Toxigenic bacteria and sudden infant death syndrome (SIDS): nasopharyngeal flora during the first year of life

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Received 1 October 1998; accepted 24 February 1999

Abstract

Many developmental and environmental risk factors for sudden infant death syndrome (SIDS) are similar to those for susceptibility to respiratory tract infection, and toxigenic bacteria have been implicated in some SIDS cases. We assessed nasopharyngeal flora of healthy infants in relation to risk factors to determine which species best fit the mathematical model proposed for the common bacterial toxin hypothesis and if these findings complemented results obtained from SIDS cases which occurred during the period of the survey. Longitudinal studies were carried out between April 1993 and March 1996 on 253 healthy infants and their mothers, 150 from a multiply deprived area, 103 from an affluent area. Concurrent SIDS infants (37) were screened for nasopharyngeal flora. Among healthy infants ≤3 months of age, the predominant isolate was Staphylococcus aureus, 57% compared with 86% for SIDS infants in that age range (P < 0.02). There were significant associations between isolation of different species from both mother and baby but no association between isolation of any species with: area of residence; parental smoking habits; breast or bottle feeding; symptoms of viral infection; seasonality. We conclude that S. aureus fits the mathematical model for SIDS. Both staphylococci and/or their toxins were identified in a significant proportion of SIDS cases. Isolation of staphylococci from healthy infants was associated with the 2-4-month age range, a risk factor consistently found in all epidemiological studies of SIDS. This might reflect the developmental stage in which 80-90% of infants express the Lewis a antigen which we have shown to be one of the receptors for S. aureus. © 1999 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: Sudden infant death syndrome; Staphylococcus aureus; Risk factor

1. Introduction

Many of the risk factors associated with sudden infant death syndrome (SIDS) are also risk factors for respiratory tract infections [1-5]; however, the most consistent and unique characteristic of SIDS is its age distribution with the peak incidence between 2 and 4 months. Although there has been a dramatic decline in the numbers of SIDS in Britain during the past 8 years, the peak incidence is still in this age group with the majority occurring among infants 3 months of age or younger [6]. It has been
suggested that toxins associated with bacteria that infants commonly encounter during the first few months of life might be adsorbed through the respiratory tract and precipitate the series of events that lead to SIDS [1–5]. There is evidence for involvement of both endotoxins of Gram-negative bacteria [7] and powerful exotoxins produced by Staphylococcus aureus [8–10]. Epidemiological studies have also implicated Bordetella pertussis [11,12].

Morris et al. [1] proposed a mathematical model which closely predicted the age distribution of SIDS if these deaths were due to a single common toxin which 50% of the population met in any 50-day period. Expression of the Lewis blood group antigen in infants also parallels the age distribution of SIDS [2,13,14], and our group has demonstrated that there is an adhesin on S. aureus that binds to this antigen on epithelial cells [14,15]. We have also demonstrated in vitro that smoking and virus infection enhance the ability of epithelial cells to bind some of the bacteria isolated from SIDS infants or implicated by epidemiological studies [14,16]. In this study we examined the nasopharyngeal flora of infants to determine which bacterial species most closely fit the pattern predicted by Morris et al. [1]. The objectives of the study were:

1. to assess ‘normal’ flora of healthy infants and their mothers to identify bacteria for more detailed study in relation to their role in SIDS;
2. to determine if risk factors associated with SIDS influenced isolation of potentially pathogenic bacteria, e.g., age range, formula rather than breast feeding [17], maternal smoking [18–20], viral infection [21,22];
3. to compare isolation rates for the different species for healthy infants and SIDS cases.

2. Subjects and methods


From April 1993 to March 1996 infants \( (n = 253) \) and their mothers from two areas of Edinburgh were recruited into the study; one group \( (n = 150) \) was from a multiply deprived area (Muirhouse) and the other \( (n = 103) \) from an affluent area (Stockbridge/New Town). In Scotland, a method for assessment of social deprivation has been developed based on the following factors: overcrowding; male unemployment; low social class (based on the proportion of persons in private households in which the head of the household is in semi-skilled or unskilled employment); lack of a car. Areas identified by the postcodes in the major towns and cities have been assigned to categories designated 1 (affluent) to 7 (deprived) [23]. The postcode for the family home was used in this study to complement other socioeconomic data obtained.

