Characterization of beta-hemolytic streptococci isolated from southern sea otters (Enhydra lutris nereis) stranded along the California coast

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1. Introduction

In marine mammals, beta-hemolytic streptococci have been implicated in debilitating disease processes such as pneumonia, septicaemia, and opportunistic infections (Howard et al., 1983; Skaar et al., 1994; Swenshon et al., 1998; Thornton et al., 1998; Johnson et al., 2006; Kuiken et al., 2006). These marine mammal isolates of beta-hemolytic streptococci have been identified as Streptococcus phocae, S. dysgalactiae subsp. dysgalactiae, S. marinmammalium, S. halichoeri, S. iniae, S. canis, and S. zooepidemicus (Pier and Madin, 1976; Skaar et al., 1994; Swenshon et al., 1998; Vossen et al., 2004; Lawson et al., 2005; Johnson et al., 2006; Kuiken et al., 2006). In particular, S. phocae has been isolated from harbor seals (Phoca vitulina), grey seals (Halichoerus grypus), California sea lions (Zalophus californianus), Caspian seals (Phoca caspica) and more recently, from Atlantic salmon (Salmo salar) (Skaar et al., 1994; Vossen et al., 2004; Gibello et al., 2005; Johnson et al., 2006; Kuiken et al., 2006; Romalde et al., 2008).

Due to similar antimicrobial sensitivity, characterization is not routinely pursued to the species level. Rather, identification of beta-hemolytic streptococcal isolates is typically based on the Lancefield system. Using this system, marine mammal isolates belonging to Lancefield group G and F or those that are untypeable are presumptively identified as S. dysgalactiae subsp. equisimilis (2 of 12; 16.7%). This is the first report of S. phocae in southern sea otters and further evidence of S. phocae expressing cell surface antigens compatible with Lancefield group G typing.
system. Thirty-five beta-hemolytic streptococcal isolates were characterized by Lancefield grouping, biochemical analysis and 16 s rRNA gene sequencing.

2. Materials and methods

Sterile culturetttes (BBL CultureSwab, Becton Dickinson and Co., Sparks, MD) were used for aseptic sample collection and transport to the Microbiology Laboratory, at the Veterinary Medical Teaching Hospital, University of California, Davis. Swabs of orogenital mucosa as well as lesions in live and dead stranded sea otters were collected, streaked for isolation on 5% sheep blood agar plates and incubated at 37 °C at 5% CO₂ for 24 h. Small, beta-hemolytic colonies of catalase negative, Gram-positive cocci were isolated, inoculated into Microbank cryovials (Pro-Lab Diagnostics, Richmond Hill, Canada) and stored at −80 °C.

Lancefield serotyping was performed using a Streptex kit (Wellcome Diagnostics, Dartford, England) into Lancefield group A, B, C, D, F and G by latex bead antibody agglutination (Skaar et al., 1994). Biochemical characterization was performed using an API (analytical profile index) 20 Strep identification system (bioMerieux, Inc., Hazelwood, Missouri), as described (Skaar et al., 1994; Lawson et al., 2005). All isolates were simultaneously incubated with BBL Bacitracin discs (Becton Dickinson Microbiology Systems, Cockeysville, MD) and any zone of inhibition was interpreted to indicate susceptibility (Skaar et al., 1994; Swenshon et al., 1998).

Sequence analysis of the 16 s ribosomal RNA gene was performed on a representative sample (N = 12) from groups G, F and untypeable. A single colony from each isolate was inoculated onto a 5% sheep blood agar plate and the resulting pure culture was used for DNA extraction. DNA templates were extracted from a crude lysis of the resulting pure culture was used for DNA extraction. Isolate was inoculated onto a 5% sheep blood agar plate and performed on a representative sample (Skaar et al., 1994; Swenshon et al., 1998). Incubation with BBL Bacitracin discs (Becton Dickson Lawson et al., 2005). All isolates were simultaneously considered very good, based on criteria defined by the manufacturer of the API Strep 20 system. Group F, C and untypeable isolates were variably identified as S. dysgalactiae subsp. equisimilis, Gemella haemolytica or untypeable.

Sequence analysis of a portion of the 16 s RNA gene from 12 selected isolates within Lancefield groups G, F and untypeable resulted in identification of 1 (100%) untypeable isolate, 4/4 (100%) group F isolates and 5/7 (71.4%) group G isolates were S. phocae (see Table 1). Sequence similarity between these isolates and the reference strain (ATCC# AJ621053.1) was high, between 98 and 100%. The remaining two group G isolates were identified as S. dysgalactiae subsp. equisimilis and were 99% similar to the reference strain (ATCC® EU075065). All sequences were submitted to GenBank (see below). Biochemical profiles of the select isolates identified by sequence analysis were compared to the previously published profiles of S. phocae, S. canis and S. dysgalactiae subsp. equisimilis in Table 2.

