of resistance genes that can also be detected in other *Streptococci,* encoding either efflux or site modification protective mechanism.[17] The high prevalence of tetracycline resistance found in the present study can probably be attributed to extensive use of tetracycline in Zimbabwe in medicine and in preventive poultry farming. The results of this study support critical consideration of such usage in the future.

In conclusion, this study, on sensitivity testing of GBS isolates of serotypes commonly found in Zimbabwe, has demonstrated that 14% of GBS isolated from humans in Zimbabwe were resistant to erythromycin and that all of the 113 isolates tested were tetracycline resistant. All isolates were sensitive to clindamycin. For penicillin, 98% of the isolates showed susceptibility to this antibiotic and 2% indicated intermediate susceptibility.

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