The study was approved by the Lothian Health ethics committee. Informed consent was obtained from mothers who were recruited by the research nurse and health visitors at the 6-week postnatal medical check up. Information on socioeconomic background and medical history was obtained by the research nurse who helped the mother fill in a standardised questionnaire at the first interview.

At each visit, saliva samples were collected from the infants with sterile plastic pipettes and nasal and throat swabs were obtained from both mother and infant. Both mother and infant were screened four times during the year following recruitment: visit 1 when the infant was 6–8 weeks old; visit 2 between 3 and 6 months; visit 3 between 7 and 9 months; visit 4 between 10 and 13 months.

Information from the questionnaire was coded to ensure confidentiality and entered into a database to which results of the bacterial cultures were added.

Randomly selected saliva specimens from the infants \( (n = 85) \) were examined by Clinical Biochemistry, Royal Infirmary, Edinburgh using the method described by Feyerabend and Russell [24] for presence of cotinine (a metabolite of nicotine) to compare with the reported number of cigarettes smoked in the household per day.

2.2. Screening of SIDS infants for respiratory flora

Nasopharyngeal swabs and secretions were obtained from 37 SIDS infants during the period of the survey. These were provided by Dr J.W. Keeling and Dr N. Smith from autopsies at the Royal Hospital for Sick Children, Edinburgh. The swabs were
cultured on the same selective media used in the survey of healthy infants.

2.3. Isolation and identification of bacterial species

The swabs were cultured on selective media for the following: *S. aureus* (nutrient agar with 5% NaCl, Oxoid); β-haemolytic *Streptococcus pyogenes* (crystal violet blood agar, Oxoid); *Neisseria* species (Modified New York City medium, Cherwell Laboratories); *B. pertussis* (charcoal agar with cepalexin, Oxoid); and *Haemophilus influenzae* (Columbia agar with chocolate horse blood and bacitracin, Oxoid). The bacterial isolates obtained from the selective medium were characterised by standard methods.

2.4. Statistical methods

Association between prevalence of bacterial isolation and other factors was tested by the χ² or Mantel-Haenzel tests. McNemar’s test was used to compare prevalence of isolation at different visits. Seasonal and time trends were tested by logistic regression. Spearman’s correlation coefficient was used to assess salivary cotinine levels in relation to the reported number of cigarettes smoked in the household per day.

3. Results

3.1. Isolation of bacterial species from healthy infants and their mothers

There were significant differences in deprivation categories between the two areas of the city which are summarised in Fig. 1. Two of the risk factors associated with SIDS, exposure to cigarette smoke and formula feeding, were significantly more prevalent among the residents in Muirhouse (Table 1). There was a significant correlation between the numbers of cigarettes smoked in the household per day and the levels of cotinine in the infant saliva specimens \((r = 0.71, P < 0.001)\).

In Britain, most SIDS deaths now occur during the first 3 months of life during which the predominant nasopharyngeal isolate was *S. aureus* (Fig. 2).

The proportion of *S. aureus* isolates declined significantly with age but there were significant increases in the proportions of β-haemolytic streptococci and haemophilus isolates during the year. Isolation of *Neisseria lactamica* did not vary significantly and *B. pertussis* was not isolated from any of the infants (Table 2). There was only one isolate of *B. pertussis* and this was from a mother.

Until the last visit, there was a significant correlation between isolation of most species from both infant and mother (Table 3). There were no significant correlations between isolation of any of the bacterial species and area of the city in which the infant lived, seasonality, maternal smoking or number of cigarettes smoked per day in the household.