4. Discussion

Thirty-five isolates of beta-hemolytic streptococci obtained from southern sea otters of the coast of California between 1998 and 2002 were characterized in this study. Results indicated that the majority of isolates were S. phocae, that the S. phocae isolates expressed cell surface antigens of Lancefield groups G, F, C or were untypeable, and that acidification of sugars varied slightly from

### Table 1

<table>
<thead>
<tr>
<th>Streptococcus sp.</th>
<th>ID#</th>
<th>Lancefield group</th>
<th>Similarity (%)</th>
<th>Non-identities</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. phocae</td>
<td>37</td>
<td>G</td>
<td>99</td>
<td>2/240</td>
</tr>
<tr>
<td></td>
<td>67</td>
<td>F</td>
<td>99</td>
<td>2/876</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>F</td>
<td>99</td>
<td>2/866</td>
</tr>
<tr>
<td></td>
<td>88</td>
<td>F</td>
<td>98</td>
<td>6/452</td>
</tr>
<tr>
<td></td>
<td>91</td>
<td>F</td>
<td>100</td>
<td>0/344</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>G</td>
<td>99</td>
<td>1/819</td>
</tr>
<tr>
<td></td>
<td>63</td>
<td>G</td>
<td>99</td>
<td>2/879</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>G</td>
<td>100</td>
<td>0/369</td>
</tr>
<tr>
<td></td>
<td>79</td>
<td>G</td>
<td>100</td>
<td>0/804</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>G</td>
<td>99</td>
<td>2/435</td>
</tr>
<tr>
<td>S. dysgalactiae</td>
<td>5</td>
<td>G</td>
<td>99</td>
<td>3/903</td>
</tr>
<tr>
<td>subsp. equisimilis</td>
<td>70</td>
<td>G</td>
<td>99</td>
<td>2/888</td>
</tr>
</tbody>
</table>

a 16s rRNA gene sequence to ATCC strain AJ621053.1 or EU075065.
b Number of dissimilar nucleotides/total number of nucleotides compared to ATCC strain.
Table 2
Biochemical characteristics which differentiate Lancefield group G Streptococcus phocae from other streptococcal isolates in marine mammals.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>S. phocae (N = 10)</th>
<th>S. dysgalactiae sp. (N = 2)</th>
<th>S. phocae</th>
<th>S. canis</th>
<th>S. dysgalactiae subsp. equisimilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lancefield group</td>
<td>G</td>
<td>F</td>
<td>U</td>
<td>G</td>
<td>F, C, U</td>
</tr>
<tr>
<td>Beta-hemolysis</td>
<td>+*</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bacitracin susceptibility</td>
<td>v</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Production of:
- Alkaline phosphatase: +, +, +, +, +, +, ND, +, +
- Leucine arylamidase: +, +, +, +, ND, ±, ±, +, ±
- Arginine dihydrolase: v, +, +, +, +, +, +, ±, ±
- Alpha-galactosidase: –, –, –, –, ND, ±, ±, +, ±
- Beta-galactosidase: –, –, –, –, ND, ±, ±, +, ±
- Beta-glucuronidase: –, –, –, –, ND, ±, ±, +, ±
- Acid produced from:
  - Ribose: v, v, +, +, +, +, +, +, +
  - Lactose: v, v, –, –, –, –, +, –, –
  - Starch: +, v, +, +, –, –, –, –, –
  - Glycogen: +, v, +, +, ±, ±, ±, ±, ±
  - Hippurate: v, –, –, v, ND, ND, ND, ND, ND
  - Trehalose: v, v, –, –, v, ND, ND, ND, ND

* Skaar et al. (1994).
* Devriese et al. (1886), Efstratiou et al. (1994).
* (U) Untypeable.
* (+) More than 90% positive, (−) more than 90% negative, (v) 11–89% positive, (±) weak reaction, (ND) not done.

Previously published reports of S. phocae. Fewer isolates were identified as S. dysgalactiae subsp. equisimilis and were typed as Lancefield group G. This is the first report of S. phocae in sea otters and further demonstration of Lancefield group G antigen expression by S. phocae.

Standard bacteriological methods were not adequate for identification of the isolates of beta-hemolytic streptococci from the sea otters sampled. Initial identification based on Lancefield grouping was not consistent with the biochemical analysis using the Strep API 20 system. Similar difficulties in identifying beta-hemolytic streptococci from marine mammals and fish have been reported (Gibello et al., 2005; Kuiken et al., 2006; Romalde et al., 2008). Sequencing of the 16 s rRNA gene revealed that a majority of the group G isolates were S. phocae and the remainder were S. dysgalactiae subsp. equisimilis. Sequence analysis of the 16 s rRNA gene is considered a more reliable means for species identification and most consider a minimum of 97–98% sequence similarity as adequate for identification (Kolbert and Persing, 1999; Johnson et al., 2003; Gibello et al., 2005).

Reactions with Lancefield group G antiserum was not observed in the original description of S. phocae (Skaar et al., 1994) or in subsequent studies (Henton et al., 1999; Vossen et al., 2004); however, group G isolates of S. phocae from California sea lions had been observed by our group (Johnson et al., 2006) and in Atlantic salmon (Romalde et al., 2008). Group C isolates were not sequenced in the present study because of their low prevalence (2/35 isolates) and Lancefield group and biochemical similarity to previous reports of S. phocae (Skaar et al., 1994; Vossen et al., 2004; Lawson et al., 2005).

In summary, S. phocae groups G and F were the predominate isolates of beta-hemolytic streptococci cultured from stranded southern sea otters. Sequencing of the 16 s rRNA gene was required for identification of isolates as S. phocae. Further work is in progress to characterize other bacterial pathogens of sea otters.

5. Nucleotide sequence accession numbers

The nucleotide sequences corresponding to the 16 s rRNA gene of S. phocae 37, 67, 72, 88, 91, 7, 63, 64, 79 and 90 have been assigned the respective accession numbers FJ429782–FJ429791 in the GenBank database. Nucleotide sequences corresponding to the 16 s rRNA gene of S. dysgalactiae subsp. equisimilis 5 and 70 have been accessioned as FJ429792 and FJ429793 in the GenBank database.

Acknowledgments

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References


