Reservoirs of coagulase negative staphylococci in preterm infants

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Abstract

This investigation was undertaken to determine the magnitude of, and inter-relations between, reservoirs of coagulase negative staphylococci on infants' skin at various sites (including sites used for insertion of intravascular catheters) and in faeces during the first six months of life. Sites with large numbers of coagulase negative staphylococci were identified by sampling 16 skin sites and stools from 20 preterm neonates at 8-30 days of life. A more detailed survey of numbers and types of coagulase negative staphylococci in stool and at six skin sites of 10 preterm infants was then performed over the first six months of life. Isolates of coagulase negative staphylococci were collected and characterised by speciation, antibiotic susceptibility profiling, and plasmid restriction fragment length polymorphism analysis. Large, relatively stable reservoirs were identified in the faeces, around the ear, and in the axilla and nares. Skin on the forearm and leg, sites at which peripheral catheters are frequently sited, carried small unstable numbers of coagulase negative staphylococci, which were usually indistinguishable from coagulase negative staphylococci isolated from other body sites on the same baby. Contamination of catheter insertion sites with coagulase negative staphylococci from reservoir sites on the same baby could explain these observations.

These data suggest that interventions reducing cross-contamination between sites on the same baby might be as important in preventing coagulase negative staphylococcal bacteraemias as measures taken to prevent cross infection between babies. Procedures which are likely to result in heavy coagulase negative staphylococcal contamination of the hands of healthcare staff, such as changing soiled nappies, should receive particular attention.

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rarely associated with death, coagulase negative staphylococcal bacteraemia may considerably increase the duration of hospital stay.⁷

Most systemic infections with coagulase negative staphylococci are intravascular catheter associated infections.⁸⁻¹⁴ Infection may arise as a result of contamination of the outer surface of the catheter at the time of insertion, or by subsequent invasion of the catheter tunnel by coagulase negative staphylococci from the skin. Alternatively, the lumen of the catheter may become colonised, usually via the catheter hub, resulting in infusion of bacteria directly into the bloodstream. Thin, immature skin provides a limited barrier to the ingress of bacteria around catheters, and immunological deficits, including poor opsonic activity,¹⁵ increase the probability that invading bacteria will survive. These factors predispose preterm infants to bacteraemia with coagulase negative staphylococci.

Coagulase negative staphylococci can be isolated from the skin of most preterm infants on special care units. Most investigators have reported low numbers, however, on the skin of premature infants relative to those found on adult skin. Several authors have observed unpredictable day to day variation in the numbers and antibiotic resistance profiles of coagulase negative staphylococci isolated from neonatal skin.¹⁶⁻¹⁸ These data suggest that these staphylococci at the sites studied, including sites used for intravascular catheter insertion, constitute transient rather than stable resident populations.

Although coagulase negative staphylococci on adult skin may be stable over many years, the transient residence of microflora is well established for many other bacteria.¹⁹ Transient micro-organisms are thought to be contaminants of the skin which are rapidly displaced because they are unable to establish a stable ecological niche. The source of the transient coagulase negative staphylococci could either be exogenous (derived from other infants or adults, probably from the hands of carers) or endogenous (spread from elsewhere on the same infant, again probably through handling by adults). The latter possibility is more consistent with the observation that in the first one to two weeks of life a trend of increasing coagulase negative staphylococcal counts is superimposed on the fluctuations in bacterial numbers.^{16-18 20} If this is so, it should be possible to identify one or more reservoirs with high and relatively stable populations of coagulase negative staphylococci, and to show that the bacteria on other body sites constitute, to some extent, a subset of the organisms present at the reservoir sites, probably combined with some coagulase

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Coagulase negative staphylococci are a frequent cause of bacteraemia in infants in intensive care units.¹⁻⁶ Treatment may require the use of expensive and potentially toxic glycopeptide antibiotics, because isolates of coagulase negative staphylococci are often resistant to multiple antibiotics. Although

negative staphylococci from exogenous contamination. In this study we attempted to identify large reservoirs of coagulase negative staphylococci in preterm infants, to investigate the stability of these reservoirs over the first six months of life, and to compare reservoir isolates with those at transient colonisation sites such as catheter insertion sites.

Methods

PRELIMINARY LOCATION OF MAJOR RESERVOIRS OF COAGULASE NEGATIVE STAPHYLOCOCCI

Sites harbouring large reservoirs were identified in a pilot survey of 20 neonates (gestational age range 28–33 weeks and age at sampling 8–31 days). Each was sampled on one occasion at nine to 13 of 18 sites, including the ear (anterior and posterior pinna separately); anterior nares, nose, axilla, umbilicus/periumbilicus, neck, antecubital fossa, upper arm, forearm, chest, popliteal fossa, inguinal folds, outer thigh, inner thigh, lower leg, toe clefts and faeces. Sampling and enumeration methods were as described below.