### Table 1

<table>
<thead>
<tr>
<th>Factor</th>
<th>Stockbridge</th>
<th>Muirhouse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Mother smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>nil</td>
<td>91 (89)</td>
<td>52 (35)</td>
</tr>
<tr>
<td>1–10</td>
<td>11 (11)</td>
<td>39 (26)</td>
</tr>
<tr>
<td>11+</td>
<td>0 (0)</td>
<td>58 (40)</td>
</tr>
<tr>
<td>Father smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>nil</td>
<td>73 (72)</td>
<td>68 (46)</td>
</tr>
<tr>
<td>1–10</td>
<td>20 (20)</td>
<td>20 (13)</td>
</tr>
<tr>
<td>11+</td>
<td>9 (9)</td>
<td>61 (41)</td>
</tr>
<tr>
<td>Other smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>nil</td>
<td>100 (98)</td>
<td>113 (76)</td>
</tr>
<tr>
<td>1–10</td>
<td>2 (2)</td>
<td>21 (14)</td>
</tr>
<tr>
<td>11+</td>
<td>0 (0)</td>
<td>15 (10)</td>
</tr>
<tr>
<td>Infant feeding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>breast</td>
<td>72 (74)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>bottle</td>
<td>25 (26)</td>
<td>150 (99)</td>
</tr>
</tbody>
</table>

\(P < 0.001\) for each category for the two areas.
method of feeding (formula or breast feeding) or symptoms of respiratory or gastrointestinal infection.

3.2. Isolation of bacterial species from SIDS infants

The predominant species isolated from 37 SIDS infants during the period in which the healthy infants were studied was also \textit{S. aureus}. These bacteria were isolated from 19/22 (86.4\%) SIDS infants 3 months or younger compared with 143/253 (56\%) of the healthy infants sampled in this age range ($\chi^2 = 5.32$, $P = 0.02$) and 7/15 (46.6\%) of those over 3 months of age compared with 235/672 (35.2\%) samples obtained from healthy infants at the second, third and fourth visits.

### Discussion

In the mathematical model for SIDS proposed by Morris et al. [1], the infant population would encounter the toxigenic bacteria involved in precipitating the series of events leading to death within the first 50 days of life. The present study suggests that \textit{S. aureus} is the species that most closely fits this model. It was the predominant isolate from healthy infants in the survey during the first 3 months of life (57\%); the majority of the infants were screened at the age of 6 weeks. This is the period in which most SIDS cases in Britain now occur [6] and \textit{S. aureus} was isolated from a significantly higher proportion of SIDS infants in the same age range (86.4\%). These figures for isolation of \textit{S. aureus} are higher than those reported in an earlier series (41\%) [26]. Use of the selective medium (5\% NaCl) might have increased the number of positive cultures in the present study.

In addition, in a complementary study, we identified pyrogenic staphylococcal toxins in the tissues of 50\% of local SIDS infants from whom fresh tissues were available and a similar proportion of French (55\%) and Australian (53\%) SIDS infants from whom formalin-fixed tissues were available. In the Australian series, only 16\% of the comparison group of infants who died of other causes had evidence of pyrogenic toxins, two from infants who died of pneumonia and one who died of complications of cystic fibrosis [27].

The significant association between isolation of the bacteria from both infant and mother indicates that
the mother is the primary source of the infant's 'normal flora' which would be expected as she has the most frequent and most intimate contact. Except for age range, the other known risk factors investigated did not affect frequency of isolation of \textit{S. aureus} or other bacteria. Infants in poorer socioeconomic conditions are much more likely to have families in which one or more members smoke (Table 1); but neither poorer conditions nor maternal smoking was associated with isolation of any of the species examined. Symptoms of respiratory or gastrointestinal infection did not affect isolation rates. Although human milk contains components thought to reduce colonisation of infants by potentially pathogenic bacteria [28], there was no difference in isolation of any of the species from bottle-fed infants compared with those who were breast-fed.

