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Distinctive Features of Surface-Anchored Proteins of *Streptococcus agalactiae* Strains from Zimbabwe Revealed by PCR and Dot Blotting▽

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The distribution of capsular polysaccharide (CPS) types and subtypes (serovariants) among 121 group B streptococcus (GBS) strains from Zimbabwe was examined. PCR was used for the detection of both CPS types and the surface-anchored and strain-variable proteins Cα, Cβ, Alp1, Alp2, Alp3, R4/Rib, and Alp4. The R3 protein was detected by an antibody-based method using monoclonal anti-R3 antibody in dot blotting. The CPS types detected, Ia (15.7% of strains), Ib (11.6%), II (8.3%), III (38.8%), V (24.0%), and nontypeable (1.7%), were essentially as expected on the basis of data from Western countries. The type V strains showed distinctive features with respect to protein markers in that Alp3 was detected in only 6.9% of the isolates while R3 occurred in 75.9% and R4/Rib occurred in 37.9% of the isolates. R3 occurred nearly always in combination with one of the alpha-like (Alp) proteins, and it was the third most common of the proteins studied. These results show that type V GBS strains from Zimbabwe differed from type V strains from other geographical areas and also emphasize the importance of the R3 protein in GBS serotyping and its potential importance in the immunobiology of GBS, including a potential role in a future GBS vaccine.

Group B streptococci (GBS) are a major cause of neonatal disease and may also affect adults, notably immunocompromised individuals. In many Western countries, much information on incidence and other epidemiological data on infectious diseases caused by GBS are available. Less is known with respect to incidence and other aspects of GBS disease in African countries, but experienced clinicians consider GBS infections an important cause of neonatal disease, for instance, in Zimbabwe (K. J. Nathoo, personal communication). In South Africa, the burden of neonatal GBS disease during the period from 1997 to 1999 was 2.06 cases/1,000 live births and 1 case/1,000 live births for early- and late-onset disease, respectively (19). In epidemiological settings, serotyping has been extensively used to identify and trace GBS variants and, in more recent years, has been supplemented or replaced with gene-based methods, such as multilocus sequence typing, pulsed-field gel electrophoresis, restriction endonuclease digestion pattern determination, and multilocus enzyme electrophoresis, albeit some investigators have considered these methods inadequate for determining GBS relatedness as described by whole-genome analysis (29).

The serotyping of GBS is based primarily on nine different capsular polysaccharide (CPS) antigens, called Ia, Ib, and II through VIII. Recently, a new CPS type, serotype IX, has been detected (25). Strains within each CPS type may also be subdivided into subtypes or serovariants on the basis of the expression of strain-variable and surface-anchored protein antigens or the detection of the genes encoding these proteins. These antigens include members of the alpha-like (Alp) protein family, Cα (*bca*), Alp1 (*alp1*), which is closely related to Cα and was previously called epsilon (GenBank accession no. U33554), Alp2 (*alp2*), Alp3 (*alp3*), and Rib (*rib*) (18), the latter being considered identical to the classical R4 protein (26). Alp proteins possess chimeric sequences, show variable immunological cross-reactivities, have repetitive structures, show ladder-like banding patterns on Western blots, and induce antibodies which are protective in experimental models (2, 16, 18, 28). The recently defined Alp4 (14), R5 (group B streptococcal protective surface protein) (9), and R3 (31) proteins have not been characterized to the same extent as the proteins mentioned above. In addition, the nonladdering Cβ protein, known for its ability to bind to immunoglobulin A (IgA) Fc fragments, also shows strain-variable expression (18).

In an earlier study, the serotype and serovariant distribution of GBS from Zimbabwe was determined by using antibody-based methods (23). The study disclosed that the CPS type distribution is essentially similar to that recorded previously for GBS from many Western countries, with some exceptions. A very low prevalence of strains which expressed the R1 protein, which probably includes the recently named Alp2 and Alp3 (16, 21), and a high prevalence of isolates which expressed the R3 protein were noticeable (23). The isolates studied had been collected in the early 1990s. In the present study, more recently collected Zimbabwean GBS isolates were serotyped, mostly by using molecular methods, to confirm or invalidate the conclusion that GBS from Zimbabwe have serotype marker characteristics somewhat different from those of GBS from many other areas. Such mapping is important

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both in epidemiological contexts and in the context of possible future GBS vaccine formulations.

