Flavobacterium meningosepticum infection: an epidemiological study in a newborn nursery

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SUMMARY After an outbreak of flavobacterium meningitis in the newborn nursery of the University Hospital, an investigation was carried out to determine the possible sources of this organism in the nursery. Various serotypes of the organism were recovered from a variety of sources such as wash basins, sinks, suction apparatus, and disinfectants in the nursery as well as the neighbouring wards. Colonisation of the pharynx with this organism was demonstrated in several clinically healthy babies in the nursery. The possible role of fomites, hospital staff, newborn babies, and their mothers in the transmission of the organism is considered together with the measures taken to reduce environmental contamination.

Flavobacterium meningosepticum, a Gram-negative water bacillus, is sometimes responsible for epidemic meningitis in newborn infants in hospital nurseries.1–6 The infection in babies tends to be severe with a high mortality rate and serious neurological sequelae in the surviving patients. Brody et al.1 described two outbreaks of meningitis caused by F. meningosepticum affecting primarily premature infants in a hospital nursery. This outbreak was described as hospital-acquired, but attempts to isolate the organism from human contacts were unsuccessful. Cabrera and Galen2 reported an outbreak of 14 cases of neonatal meningitis due to F. meningosepticum. The source was traced to a leaking sink trap in an anteroom of the premature nursery. Repair of the faulty leaky trap eradicated the reservoir of this infection and terminated the outbreak. George et al.3 also reported 14 cases of meningitis and 30 asymptomatic nasopharyngeal carriers during an outbreak in their newborn nursery. Plotkin and McKitrick4 described two cases of flavobacterium meningitis which were traced to a saline solution. This saline solution containing flavobacteria was stored in a glass bottle that was seldom sterilised but refilled with sterile saline as needed. The solution was aspirated into a rubber bulb and used to flush the eyes of infants after the application of silver nitrate solution. More recently, Coyle-Gilchrist et al.7 isolated F. meningosepticum from aqueous chlorhexidine gluconate (Hibitane) solutions used in the wards for the storage of thermometers and for routine disinfection.

King,8 at the Center for Disease Control, Atlanta, USA, used serological procedures for typing strains isolated in epidemiological studies. Six serotypes (A to F) have been described, type C being responsible for most of the cases of meningitis.4 8 9

Clinical cases

Table 1 presents the relevant data on seven confirmed cases of flavobacterium meningitis acquired in the University Hospital since 1972. F. meningosepticum was isolated from the cerebrospinal fluid in all seven infants, from blood in three infants, and from peritoneal fluid in one infant (case 6). Four strains were found to be serotype C, two were of serotype F (cross-reacting with serotype E), while one strain could not be classified into any of the six serotypes.

Cases 1 and 2 who died did not receive intraventricular chemotherapy. The other babies received appropriate antibiotics such as rifampicin SV, erythromycin, or novobiocin, for it has been shown that these organisms have an unusual pattern of antibiotic sensitivity, being generally resistant to the conventional chemotherapeutic agents used in the treatment of neonatal meningitis, that is, ampicillin, gentamicin, kanamycin, and chloramphenicol.10 11 The treated babies who survived the infection are being followed up. Cases 3, 4, and 5 are normal
neurologically. Case 6 has hydrocephalus with severe brain damage; the infection in this infant began with umbilical sepsis and peritonitis. The infecting organism was isolated from the peritoneal fluid two days before the cerebrospinal fluid yielded the same organism. Case 7 also has hydrocephalus without showing any other evidence of neurological handicap. This infant, the elder of twin boys, was nursed in a cot alongside his twin who was in an incubator in the nursery. The younger twin did not become ill, and *F. meningosepticum* was not isolated from his throat.

The first four cases were widely separated in time and were not thought to be related. However, case 5 occurred within three months of case 4, and the infecting organisms were found to be identical biochemically as well as serologically. It was also noted that case 5 occupied an incubator used earlier by case 4. Accordingly, an investigation was undertaken to discover the possible source of the infections. When case 6 and, later, case 7 occurred due to other serotypes, the epidemiology became more complex than that involving a single serotype.