A Swedish study of nasopharyngeal flora of infants found a pattern similar to that observed in our local population in which \textit{S. aureus} was the predominant isolate during the first 3 months of life [29]. In the paper by Harrison et al. [30] the isolation rate for \textit{S. aureus} from infants in the age range 0–3 months was similar. During the age range in which infants appear to be most vulnerable to SIDS, most express the oligosaccharide Lewis\textsuperscript{a} antigen to which both staphylococci and \textit{B. pertussis} bind [14–16]. The peak incidence of SIDS appears to coincide with the expression of Lewis\textsuperscript{a} and isolation of \textit{S. aureus} (Fig. 3). Binding of toxigenic staphylococcal strain NCTC 10655 was demonstrated to be associated with the level of Lewis\textsuperscript{a} expressed on epithelial cells [14]. These observations suggest a parallel with the vulnerability of calves to colonisation by the enterotoxigenic K99 strains of \textit{Escherichia coli}. The receptor most avidly recognised by the adhesins on the \textit{E. coli} K99, the oligosaccharide N-glycolyl neuraminic acid 2,3-galactose, is expressed abundantly in the first weeks of life during which the calves are susceptible to the diarrhoeal disease caused by these bacteria. As the expression of antigen declines, so does susceptibility to colonisation and disease [31].

Factors such as expression of bacterial adhesins and host cell receptors that enhance density of colonisation of epithelial cells are associated with development of disease by both invasive and toxigenic bacteria [32]. Moderate to heavy growth of potentially toxigenic bacteria was observed in the nasopharyngeal cultures of SIDS infants [1,26]. Some indigenous populations have high incidences of SIDS, e.g., Native Americans, Australian Aborigines and the Maori of New Zealand [33–35]. Other studies on susceptibility to infectious agents indicate that these groups are colonised earlier and more densely by potentially pathogenic bacteria [36,37].

Evidence from laboratory investigations indicates

<table>
<thead>
<tr>
<th>Sample</th>
<th>\textit{S. aureus}</th>
<th>\textit{S. pyogenes}</th>
<th>\textit{N. lactamica}</th>
<th>\textit{N. meningitidis}</th>
<th>\textit{H. influenzae}</th>
</tr>
</thead>
<tbody>
<tr>
<td>mother</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>65 (50)</td>
<td>24 (10)</td>
<td>10 (4)</td>
<td>0 (0)</td>
<td>16 (10)</td>
</tr>
<tr>
<td>+</td>
<td>78 (66)</td>
<td>9 (41)</td>
<td>4 (100)</td>
<td>1 (50)</td>
<td>41 (43)</td>
</tr>
<tr>
<td>(P)</td>
<td>(&lt; 0.05)</td>
<td>(&lt; 0.001)</td>
<td>(&lt; 0.001)</td>
<td>(&lt; 0.001)</td>
<td>(&lt; 0.001)</td>
</tr>
<tr>
<td>2</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>mother</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>35 (29)</td>
<td>38 (18)</td>
<td>11 (5)</td>
<td>0 (0)</td>
<td>36 (23)</td>
</tr>
<tr>
<td>+</td>
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<td>15 (56)</td>
<td>3 (100)</td>
<td>0 (0)</td>
<td>52 (60)</td>
</tr>
<tr>
<td>(P)</td>
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<td>(&lt; 0.001)</td>
<td>(&lt; 0.001)</td>
<td>NS</td>
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</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>mother</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>28 (25)</td>
<td>38 (19)</td>
<td>9 (4)</td>
<td>1 (0)</td>
<td>53 (36)</td>
</tr>
<tr>
<td>+</td>
<td>45 (40)</td>
<td>21 (70)</td>
<td>2 (33)</td>
<td>1 (100)</td>
<td>55 (66)</td>
</tr>
<tr>
<td>(P)</td>
<td>(&lt; 0.05)</td>
<td>(&lt; 0.001)</td>
<td>(&lt; 0.05)</td>
<td>(&lt; 0.001)</td>
<td>(&lt; 0.001)</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mother</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>17 (18)</td>
<td>40 (24)</td>
<td>10 (5)</td>
<td>1 (1)</td>
<td>44 (33)</td>
</tr>
<tr>
<td>+</td>
<td>43 (43)</td>
<td>10 (33)</td>
<td>3 (50)</td>
<td>0 (0)</td>
<td>48 (79)</td>
</tr>
<tr>
<td>(P)</td>
<td>(&lt; 0.001)</td>
<td>NS</td>
<td>(&lt; 0.001)</td>
<td>NS</td>
<td>(&lt; 0.001)</td>
</tr>
</tbody>
</table>