MATERIALS AND METHODS

Bacterial strains. The reference and prototype strains used in this study have been described previously (15, 22). The isolates included strains of all known CPS types except serotype IX and isolates which expressed one or more of the GBS proteins Cβ, Cα, Alp1, Alp2, Alp3, Alp4, R4/Rib, and R3. All of the strains belonged to the strain collection of the Laboratory of Medical Microbiology, St. Olavs Hospital, Trondheim, Norway. The 121 clinical Zimbabwean GBS strains under investigation were vaginal isolates from pregnant women ($n = 109$) and isolates from colonized neonates ($n = 12$), 6 of the latter from the ear and 6 from the umbilicus. All individuals were carriers without signs of GBS disease. The sampling and culturing were undertaken during the period from 2003 to 2005. Swabs were transported in Stuart's transport medium and were cultured on tryptose blood agar base with 10 mg of colistin sulfate/liter and 15 mg of nalidixic acid/liter at 37°C for 18 h, after which serogroup determination was performed by means of the Pastorex grouping kit according to the instructions of the kit manufacturer (Bio-Rad, Marnes-la-Coquette, France). Isolates were preserved in Greave's medium at −70°C. The isolates were collected at the Chitsungo ($n = 25$), Harare ($n = 47$), and Guruve ($n = 49$) health centers in Zimbabwe, meaning that they came from mainly rural, urban, and rural-urban areas, respectively.

Oligonucleotide primers. All oligonucleotide primer pairs for the identification of CPS-synthesizing genes (*cps*) were constructed by Eurogentech, S.A. (Liege, Belgium), as described by other investigators (4, 13). Primer sets constructed as described by Kong et al. (13) were as follows: for CPS Ia detection, the pair CpsIA-IacpsHS1 (amplicon size, 354 bp); for CPS type Ib detection, the set IbcpsIS-IbbcpsIA1 (523 bp); for CPS type III detection, IIIcpsHS-cpsIA (641 bp); for CPS type IV detection, IVcpsHS1-IVcpsMA (379 bp); for CPS type V detection, VcpsHS2-VcpsMA (374 bp); and for CPS type VI detection, VIcpsHS1-VIcpsIA (360 bp). For PCRs to detect the CPS types II, VII, and VIII, primer pairs as described by Borchardt et al. (4) were constructed. The sizes of the amplicons generated by these PCRs were 526, 293, and 570 bp, respectively. Primers for use in multiplex PCR for the detection of protein antigen genes were constructed as described by Creti et al. (5). The target genes included *bca*, *rib*, *alp1*, *alp2*, *alp3*, and *alp4*, encoding the proteins Cα, R4/Rib, Alp1, Alp2, Alp3, and Alp4, respectively. In these PCRs, a single forward primer targeted a conserved site in the genes and the reverse primers targeted gene-specific sites (5). The primer pair GBS1360S-GBS1937A (amplicon size, 652 bp) was used for the detection of *bac*, encoding the Cβ protein (14), and the pairs bal23S1-bal2A2 (426 bp) and bal23S1-bal3A (321 bp) were used for the separate detection of *alp2* and *alp3* (14).

PCR. Bacterial lysates were prepared as described previously (20), and the multiplex PCR was performed as described previously (5) except that the number of cycles was increased from 30 to 35 to increase the amounts of the PCR products. The remaining PCRs, designed to detect the *cps* genes, *bac*, and the individual *alp2* and *alp3* genes, were performed as described earlier (20), with some modifications. The final volume of 50 μl contained 2 μl of the template, 1× PCR buffer, 1.5 mM $MgCl_2$, 50 μM deoxynucleoside triphosphates (dATP, dCTP, dGTP, and dTTP), a 0.4 μM concentration of each primer, and 1.5 U of AmpliTaq Gold polymerase (Applied Biosystems). The PerkinElmer GeneAmp PCR system 2400 was used to carry out 35 cycles (denaturation at 94°C for 1 min, annealing at 55°C for 1.5 min, and extension at 72°C for 2 min), with a terminating delay at 72°C for 7 min. The PCR products were detected using a bioanalyzer 2100 as recommended by the manufacturer (Agilent Technologies).

Sequencing. *bac* (Cβ) PCR amplicons generated from selected GBS strains were sequenced after PCR by using the primers diluted to 3.2 pmol. The PCR products were purified with the QIAquick PCR purification kit (Qiagen). Sequencing was performed using the BigDye Terminator cycle sequencing kit version 3.1 (Applied Biosystems) and subsequent capillary electrophoresis on an ABI 313Ox genetic analyzer according to the protocols of the manufacturers. Sequence alignment analyses were done using the program Sequencher 4.2 (Gene Code Corporation, MI).

