Comparison of streptococci of serological group B isolated from healthy carriers and active disease in Chile

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Summary. Serotyping of 50 streptococcal strains of serological group B isolated from human clinical specimens in Chile revealed mainly the serotypes Ia, II and III, either alone or in combination with protein antigens c or R. No significant difference in serotype distribution was detected between group B streptococci isolated from cervical swabs from clinically healthy women and those isolated from various pathological processes. Determination of antibiotic susceptibility of the bacteria demonstrated resistance to tetracycline and minocycline in 29 isolates. All 29 tetracycline-resistant cultures hybridised with a gene probe for tet(M). Again, no differences were detected between the group B streptococcal isolates of various origins.

Introduction

Streptococci of serological group B were originally described as causative agents of bovine mastitis. Because of this historical association with the bovine udder, these organisms were taxonomically classified as Streptococcus agalactiae. Group B streptococci are also a major cause of neonatal sepsis, late onset meningitis in infants and post-partum endometritis in women. This organism may also be carried in the vagina of pregnant women without apparent clinical consequences for the newborn.1-4 Other, less common infections caused by group B streptococci include bacteraemia, pneumonia, meningitis, endocarditis and urinary tract infections.5-12 Current knowledge of the epidemiology of group B streptococcal disease in man and the relationship between infections in man and cattle has been obtained by serotyping this organism on the basis of polysaccharide and protein antigens. Serotyping of group B streptococci from human sources has shown a predominance of the type antigen patterns Ia/c and III/R.12 Differences in serotype together with cultural and biochemical properties supported the conclusion that human and bovine isolates form two distinct biotypes.12,13 The present study was performed to serotype and further characterise group B streptococci isolated in Chile from cervical swabs of clinically healthy women and from various pathological processes.

Materials and methods

Bacterial cultures

A total of 50 group B streptococci was examined in this study. The strains, collected in two different hospitals in Santiago de Chile, were isolated from cervical swabs of clinically healthy women (28 isolates) and from pathological processes. The latter were from urinary tract infections (six isolates), foot wounds (seven isolates), meningitis (three isolates) and septicaemia (two isolates) of neonates, endometritis (two isolates), peritoneal fluid (one isolate) and a gallbladder (one isolate). Isolates were presumptively identified in Chile on the basis of their β-haemolysis and CAMP reactivity and stored on sheep blood agar at 4°C until transported to Germany for further characterisation.

Serological properties and further characteristics

Serogrouping was performed with autoclaved extracts and group B streptococcal antisera (Wellcome, Burgwedel, Germany) in immunodiffusion reactions.14 The cultures were tested additionally for sugar fermentation, for CAMP reactivity and pigment production as described previously.14 The pigment production was determined in GBS Islam Agar (Oxoid, Wesel, Germany) supplemented with sterile horse serum 50 ml/L as stab cultures in screw capped tubes in a candle jar. For serotyping, the type-specific
Antigens were obtained by extraction of the bacteria with 0.2 M HCl for 2 h at 52°C. The antigens were tested with monospecific antisera in immunodiffusion reactions as described previously. The monospecific antisera were prepared with the group B streptococcal reference strains 090 (type Ia), H36B (type Ib), 18RS21 (type II), 6313 (type III), 3139 (type IV), SS1169 (type V), NT6 (provisional type VI), 7271 (provisional type VII), Compton 24/60 (antigen X), Compton 25/60 (antigen R), A 909 (antigens Ia/c), 335 (antigen ca) and 70339 (antigen cp) by immunising rabbits. The monospecificity of the antisera was achieved by absorption of the antisera with cross-reacting antigens.

In addition, antisera against group B streptococcal reference strain JM9 (provisional type VIII) was kindly provided by M. Stilhammar-Carlemalm and G. Lindahl (Institute for Medical Microbiology, Lund, Sweden).

**Antibiotic susceptibility**

Antibiotic susceptibility of the isolates was determined on Müller-Hinton Agar (Oxoid) containing sheep blood 5% according to the Kirby-Bauer disk diffusion method with disks containing erythromycin 15 μg, clindamycin 2 μg, bacitracin 10 U, penicillin 10 U, ampicillin 10 μg, tetracycline 30 μg and minocycline 30 μg, as described previously.