Figures are number (%) of babies from whom the bacteria were isolated.
that some of the other risk factors might affect density of bacterial colonisation or the host’s inflammatory responses to bacterial toxins. Cells from the mouths of smokers bind greater numbers of staphylococci, B. pertussis and several Gram-negative species than cells from non-smokers [16,38]. In an in vitro model, the epithelial cell line HEp-2 infected with either serotype A or serotype B of respiratory syncytial virus (RSV) bound significantly more staphylococci, B. pertussis and several species of Gram-negative bacteria than the uninfected HEp-2 cells [14,39,40]. It has been suggested that the prone sleeping position might enhance density of colonisation by nasopharyngeal flora, particularly in the presence of upper respiratory tract infection [41]. Harrison et al. [30] found both viral infection and prone sleeping position in the 12–18-month age range significantly enhanced both the numbers of species and the total bacterial count in nasopharyngeal swabs obtained from infants in their study.

In contrast to Harrison et al. [30], we observed no seasonal variation in isolation of the bacterial species examined, the winter peak of SIDS still observed in Scotland suggests that respiratory virus infection might be an important cofactor. Many SIDS infants are reported to have had symptoms of an upper respiratory tract infection prior to death. The significant associations reported between maternal smoking and SIDS [17–19] might reflect increased susceptibility to upper respiratory tract infections. In studies on exposure to cigarette smoke and respiratory infection in children, the strongest correlation is usually with the mother’s smoking [42]. In model systems, both components of cigarette smoke and viral infection have been demonstrated to enhance the lethal effects of bacterial toxins [43] or the inflammatory responses to them [44–47].

Although it has been suggested that some cases of SIDS might be due to asymptomatic whooping cough [11], we did not isolate B. pertussis from any of the healthy or SIDS infants tested. We did not take pernasal swabs which are thought to be the best samples as these are uncomfortable for the infant. These bacteria are difficult to culture, even from patients with symptomatic whooping cough; however, the isolate obtained from one of the mothers suggests our selective medium was adequate. Our studies indicate that S. aureus is the most likely candidate for the model proposed by Morris et al. [1]. We have identified staphylococcal toxins reported by other groups, TSST-1 and SEC1 [8,9], in tissues of the majority of SIDS infants tested since the technique was developed. The toxins are produced by the bacteria only at temperatures between 37 and 40°C [48], and the normal temperature of the nasopharynx is below this range [49]; therefore, even if infants are colonised by these bacteria, there must be other cofactors such as viral infection or the prone sleeping position which create a suitable environment in which the toxin can be induced. Recent studies by our group indicated that in the prone position, the nasal temperature of children can reach 37°C [50].

Staphylococcal toxins, TSST and SEC1 act as superantigens and can induce toxic shock and death in previously healthy adults [51] and we have demonstrated TSST-1 in tissues of a 6-year-old who died suddenly and unexpectedly following parainfluenza infection [52]. These toxins have been identified among SIDS infants in Britain, France and Australia. In our local population there was a decline in antibodies to TSST and SEC1 during late pregnancy but not SEA or SEB [53]. Methods to reduce colonisation by S. aureus or methods to boost passive or active immunity to these toxins during the early months of life might further reduce the numbers of SIDS deaths.

Acknowledgements

This study was supported by a grant from the Scottish Cot Death Trust. We are grateful to the families who participated in this study, to the physicians and health visitors who helped with recruitment and follow-up, to D. Jarvie and D. Simpson of the Department of Clinical Biochemistry, Royal Infirmary, Edinburgh for analysis of cotinine levels in saliva, and to A.T. Saadi, M.W. Raza, S.D. Essery, A.E. Gordon and O. Al Madani for helpful discussions in relation to this work.

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