Anti-R3 protein antibodies. A previously described murine anti-R3 monoclonal antibody (R3 MAb) (15) was used. This antibody was raised by immunization with the R3 reference strain 10/84 (ATCC 49447; a type V strain expressing R3 [serotype V/R3]), was of the IgM isotype, and was R3 protein specific as deduced from immunofluorescent-antibody testing results (15). In some experiments, rabbit anti-R3 antibodies raised by immunization with whole cells of strain 10/84 were used. The whole-cell antiserum was cross-absorbed with strain 161757 (V/Alp3) and was R3 specific according to the results of testing as for the R3 MAb. The R3 MAb (ascitic fluid) and rabbit anti-R3 serum were used diluted 1:1,000 in dot blotting and Western blotting.

Immunological techniques. For dot blotting, bacteria were cultured, applied to polyvinylidene difluoride membrane strips (Bio-Rad, Richmond, CA), and tested largely as described previously (24) but with some modifications: the bacterial pellet was suspended in a volume of phosphate-buffered saline four times the pellet volume instead of nine times the volume, the volume of the bacterial suspension applied to the paper strips was increased from 3 to 5 μl, and membrane strips were first processed by being prewet briefly in 100% methanol and then in water. Peroxidase-conjugated anti-mouse IgM (Dako A/S, Glostrup, Denmark) was used to detect R3 MAb binding, and 3,3′-diaminobenzidine tetrahydrochloride (Dako) was the substrate. In all of the MAb-based testing, either distinctly colored spots corresponding to the applied bacteria or no color was developed. Testing using a nonsense MAb was always included as a control.

Western blotting was performed essentially as described previously (24). Material solubilized from whole cells of GBS with hot dodecyl sulfate was applied to 10% (wt/vol) polyacrylamide separating gel with 4% stacking gel and, after electrophoresis, transferred onto polyvinylidene difluoride membrane and probed with the R3 MAb or the rabbit anti-R3 serum (1: 1,000). Peroxidase-conjugated anti-mouse IgM antibodies (Dako) or anti-rabbit IgG antibodies (Dako) were used to detect primary antibody binding as in the dot blotting.

RESULTS AND DISCUSSION

Performance of the tests. The performance of the CPS and protein gene PCRs was evaluated by testing isolates of our collection of reference and prototype GBS strains. Isolates of all GBS CPS types except the lately described type IX (25) and isolates expressing one or more of the strain-variable proteins searched for in this study were tested. These strains showed PCR results as expected on the basis of known genotypic and phenotypic traits of the isolates. Thus, the testing confirmed the overall agreement between the results obtained by antibody-based serotyping of GBS and those obtained by molecular serotyping methods described by other investigators (4, 5, 6, 13, 14). For this reason, GBS markers defined by serology and those defined by the identification of genes encoding the markers are not distinguished in this work.

The gene encoding the R3 protein has not been sequenced. Consequently, R3 expression was detected mostly by using the anti-R3 MAb in dot blotting. The MAb showed positive results in dot blotting for the R3 reference strain ATCC 49447 (10/84; V/R3) and strain ATCC 9828 (Compton, or Prague 25/60; nontypeable [NT]/R3, Alp4) and negative results for all of our other prototype and reference strains. In whole-cell-based Western blotting, the binding of the R3 MAb was recorded only when probing was done against the R3-expressing isolate (Fig. 1, lane 4). This was also the case for the polyclonal anti-R3 antibody which had been rendered R3-specific by cross-absorption (data not shown). These results confirmed the R3 specificity of both of the antibodies described previously (15).

CPS type distribution. The distribution of CPS types among 121 Zimbabwean carrier GBS isolates is shown in Table 1. A total of 119 (98.3%) of the strains turned out to belong to one of five CPS types, Ia (15.7%), Ib (11.5%), II (8.3%), III (38.8%), and V (24.0%). Only two (1.6%) of the isolates were CPS NT. Overall, the CPS type distribution was similar to that recorded in previous testing of GBS from Zimbabwe (23) and also matches that among GBS from other geographical areas (6, 11, 12, 14). Type IV and type VI strains were not detected, as strains of these CPS types occur rarely (6, 11, 12, 14). Type VIII strains, frequently seen in Japan (17) and recently also in other areas (4, 8, 12), were not detected in the Zimbabwean

