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ORIGINAL RESEARCH

Nonpathogenic bacterial flora and immunoglobulin A in the oral cavity inhibit colonization of methicillin-resistant *Staphylococcus aureus* in very low birth weight infants

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Background: The aim of this prospective study was to investigate if nonpathogenic bacterial flora and high concentrations of immunoglobulin A in the oral cavity inhibit colonization with methicillin-resistant *Staphylococcus aureus* (MRSA) in very low birth weight infants.

Methods: We retrospectively analyzed MRSA colonization during hospitalization in 29 preterm infants with a birth weight < 1500 g who were admitted to the neonatal intensive care unit at Nagano Children's Hospital. We compared the incidence of MRSA colonization in 12 infants who had nonpathogenic bacterial flora and high concentrations of IgA (>2 mg/dL) in the oral cavity with 17 infants who did not.

Results: MRSA colonization in infants who had nonpathogenic bacterial flora and high concentrations of immunoglobulin A was significantly lower than in other infants ($P < 0.01$).

Conclusion: These results indicate that nonpathogenic bacterial flora and high concentrations of immunoglobulin A in the oral cavity may protect against MRSA colonization in very low birth weight infants.

Keywords: nonpathogenic bacterial flora, immunoglobulin A, methicillin-resistant *Staphylococcus aureus*, very low birth weight infants

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is considered a nosocomial pathogen. However, MRSA infection, especially neonatal toxic shock syndrome, has become a large problem in neonates.[1] Therefore, it is important to inhibit MRSA spread, colonization, and infection within neonatal intensive care units. Although many control measures have been introduced, including handwashing, reducing overcrowding, increasing nursing staff, and treating staff and carriers with mupirocin, the spread of MRSA has not been stopped.[2–4] An exponential increase in the isolation rate of MRSA is one of the most serious problems in neonatal intensive care units in Japan.[5]

Nonpathogenic bacterial flora can inhibit colonization with pathogenic bacteria in older people.[6–9] The birth canal of the mother may play an important role in the formation of normal bacterial flora in newborns. Coagulase-negative *Staphylococcus* is usually isolated from the skin of the newborn within a few hours of vaginal birth. However, neonates have no detectable normal bacterial flora in their nasal and oral cavities during the first several days after birth.[10,11] *Corynebacterium* species eliminate MRSA colonization in adult nasal cavities.[12] Recently we reported that nonpathogenic bacterial flora may inhibit MRSA colonization in newborns.[13]

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Salivary immunoglobulin A (IgA) secreted by plasma cells in the submucosa of the upper respiratory tract is a characteristic humoral factor of the local immune system. It is thought that synthesis of salivary IgA starts in early childhood. Many researchers report a progressive increase in salivary IgA levels during the first year of life.[14,15] Epidemiological studies emphasize the importance of salivary IgA in protection against infections in the upper respiratory tract.[16,17] Pathogenic bacteria, such as *Streptococcus pyogenes*, *S. aureus,* and enteropathogenic *Escherichia coli* can be opsonized by salivary IgA.[18–21] According to these observations, salivary IgA in neonates may interfere with MRSA colonization in the neonatal oral cavity. The objective of this study was to determine the role of nonpathogenic bacterial flora colonization and IgA levels in the oral cavity on later MRSA colonization in very low birth weight infants.

Materials and methods

Twenty-nine infants were enrolled in this study. All infants underwent oral bacterial sampling and oral saliva IgA assays every three days for three months. All infants were tube-fed. We compared the rate of MRSA colonization in infants who had nonpathogenic bacterial flora and oral IgA levels $>$ 2 mg/dL during the first seven days of life (Group 1) with the rate of MRSA colonization in other infants (Group 2). Parental consent was obtained for all infants to participate in the study, which was approved by the hospital ethics committee.