LONGITUDINAL SURVEY

Ten infants admitted within 24 hours of birth to the neonatal intensive care unit at St Michael's Hospital, Bristol, were recruited between September 1993 and March 1994. Criteria for inclusion in the study were gestational age 30 weeks or less, informed consent of parents, and a sufficiently stable clinical condition to allow sampling at 4 days of age.

Each infant was sampled on days 4, 8, 12, 16, 21, and 28 of age and monthly thereafter until discharged home, and then at one to two monthly intervals until 6 months of age. Sites sampled (selected on the basis of the pilot study data) were the ear (anterior and posterior pinna); anterior nares, and axilla. The forearm and lower leg were also sampled because these are common sites for peripheral catheter insertion.

Rayon swabs moistened in 1 ml phosphate buffered 0.1% Triton X100 solution were used to sample 1–3 cm² of the skin; a 3 cm² plastic template was used when sampling the forearm and lower leg. The swab tip was immediately broken off into the wash solution and vortex mixed for 30 seconds.¹⁸ Viable counts were made²¹ on milk agar (CM21; Oxoid, Basingstoke), malassezia agar²² and Reinforced Clostridial Agar (CM151; Oxoid) with 6 mg/l furazolidone for the enumeration of *Propionibacterium acnes*.²³

Stool samples were collected when available, dispersed in 9 parts glycerol broth (1% Lab Lemco powder (L29; Oxoid), 10% glycerol) and stored at –70°C. Coagulase negative staphylococci in stool samples collected closest to the skin sampling day were counted on Diagnostic Sensitivity Test agar (CM261; Oxoid) supplemented with (1⁻¹) glycine (0.5 g), lithium chloride (2 g), potassium thiocyanate (22.5 g), and aztreonam (15 mg, Bristol-Myers Squibb).

During the course of the study, we collected a number of clinical isolates of coagulase negative staphylococci from catheter tips and blood cultures of infants included in the study. Catheter tips were processed using the Maki roll method.²⁴ Isolates were regarded as clinically important if more than 15 colonies were recovered.

A representative of each coagulase negative staphylococcal colony type isolated from each sampling site was stored in glycerol-citrate medium (40% glycerol, 60% 50 g/l trisodium citrate) at –70°C for subsequent characterisation.

CHARACTERISATION OF COAGULASE NEGATIVE STAPHYLOCOCCAL ISOLATES

All coagulase negative staphylococci isolates from the faeces, blood cultures, and line tips and from 16 day skin samples were characterised by a variety of methods.

Antibiogram determination Susceptibility to penicillin G, tetracycline, erythromycin, trimethoprim, chloramphenicol, gentamicin, netilmicin and streptomycin was determined by the modified rotary plating Stokes method.²⁵

Speciation Isolates were tested for acid production from mannitol, trehalose, and *N*-acetylglucosamine, for susceptibility to desferrioxamine (1000 µg disc)²⁶ and novobiocin (5 µg disc), and production of β-glucosidase (fluorescence at 366 nm after growth on P agar containing 150 mg/l 4-methyl-umbelliferyl-β-D-glucopyranoside) and urease.²⁷ Any isolates not speciated by these tests were inoculated into an API Staph strip (bioMérieux).

Plasmid RFLP determination Plasmids were extracted and purified by a modification of the protocol of Voskuil and Chambliss,²⁸ with 50 U/ml lysostaphin added to the lysis mix to improve cell lysis. Purified plasmids were cut with *Cla* I restriction endonuclease. The resultant fragments were separated by electrophoresis in 0.8% agarose, stained with ethidium bromide, and photographed under ultraviolet illumination.

Table 1 Median coagulase negative staphylococcal counts obtained from 20 infants of gestational age of less than 35 weeks and postnatal age range 8–30 days

Site	Count (cfu)
Faeces	1.1 × 10 ⁷ g ⁻¹
Ear (posterior pinna)	4.6 × 10 ⁶ ml ⁻¹
Ear (anterior pinna)	4.7 × 10 ⁴ ml ⁻¹
Anterior nares	1.7 × 10 ⁴ ml ⁻¹
Axilla	1.7 × 10 ⁴ ml ⁻¹
Nose (external)	3.0 × 10 ³ ml ⁻¹
Neck	2.0 × 10 ³ ml ⁻¹
Antecubital fossa	5.1 × 10 ² ml ⁻¹
Popliteal fossa	4.7 × 10 ² ml ⁻¹
Upper arm	3.5 × 10 ² cm ⁻²
Toe webs	5.0 × 10 ¹ ml ⁻¹
Outer thigh	2.7 × 10 ¹ cm ⁻²
Inguinal folds	1.6 × 10 ¹ ml ⁻¹
Chest	1.1 × 10 ¹ cm ⁻²
Forearm	6 cm ⁻²
Inner thigh	6 cm ⁻²
Lower leg	6 cm ⁻²

To facilitate quantitative assessment bacteria were washed from swabs by vigorous agitation in 0.1% Triton X100 solution. cfu=colony forming units; g⁻¹=per g faeces; ml⁻¹=per ml wash solution, area of skin not defined; cm⁻²=per cm² skin, area of swabbed skin defined by a plastic template.