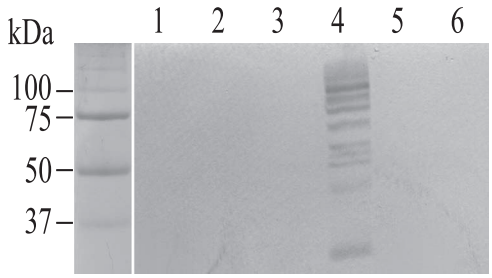

FIG. 1. Western blots of sodium dodecyl sulfate lysates of whole cells of GBS strains. Lysates from BM110 (III/R4/Rib) in lane 1, 335 (Ia/Alp1) in lane 2, ATCC 12403 (III/Alp2) in lane 3, ATCC 49447 (strain 10/84; V/R3) in lane 4, A909 (Ia/Cα, Cβ) in lane 5, and 7271 (VII/Alp3) in lane 6 were probed with the anti-R3 MAb. Protein size standards are shown to the left.

strain collection. However, this result does not exclude the existence of GBS of these CPS types in the Zimbabwean population, as only 121 isolates were serotyped. It is important that a large proportion of the isolates were type III strains, which are major cause of neonatal GBS disease (7, 19).

Serovariants and serovariant markers. GBS strains of all of the CPS types can be divided into subtypes or serovariants on the basis of surface-anchored and strain-variable proteins. In this study, we chose to search by PCR for the genes encoding the proteins Cβ, Cα, Alp1, Alp2, Alp3, R4/Rib, and Alp4. Testing for R3 was MAb based. The testing resulted in the division of the 121 GBS strains from Zimbabwe into 25 serovariants, with the highest number of variants occurring among isolates of the CPS types Ia and V, which each included 6 variants (Table 1). Alp1 (epsilon) predominated among the type Ia strains, the Cα-Cβ combination predominated among the type Ib and type II strains, and R4/Rib predominated among the type III strains, essentially similar to earlier findings for Zimbabwean GBS (23) and GBS from other areas (5, 11, 14). *alp4*, encoding Alp4, was not detected except in the reference strain 9828, in which Alp4 was originally identified (14).

Distinctive features of the Zimbabwean GBS were related to the CPS type V strains and concerned the R3 protein and the genes *alp3*, encoding Alp3, and *rib*, encoding the R4/Rib protein. The gene *alp2*, encoding Alp2, was not detected in this strain collection, but this gene and its product occur rarely (14, 18). Studies from other geographical areas have demonstrated a high prevalence of the gene *alp3* or its product, Alp3, among type V GBS, with up to 92% of type V strains carrying this marker (14). Of 33 invasive type V GBS strains isolated in Norway during the period from January to October 2007, 26 strains (78.8%) possessed *alp3* (R. V. Lyng, personal communication). Only 2 (6.9%) of the 29 Zimbabwean type V GBS strains and only 6 (5.0%) of the strains in the whole collection possessed *alp3* (Table 1). Another feature of the Zimbabwean type V strains was a comparatively high frequency (37.9%) of *rib* possession. The *rib* gene was detected in only 1 of 38 Australasian type V GBS strains (14), and R4/Rib expression in type V strains is considered to be rare (18). Also, recent observations (21, 22) have generated suspicion that R4/Rib expression by type V isolates may be overestimated when testing is performed by means of antibody-based methods. This question is due to the strong immunological cross-reactivity between R4/Rib and Alp3 and the fact that the latter protein is frequently expressed by type V strains from many geographical areas (2, 11, 16, 18). Alp3 itself probably has no protein-specific antigenic determinant, only determinants shared with other Alps (21), although the encoding gene (*alp3*) possesses at least one gene-specific primer binding site (14). Thus, available information on R4/Rib expression by type V GBS may not be reliable due to the disturbing existence of cross-reacting antibodies.

A striking feature of the Zimbabwean type V GBS was their frequent expression of the R3 protein, which was detected in 22 (75.9%) of 29 isolates, results which matched the findings for isolates collected a few years earlier (23). R3 was the third most common of the protein markers tested in this study (Table 2) and was almost restricted to the CPS type V GBS. Only four (4.3%) of the non-type V isolates expressed R3. These results show that R3, not Alp3, is a predominating surface-anchored protein in Zimbabwean type V GBS. It is possible that this is the case in larger areas of southern Africa, but this possibility remains to be verified. Although R3 was defined in 1972 (31), it has mostly been neglected in GBS serotyping, though not entirely. The protein was not detected previously among 131 U.S. strains (10). It was expressed by 6.5% of GBS isolates from Norway (15). In the earlier study of GBS from Zimbabwe, 24% of all isolates examined and 84% of the type