**Detection of tet genes**

The genetic basis for combined resistance to tetracycline and minocycline was investigated by Southern blot hybridisation as described recently. Briefly, single colonies were cultured for 18 h at 37°C in 10 ml of Todd Hewitt Broth (THB, Oxoid) supplemented with tetracycline 15 mg/L. Total cellular DNA was prepared by repeated phenol-chloroform extraction of mutanolysin- (Sigma) and SDS-treated streptococcal cultures. After isopropanol precipitation, the DNA pellet was briefly dried in a dessicator and dissolved in 40 μl of distilled water. A 10-μl sample was checked for degradation by agarose gel electrophoresis. Samples of 10-20 μl, containing c. 1 μg of DNA, were incubated overnight at 37°C in the presence of EcoRI (Boehringer, Mannheim, Germany) 20 U and the reaction buffer supplied by the manufacturer. The resulting DNA fragments were separated in agarose 0.8% w/v gels in Tris-acetate-EDTA buffer. HindIII fragments of bacteriophage λ DNA (Gibco BRL, Paisley) served as a size standard. EcoRI-digested DNA was transferred from agarose gels to nitrocellulose membranes (Hybond ECL, Amersham-Buchler, Braunschweig, Germany) by the capillary blot procedure. Three tet gene probes were used for the identification of genes conferring combined resistance to tetracycline and minocycline. The tet(M) gene probe consisted of the 0.87-kb EcoRI-HindIII fragment of plasmid pAT101 containing 0.85 kb of the tet(M) gene from transposon Tn1545. A 1-67 kb NdeI-NdeI fragment of the tet(O) gene from Campylobacter coli, derived from plasmid pAT121, was used as a tet(O) gene probe and the tet(Q) gene probe consisted of the 1-54 kb EcoRI-PvuII fragment of plasmid pBSK1.2-5. These gene probes were labelled with the non-radioactive ECL-system (Amersham-Buchler). Hybridisation experiments and signal detection were performed strictly according to the manufacturer’s recommendations with the detection solutions supplied with the ECL kit.

The presence of plasmids in the group B streptococci was determined by a modification of the alkaline lysis method in which mutanolysin was included to aid removal of the streptococcal cell wall.

**Results**

**Serological properties and further characteristics**

All 50 cultures used in the present investigation reacted with group B specific antisera and 49 cultures gave a positive CAMP reaction in the zone of staphylococcal β lysin. In GBS Islam agar, 46 of the cultures were clearly pigmented.

The serotyping patterns of the 50 group B streptococci are summarised in the table. Eleven isolates (22%) had antigen II alone. Antigen patterns Ia/czβ and III/R each accounted for six isolates (12%) and antigen patterns Ia and III for five isolates (10%). Antigen patterns Ia/cz and II/R were each detected in four cultures (8%) and antigens II/czβ in three cultures (6%). The remaining antigen patterns Ib/czβ, II/cz, V, V/R, NT/cz and NT/R accounted for single isolates.

None of the cultures reacted with type IV, provisional type VI, VII, VIII or protein antigen X specific antisera. All cultures reacting with protein R specific antisera also reacted with antiserum produced against the purified protein antigen Rib.

**Antibiotic susceptibility and detection of tet genes**

All the group B streptococci were susceptible to clindamycin, bacitracin, penicillin and ampicillin al-

**Table.** Type antigen patterns of 50 group B streptococcal strains

<table>
<thead>
<tr>
<th>Protein antigens</th>
<th>Polysaccharide antigens</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ia</td>
<td>Ib</td>
</tr>
<tr>
<td><strong>None</strong></td>
<td>5*</td>
<td>0</td>
</tr>
<tr>
<td><strong>cz</strong></td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td><strong>czβ</strong></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>czβ</strong></td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td><strong>R</strong></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>15</td>
<td>1</td>
</tr>
</tbody>
</table>

NT, non-typable.