### Epidemiological Investigations

**Background**

Approximately 400 live babies delivered in this hospital each month are accommodated in three areas of the maternity block. Infants who appear normal at birth are nursed next to their mothers in one of two postnatal wards. Those from premature or complicated and septic deliveries (about 60 a month) are admitted to the special care nursery which can accommodate a maximum of 19 babies. The special care nursery has two big rooms, nursery I and nursery II, to accommodate eight babies each, and three single isolation rooms for infected babies.

**Bacteriology**

In order to detect *F. meningosepticum* in the environment, equipment, and various fomites and throat swabs of staff and patients, a large number of samples were collected for examination from the nursery and neighbouring wards. These materials were plated on to ox or human blood agar (blood agar base (Oxoid) with 10% blood), McConkey agar (Oxoid), and modified Thayer Martin medium (BBL) containing vancomycin 3 μg/ml, colistin 7.5 μg/ml, nystatin 1250 units/ml, and trimethoprim lactate 5 μg/ml.

Surface areas in the nursery, such as incubators, which do not contain any water for humidification, bassinets, weighing machines, suction tubings, and sinks, were sampled using swabs premoistened with sterile nutrient broth. After collection, the swabs were inoculated on to agar plates and/or into nutrient broth (Oxoid). Aliquots of fluids from various sources were cultured directly in nutrient broth. After one to two days' incubation, the broth cultures were plated on to blood agar and McConkey agar. Disinfectants were examined using the in-use test of Kelsey and Maurer. Culture plates were exposed for air sampling for 4 hours at different times of the day in various locations in the nursery. The ward air was also cultured using a slit sampler on several occasions. More than 1450 culture specimens were examined between August 1976 and June 1977, including 91 throat swabs from 46 newborn babies in the nursery. In addition, 585 sputum specimens and 475 vaginal swabs sent for routine culture between February and August 1977 were also plated on modified Thayer Martin medium to detect *F. meningosepticum* as a supplement to our search for the organisms biologically identical with the strains causing meningitis in our newborn babies.

Tap water from several wards was collected for filtration through sterile disposable membrane filters of 0.45 μm pore size (Falcon). After filtration the membranes were transferred to agar plates. The agar plates and broth cultures were incubated aerobically at 37°C for two to seven days. Suspicious-looking colonies were picked out and inoculated into tubes of Kliger iron agar (Oxoid). Organisms not ferment-
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ing glucose were subcultured on blood agar and identified specifically using the methods of Cowan and Steel.14

The strains were serotyped by the rapid slide agglutination test using absorbed rabbit antiserum against *Flavobacterium meningosepticum* sera (Difco). The polyvalent antiserum was used first, and if the strain was found to be positive then the individual group (A to F) sera were used.

Results

*Flavobacterium meningosepticum* was recovered from various sources in the nursery as well as in the neighbouring paediatric and obstetric wards. These sources are shown in Table 2. The organism was recovered mainly from the outlets of hand basins, sinks, and babies' bath basins as well as from stock bottles of aqueous chlorhexidine. Freshly distilled water gave negative results but stored distilled water in the pharmacy unit yielded one strain serotype E and one unclassified strain. The organism was also detected in the throat cultures of four neonates and of two postnatal mothers. In two of the babies the organism was recovered on several occasions. All four babies and the two mothers were clinically healthy.

*Flavobacterium meningosepticum* was recovered from 67 (11%) of 585 routine sputum specimens cultured on modified Thayer Martin medium. These specimens were collected from adult outpatients and inpatients of the hospital. Among the 475 vaginal swabs cultured, only one strain of *Flavobacterium meningosepticum* was recovered, that is, an isolation rate of 0-2%. This strain, which could not be serogrouped, was recovered from a 28-year-old outpatient with puerperal fever.

A number of other sources examined in the special care nursery failed to yield the organism. These were rectal thermometers, brushes and mops, refrigerators, and air vents of air-conditioners. *Flavobacterium meningosepticum* was not isolated from any of the incubators used by newborn babies during the 10-month period of study; 512 samples were tested. None of the incubators used in this hospital is humidified, that is, there is no water reservoir inside each incubator. Repeated throat cultures of 24 nursery staff also failed to yield the organism.