TABLE 1. Distribution of CPS types and serovariants among 121 GBS carrier isolates from Zimbabwe

CPS type (no. of strains; % of total strains)[a]	Serovariant	No. of strains	% of strains of CPS type[b]
Ia (19; 15.7)	Ia	2	10.5
	Ia/Alp1	12	63.2
	Ia/Cα, Cβ	1	5.3
	Ia/Alp1, Alp3	1	5.3
	Ia/Cβ, Alp1	1	5.3
	Ia/Alp1, R3	2	10.5
Ib (14; 11.6)	Ib/Cα	1	7.1
	Ib/Cβ	1	7.1
	Ib/Alp1, R3	1	7.1
	Ib/Cα, Cβ	11	78.6
II (10; 8.3)	II/Alp1	2	20.0
	II/R4/Rib	3	30.0
	II/Cα, Cβ	5	50.0
III (47; 38.8)	III	5	10.6
	III/R4/Rib	40	85.1
	III/Alp3, R4/Rib	1	2.1
	III/Alp3	1	2.1
V (29; 24.0)	V/R3	2	6.9
	V/Cα, R3	6	20.7
	V/R3, R4/Rib	6	20.7
	V/Alp1, R3	8	27.6
	V/Alp3	2	6.9
	V/R4/Rib	5	17.2
NT (2; 1.7)	NT/Alp3	1	50.0
	NT/R3, R4/Rib	1	50.0

[a] Calculated as the percentage of the total number of strains tested.
[b] Calculated as the percentage of isolates within each CPS type.

TABLE 2. Frequency of occurrence of each of the protein markers studied among 121 GBS carrier isolates from Zimbabwe and most favored CPS type associations

Protein	No. (%) of strains	Two most favored CPS type associations
R4/Rib	56 (46.3)	III and V
Alp1	27 (22.3)	Ia and V
R3	26 (21.5)	V and Ia
Cα	24 (19.8)	Ib and II
Cβ	19 (15.7)	Ib and II
Alp3	6 (5.0)	V and Ia

V strains in that collection expressed R3 (23), findings which match the results of the present study.

R3 presented with multiple bands on Western blots (Fig. 1 and 2) and with little strain variation in the molecular mass of the protein (Fig. 2). Whether the ladder-like pattern was due to a repetitive structure or was caused by the degradation of the protein, such as the hydrolysis of acid-labile bonds in the molecule (30), awaits clarification. We did not record immunological cross-reactivity between R3 and Cβ or Alp family members (Fig. 1), even when polyclonal anti-R3 was used for probing to confirm MAb-based results (data not shown). Alp family proteins have chimeric sequences, which explain why these proteins show immunological cross-reactivity (16, 18). The unique immunological specificity of R3 should accord with the unique sequence of this protein which, as yet, has not been analyzed by sequencing. A notable feature of R3 revealed in this study was its expression by strains which possessed one or another of the Alp family genes. In fact, 24 of the 26 R3-positive isolates showed combined R3 expression and Alp gene possession. On the other hand, only two isolates possessed more than one of the Alp genes, probably because Alp proteins are encoded by allelic genes (16, 18) and the gene encoding the R3 protein is not allelic, emphasizing its uniqueness. It is a possibility that the combined R3-Alp expression imparts advantages to GBS, for instance, in relation to pathogenic potential, notably since the combination occurred with particularly high frequency (69%) in type V strains, which have emerged as important pathogens in serious GBS infections (3, 6, 11, 13).

A protein called Fbs (type V group B surface protein), described in 1999 (2), was isolated from strain 10/84, the R3 reference strain used in the present study. Fbs had a molecular mass of ~110 kDa, close to that of the protein targeted by the R3 MAb (Fig. 1 and 2); was susceptible to trypsin and pepsin digestion, similar to the target of our R3 MAb, as shown in an earlier study (15); occurred frequently in type V strains and rarely in strains of other CPS types, similar to the R3 MAb target in the Zimbabwean GBS (Table 1); and was immunologically unrelated to other surface-anchored GBS proteins, also similar to the R3 MAb target (Fig. 1). Although Fbs failed to generate multiple bands upon Western blotting (2), unlike the MAb target (Fig. 1 and Fig. 2), the available data suggest that Fbs and the R3 MAb target may be identical. It is important that rabbit anti-Fbs antibodies were protective in an experimental model (2), which in the case of the two proteins' being identical, means that R3 is a target of protective antibodies.