Microbiological testing

Infants underwent surveillance cultures from the oral cavity with sterile rayon-tip swabs (Seed swab number 2, Eiken Kizai, Tokyo, Japan). All swabs were inoculated onto plates with 5% sheep blood agar, chocolate agar, modified Drigarsky agar, and OPA *Staphylococcus* agar; all plates were purchased from Becton Dickinson, NJ. Plates were incubated for 24 hours at 37°C in 5% CO_2 in air. MRSA was defined as *S. aureus* for which the minimum inhibitory concentration of oxacillin was $>$4 μg/mL.

Collection of saliva and measurement of IgA

Samples of whole saliva from infants were collected every three days from birth to three months after birth. Unstimulated whole saliva was collected from the mouth before tube feeding in the morning. Saliva was obtained using a sterile cotton swab. Immediately after collection, swabs were centrifuged at 3500 g for 10 minutes to obtain the saliva, and the saliva samples were kept at –80°C until assay. The concentration of IgA was measured by immunoturbidimetric assay (N-assay TIA IgA-SH Nittobo, Nittobo, Tokyo, Japan).

Statistical analysis

Data are presented as means ± standard deviations or as percentages. Outcomes were compared using the Welch's *t*-test or Fisher's Exact probability test as appropriate. Receiver operating characteristic curves were constructed to assess the sensitivity, specificity, and cutoff values of IgA to compare their abilities to detect MRSA. For all testing, $P < 0.05$ was considered significant.

Results

The clinical characteristics of the two groups are shown in Table 1. Table 2 shows the species of nonpathogenic bacterial flora cultured from the oropharynx. Figure 1 shows the cumulative rate of infants with no MRSA colonization. None of the infants with colonization of nonpathogenic bacterial flora and oral IgA $>$ 2 mg/dL had MRSA colonization later. The rate of MRSA colonization was significantly lower in these infants than in others ($P < 0.01$). Determination of oral IgA offers comparable sensitivity of 80% and specificity of 61% using a cutoff value $>$ 2 mg/dL for MRSA colonization.

Discussion

Uncontrollable spread of MRSA in newborns in neonatal intensive care units has been largely attributed to environmental risk factors. In addition, neonatal immune systems compromised by prematurity, illness, and invasive procedures

Table 1 Baseline characteristics of infants

	Group 1 n = 12	Group 2 n = 17	*P* value
Gestation (week)	29.9 ± 2.8	30.8 ± 2.8	0.4
Birth weight (g)	993.1 ± 231.7	945.7 ± 276.1	0.62
Agar score at one minute	4.4 ± 2.2	4.8 ± 2.4	0.12
Cesarean section	9/12 (75%)	12/17 (70%)	0.79
Antenatal steroid exposure	8/12 (67%)	7/17 (41%)	0.18
Premature rupture of membranes	8/12 (67%)	7/17 (41%)	0.18
Duration of incubation (days)	31.4 ± 23.4	33.8 ± 26.7	0.8
Duration of hospitalization (days)	80.2 ± 0.4	89.9 ± 28.8	0.43
Death	0/12 (0%)	0/17 (0%)	1

Table 2 Species of nonpathogenic bacterial flora

Staphylococcus epidermidis	67.6 (%)
Staphylococcus aureus (not MRSA)	9.9 (%)
Enterobacteriaceae	9.9 (%)
Corynebacterium	4.2 (%)
Lactobacillus	4.2 (%)
alpha-Streptococcus	2.8 (%)
Others	1.4 (%)

play an important role in MRSA colonization. Most nosocomial infections in neonatal intensive care unit patients result from person-to-person transmission via the hands of medical staff.[22] Colonization with MRSA is achieved via a number of continuous processes, ie, arrival of bacteria from other sources to the newborn and specific attachment of bacteria to molecules on epithelial cells on the newborn. Interruption of the continuous flow of the colonization process at any point is likely to inhibit colonization with MRSA. Current methods of prevention of colonization focus either on preventing patient-pathogen contact or on preventing growth of the colonized microorganism.