Table 3 shows the serotypes of the isolated strains by source. All the serotypes associated with the seven cases of neonatal meningitis have been recovered from the various sources examined in the study. This includes the *Flavobacterium meningosepticum* recovered from case 6 and labelled as unclassified (that is, not group A, B, C, D, E, F, or G) which was also isolated from hand basins in the paediatric and obstetric wards, bronchial suction tubings, and routine sputum specimens and routine vaginal swabs. Thus widespread possible sources of infection were found in the nursery and neighbouring wards.

Discussion

Over a period of six years (June 1971 to June 1977), 16 cases of neonatal meningitis due to *Flavobacterium meningosepticum* were seen at the University Hospital, Kuala Lumpur. Of these 16 cases, only seven were acquired in the University Hospital; the rest were from eight different hospital environments throughout West Malaysia.

*Flavobacterium meningosepticum* has high virulence and selectivity for the newborn, with a predilection for premature infants. However, not all neonates exposed to the organism became ill. Cabrera and Galen2 reported that only one twin of each of three sets became ill and died, while the other twins neither became ill

<p>| <strong>Table 2 Positive isolation of Flavobacterium meningosepticum from various sources</strong> |</p>
<table>
<thead>
<tr>
<th>Source</th>
<th>Number examined</th>
<th>Number positive</th>
<th>Percent positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suction apparatus of babies' resuscitation machines</td>
<td>2</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Babies' bath basins</td>
<td>4</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Hand basins in paediatric and obstetric wards</td>
<td>204</td>
<td>73</td>
<td>36</td>
</tr>
<tr>
<td>Aqueous solutions of chlorhexidine (0·05%, 0·5%, 1%)</td>
<td>19</td>
<td>12</td>
<td>63</td>
</tr>
<tr>
<td>Stored distilled water in pharmacy</td>
<td>1</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Special care nursery ward air</td>
<td>8</td>
<td>12·5</td>
<td></td>
</tr>
<tr>
<td>Bronchial suction tubings*</td>
<td>40</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Throat swabs of newborn babies</td>
<td>46</td>
<td>8·7</td>
<td></td>
</tr>
<tr>
<td>Throat swabs of postnatal mothers</td>
<td>49</td>
<td>4·1</td>
<td></td>
</tr>
<tr>
<td>Routine sputum specimens</td>
<td>585</td>
<td>67</td>
<td>11</td>
</tr>
<tr>
<td>Routine high vaginal swabs</td>
<td>475</td>
<td>1</td>
<td>0·2</td>
</tr>
</tbody>
</table>

*After use in special care nursery and labour ward.

<p>| <strong>Table 3 Serotypes of Flavobacterium meningosepticum by source</strong> |</p>
<table>
<thead>
<tr>
<th>Source</th>
<th>Serotype</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>Unclassified</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF</td>
<td>—</td>
<td>4</td>
<td>(2)</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Babies' resuscitation machines</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>2</td>
<td>(1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Babies' bath basins</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hand basins in paediatric and obstetric wards</td>
<td>4</td>
<td>27</td>
<td>4</td>
<td>3</td>
<td>16</td>
<td>2</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Aqueous solutions of chlorhexidine</td>
<td>—</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stored distilled water in pharmacy</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Special care nursery ward air</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronchial suction tubings</td>
<td>—</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Throat swabs of newborn babies</td>
<td>—</td>
<td>2</td>
<td>2</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Throat swabs of postnatal mothers</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Routine sputum specimens</td>
<td>3</td>
<td>2</td>
<td>7</td>
<td>47</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Routine vaginal swabs</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

( ) Serological cross-reaction,
nor were colonised by this organism. This suggests that immune mechanisms may play a role in these nosocomial infections. That compromised adults can also become ill and die as a result of infection with this opportunist organism has been reported by Mani et al.\textsuperscript{15}

Isolates from four of our cases (Table 1) were found to belong to serotype C, the commonest serotype causing infections in the newborn.\textsuperscript{4, 8, 9} One isolate (case 6) was identified as \textit{F. meningosepticum} on the basis of its morphology and biochemical properties but could not be classified as it did not react with any of the \textit{F. meningosepticum} antisera. Isolates from cases 4 and 5 were found to belong to serotype F but showed cross-reaction with serotype E. These serological reactions were confirmed by Dr RE Weaver, Center for Disease Control, Atlanta, USA.