Features of amplicons. The sizes of the amplicons generated from the various *cps* or protein antigen genes were nearly identical for each particular gene, consistent with the stability of the sequences in the amplified stretches of these genes, except for the *bac* amplicons. The *bac* gene, which encodes Cβ, gave rise to amplicons with some size variation. Since Cβ possesses at least one stretch which varies in size (1), we sequenced the PCR products from two different isolates, amplicons of 647 and 632 bp. However, both amplicons had a sequence identical to that of the corresponding stretch of the sequenced gene (*bac*) encoding Cβ (GenBank accession no. X59771). We have no explanation for the size variation of the *bac* amplicons.

Comments. This study has shown that GBS from Zimbabwe were essentially similar to GBS from other geographical areas with respect to CPS types. Strains of the CPS types Ia, Ib, II, III, and V and NT strains were detected. As expected, for the CPS types Ia, Ib, II, and III, results for the genes encoding the strain-variable and surface-anchored Alp family proteins and for *bac*, encoding the Cβ protein, were also recorded. On the other hand, serovariant protein markers of the CPS type V strains of Zimbabwean origin differed considerably from those reported for type V GBS from Western countries. As many as 38% of the Zimbabwean type V strains contained *rib*, encoding the R4/Rib protein, otherwise known to be expressed rarely by CPS type V strains (18). The R3 protein was expressed by 75.8% of the Zimbabwean type V strains, while only 6.8% of the strains possessed *alp3*, which usually predominates among type V GBS. It seems that in the type V isolates from Zimbabwe, the R3 protein occupied the position and possibly the biological function held by Alp3 in type V strains from other areas. It is tempting to speculate whether this reflects evolutionary differences among lineages of GBS carried by humans which for thousands of years have lived in separated geographical areas. Comparative genome sequencing of representative type V strains from Zimbabwe and from other areas should be of great interest.

The development of a GBS protein vaccine has been an objective for a long time. Our results show that one or two of the three most prevalent proteins, R4/Rib, Alp1 (epsilon), and R3, were present in 75.2% of the Zimbabwean GBS strains tested. Generally, surface-anchored and strain-variable GBS proteins possess protective epitopes, as evidenced by experimental models (9, 18, 26, 28). Thus, it seems possible that if

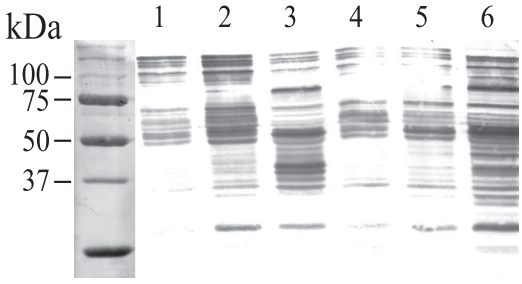

FIG. 2. Western blots of sodium dodecyl sulfate lysates of whole cells of five Zimbabwean GBS strains positive for R3 expression in dot blotting (lanes 1 to 5) and of the R3 reference strain ATCC 49447 (strain 10/84; V/R3) (lane 6), probed with the anti-R3 MAb. Protein size standards are shown to the left.

introduced in Zimbabwe, a GBS vaccine which includes these three proteins may enhance resistance against infection with a large proportion of GBS strains. Also, it seems likely that a vaccine based on these proteins may result in increased protection against invasive Cα- and Alp3-expressing GBS isolates due to Alp1-Cα cross-reactivity and R4/Rib-Alp3 cross-reactivity (2, 22, 28), offering the prospect of enhanced resistance against virtually all GBS variants found in this study. Such a vaccine also may induce protection against group A streptococcal variants which express the R28 (Alp3) protein (27, 28). The isolates under investigation were carrier strains. The serotype and serovariant distribution of invasive isolates from Zimbabwe may be different, as found by serotyping (11) or multilocus sequence typing (12) for GBS from other areas. Such data on isolates from Zimbabwe are not known but would be important. In South Africa, CPS type III strains predominate as the cause of early- and late-onset neonatal disease, while for instance, type V strains play a less prominent role (19). This pattern is different from the role of CPS type V strains in invasive GBS disease in many other areas (3, 6, 11, 13).

The results of the present study have substantiated that R3 is an important serovariant marker, at least in GBS isolated from humans in certain geographical areas. Accordingly, this protein may be important in the pathogenesis of GBS disease caused by R3-expressing strains, and as R3 may be a vaccine candidate, R3 protein detection should be pursued actively in GBS typing.

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