*Number of cultures with the respective antigen pattern.
through resistance to erythromycin was detected in one isolate. Resistance to both tetracycline and minocycline was detected in 29 isolates (58%). The genetic basis of this combined resistance to tetracycline and minocycline was investigated. No plasmids were detected in isolates that exhibited this resistance (the presence of plasmids was determined only for these 29 isolates). However, in all cases EcoRI-digested chromosomal DNA from resistant isolates hybridised with the tet(M) gene probe, whereas no hybridisation was detected with the gene probes specific for tet(O) or tet(Q). The tet(M) gene probe hybridised to EcoRI fragments of either 10.5 kb (8 isolates) or to larger fragments c. 22 kb (21 isolates) (figure). No hybridisation was detected between DNA from the tetracycline-susceptible isolates and any of the three probes.

### Discussion

Group B streptococci can be subdivided on the basis of their polysaccharide and protein surface antigens. However, at present little information is available about the distribution of serotypes among isolates of this species from human subjects in Chile. The present study was designed to serotype and further characterise group B streptococcal isolates from Chile and to compare isolates from cervical swabs of clinically healthy women with those from pathological processes. Most isolates gave a positive CAMP reaction and were pigmented in GBS Islam agar. However, one culture gave a negative CAMP reaction and four cultures showed no pigmentation. These negative reactions did not seem to be related to the origin or any other characteristics of the bacteria.

The serotyping was performed with mono-specific antisera against the currently recognised polysaccharide and protein surface antigens and antisera against the newly proposed serotype VI, strain 7271 (provisional type VII), strain JM9 (provisional type VIII) and protein antigen Rib. The serotyping results showed that most of the cultures had the polysaccharide antigens Ia, II and III, either alone or together with the protein antigens c or R. Protein antigen c predominantly occurred with its cz component alone or with its cz component in combination with the cb component. In addition, all cultures reacting with protein antigen R specific antisera also reacted with antisera produced against the purified protein Rib. This suggests at least some identity between both proteins. The relationship between Rib and group B streptococcal R proteins remains to be elucidated.

The serotyping results of the present study are in agreement with those obtained from serotyping group B streptococcal isolates from Germany and Indonesia. However, there was no significant difference between the distribution of serotypes in group B streptococci isolated from clinically healthy women and group B streptococci isolated from various pathological processes. This indicates that serotyping (even when the newly proposed polysaccharide type antigens and protein antigens are included) does not differentiate between virulent and avirulent clones of group B streptococci although the virulence of group B streptococci has been shown to be related to the release of type-specific antigens from the cell surface, in addition to the levels of the extracellular enzymes neuraminidase and protease produced.

In contrast, Musser et al. and Helmig et al. characterised group B streptococci by multilocus enzyme electrophoresis and could separate virulent and avirulent clones.

The antibiotic resistance patterns of the group B streptococci of the present study were similar to those reported previously and confirm that resistance to tetracycline and minocycline is common among group B streptococcal isolates from man and animals. The resistance determinants conferring combined resistance to tetracycline and minocycline among group B streptococci from Chile.
streptococci have been assigned to the classes M and O.\textsuperscript{17,30} Tet(Q) has been described as a novel tetracycline resistance gene from \textit{Bacteroides fragilis} which also mediates a combined resistance to tetracycline and minocycline.\textsuperscript{31} Previous studies have shown the tet(M) gene to be the predominant tet gene of group B streptococci.\textsuperscript{17} In the present investigation all isolates were shown to possess tet(M). Southern blot hybridisation showed the tet(M) genes to be located on EcoRI fragments of similar sizes to those previously reported for group B streptococci isolated from man, cattle, pigs and nutrias.\textsuperscript{11} This confirms that the distribution of the tet(M) gene among epidemiologically unrelated group B streptococci from man and animals, as well as its location within the chromosomal DNA of the respective cultures, is not related to the origin or virulence of the cultures.

The results of the present study show that group B streptococcal isolates from Chile have almost identical serotyping and antibiotic susceptibility properties to those from other parts of the world. The present study also shows there is no difference between the distribution of serotypes or antibiotic susceptibility in isolates from healthy carriers and isolates from active disease.

References