Our relatively high isolation rates of \textit{F. meningosepticum} from environmental sources were due to our use of modified Thayer Martin medium as a selective medium for this organism. These strains of \textit{F. meningosepticum} were mainly present in samples taken from babies’ bath basins, handbasins in the paediatric and obstetric wards (73 positives out of 204 samples), and aqueous solutions of chlorhexidine (12 positives out of 19 samples). All six serotypes as well as serologically untypable strains were isolated from various sources, including one from a babies’ bath basin belonging to serotype E but cross-reacting with serotype F. Type E was the commonest serotype isolated. These results strongly suggest that flavobacterium infections are acquired not from mothers or nursery personnel but from environmental contamination and accounted for the few and sporadic cases seen in our hospital. It is known that outbreaks caused by environmental contamination tend to be intermittent and affect only a few babies in a nursery.\textsuperscript{19} Our investigations also show that flavobacterium infections can become endemic in a particular hospital. Only two of our cases were proven to be related epidemiologically (cases 4 and 5).

We are unable to reach conclusions regarding the portals of entry in our patients, although in one patient (case 6) the organism probably gained entry via the infected umbilicus. Direct invasion of the organism through the mucous membranes of the respiratory tract, conjunctiva, or abraded skin are possible portals of entry.\textsuperscript{5, 15, 16}

It must be emphasised that vigorous control measures must be instituted to contain and prevent further infections. Bacteriological surveillance of equipment and fomites, disinfectants, distilled water, and anything that comes in contact with the newborn must be carried out regularly. The human factor must also be considered, and periodic supervision and observation of manoeuvres and aseptic techniques carried out by the doctors and nursing staff is also important.

**CONTROL MEASURES**

Control of infection in the newborn intensive care unit requires an understanding of the reservoirs and modes of transmission of nosocomial organisms. As a result of the investigation carried out by us, several changes in the existing facilities and procedures were made in the nursery and obstetric wards. The suction apparatus of resuscitation machines was dismantled, cleaned thoroughly with soap and water, and disinfected with 1\% chlorhexidine in 70\% spirit after each day of use. With this method of disinfection rubber tubings became brittle and had to be replaced at frequent intervals. Suction bottles were changed daily.

The current procedures for cleaning with soapy water and disinfection with Ampyl solutions for incubators and bronchial suction apparatus were found to be satisfactory. Ampyl solution, produced by Sterling Drugs, USA, is described by the manufacturer as a phenolic disinfectant, deodorant, and detergent. The concentrations of Ampyl solutions used were 0.5\% for incubators and 2\% for bronchial suction apparatus, as recommended by the manufacturer.

Nasal suction catheters used for 24 hours were soaked in aqueous chlorhexidine. Aqueous solutions of chlorhexidine were not used as disinfectants for the skin or for any equipment unless they were supplied as heat-sterilised solutions in small-volume containers. Bath basins, wash basins, and sinks were cleaned with scouring powder at least twice daily. Since flavobacteria are often found in water,\textsuperscript{2} only boiled water that has been cooled in sterile containers was used to wash premature babies in the nursery.

Handwashing\textsuperscript{17} can reduce the transmission of nosocomial disease, and it was recommended that sterile aqueous chlorhexidine be used for handwashing during doctors’ ward rounds in between examination of babies, especially high-risk neonates. Washing of hands with soap and running water was sufficient elsewhere in the wards. The use of paper towels or hot air for the drying of hands was also recommended.

Samples for bacteriological examination were taken periodically to determine the degree of contamination in the environment and equipment.

Since April 1977, when case 7 occurred, no other case of neonatal meningitis due to \textit{F. meningosepticum} has occurred in this hospital since the control measures were taken to prevent further infections.
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References


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