Cultures of the nose, nasopharynx, throat, umbilicus, and rectum are usually negative in neonates on admission. Infants are colonized by flora delivered from the body of the mother and other human contacts. The mother's birth canal may play an important role in the formation of normal flora in newborns. Coagulase-negative *Staphylococcus* is usually isolated from a newborn's skin within a few hours of vaginal birth and, simultaneously, from the vagina of the mother. *Bacteroides fragilis*, a fecal flora, can be isolated within 48 hours of birth from newborns who are vaginally delivered, but few of these bacteria are isolated from newborns who are delivered via cesarean section.[23] Breast-fed infants develop normal bacterial flora by the third day of life, with a predominance of *Staphylococcus epidermidis* in the nose and umbilicus, alpha *Streptococcus* species in the throat, and *E. coli* in the stool.[23] In addition, a full-term newborn gets normal bacterial flora from the mother's nipple and breast milk during feeding. However, many very low birth weight infants are born by cesarean section, separated from the mother immediately after birth, and undergo tube feeding by a nurse. Therefore, it is difficult for very low birth weight infants to acquire normal bacterial flora.

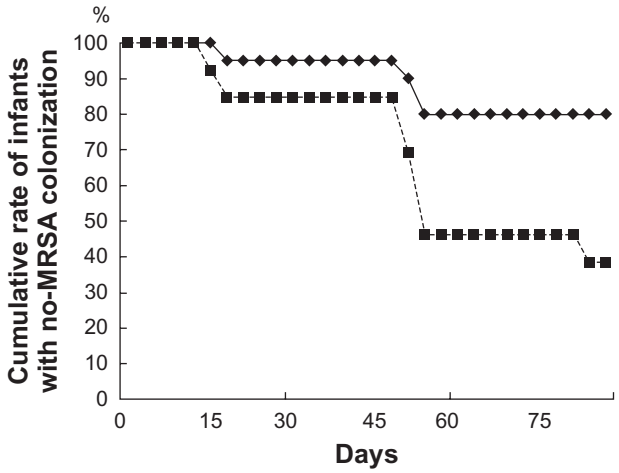

Figure 1 Cumulative rate of infants with no methicillin-resistant *Staphylococcus aureus* in Group 1 and Group 2 every three days until 90 days old. □ Group 1, infants with nonpathogenic bacterial flora and oral salivary IgA > 2 mg/dL (n = 12); ■ Group 2, all other infants (n = 17).

IgA is the principal antibody on all mucosal surfaces and in external secretions, including saliva. Salivary IgA plays an important role in the defense against viral and bacterial infections. Binding of intact IgA to antigens on the bacterial cell surface may reduce contact with the gut mucosa and facilitate elimination of excess potentially pathogenic substances of alimentary, bacterial, or viral origin. Pathogenic bacteria, such as *S. pyogenes*, *S. aureus*, and enteropathogenic *E. coli* can be opsonized by specific salivary IgA.[19–22] According to these observations, salivary IgA in neonates interferes with bacterial growth in the oral cavity. A controlled clinical study of a human IgG preparation for oral use showed that it conferred significant protection against necrotizing enterocolitis in low birth weight infants.[24] The concentration of salivary IgA depends on a number of factors, including age, stress, and cortisol levels.[25,26] In addition, salivary IgA secretion is stimulated by breast feeding and formula feeding.[27–31]

The present study has a few limitations, including being retrospective and including only a small number of cases. In addition, we could not determine if the production of salivary IgA had an independent effect on resistance to colonization. Further prospective studies are needed to clarify the potential value of using bacterial flora and salivary IgA to inhibit the spread of MRSA in neonatal intensive care units. The results of this study suggest that nonpathogenic bacterial flora and IgA may play a role in resistance to colonization by MRSA.

Disclosure

The author reports no conflicts of interest in this work.

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