Figure 1 Coagulase negative staphylococci at various sites during the first six months of life. Note that counts are displayed as mean counts per gram for faeces but mean counts per swab at all other sites.

Results

PRELIMINARY LOCATION OF MAJOR RESERVOIRS OF COAGULASE NEGATIVE STAPHYLOCOCCI
The median population sizes found at various skin sites and in faeces of neonates are given in table 1. The largest counts were found at the ear (anterior and posterior pinna) anterior nares, axilla, and in the faeces. These sites were selected for further study as were the forearm and the lower leg, where peripheral catheters are frequently sited.

LONGITUDINAL SURVEY

Population size

The mean coagulase negative staphylococcal counts at seven sites on 10 babies from 4 days to 6 months of age are shown in fig 1. As expected from the preliminary investigations, the ear, nose, and axilla consistently carried considerably higher numbers than the forearm and lower leg, which harboured low numbers throughout the study. Bowel numbers were largest around 12 days but progressively decreased thereafter. Mean bacterial counts in the ear and nose also increased substantially between 4 and 12 days, but did not change consistently beyond this age.

Composition

Most skin and faecal coagulase negative staphylococci isolates were *S. epidermidis* (76% of 191 strains speciated), *S. haemolyticus*, *S. warneri*, or *S. capitis* (table 2). The proportion sensitive to the antibiotics tested were penicillin G 3.1%, gentamicin 12.9%, netilmicin 23.2%, trimethoprim 38.7%, erythromycin 62.4%, chloramphenicol 90.7%, streptomycin 91.8% and tetracycline 96.9%. Plasmid RFLPs were useful in the subdivision of these species and revealed that many babies

carried one or more strains at several sites but that these strains were rarely shared by different babies. *Staphylococcus epidermidis* plasmid type 11 (resistant to penicillin, methicillin, gentamicin, netilmicin, erythromycin and trimethoprim) was a notable exception, being isolated from seven of the 10 babies studied. Eight of the nine line tip and blood culture isolates collected were indistinguishable from isolates recovered from the skin of the same baby; two were also recovered from faeces.

Stability

Coagulase negative staphylococcal counts at most skin sites on each individual showed wide fluctuations between sampling days. Counts around the ear fluctuated considerably less, as did faecal coagulase negative staphylococcal numbers. Plasmid typing of coagulase negative staphylococci isolated from faeces on different days showed that there was also some qualitative stability, but a succession of different isolates was also evident. For example, case 3 yielded predominantly *S. epidermidis* on days 5 and 7, *S. warneri* on days 13, 15, and 18, a mixture of both species on day 27, and exclusively *S. epidermidis* at 2, 3, and 4 months.

Table 3 shows the correlations between staphylococcal counts obtained at sites used for catheter insertion (forearm and leg) and sites with large staphylococcal populations. Counts at the axilla correlated with those on both the leg and the forearm. There were less obvious correlations between the forearm and the ear and nares. However, counts from the faeces did not correlate with counts on the forearm and were inversely related to counts on the leg.

OTHER SKIN MICRO-ORGANISMS

Propionibacterium spp and *Malassezia furfur* population densities are given in figs 2 and 3. Mean counts at all skin sites were low in the first three to four weeks of life but progressively increased thereafter at the axilla, ear, and to a lesser extent the nares. Patterns of colonisation were broadly similar at all sites for these two groups of organisms, but were different from coagulase negative staphylococcal colonisation patterns.

Discussion

The characteristic skin microbiota of adults develops at puberty and thereafter can remain stable over many years,²⁹ but the skin microflora in prepubescent children, including neonates, has not been well described. Although the numbers of micro-organisms on skin are generally low in early childhood, certain skin sites on the heads of infants in their first year of life have been observed to carry large numbers of staphylococci, propionibacteria, and *Malassezia furfur*.^{30 31} This investigation confirms that skin at certain sites (ear, nares, and axilla) can harbour all of these micro-organisms. Staphylococci predominated and colonised earlier than propionibacteria

Table 2 Species and plasmid types isolated from various sites at and around 16 days

Case	Plasmid RFLP type of staphylococci recovered from:					Tip (T) and blood culture (BC)
	Bowel	Ear	Nares	Axilla	Arm and/or leg	
1	NT ^H 1	2 4 ^H 6	4 ^H	7		
2	11	11 14	11	11	11	11 (BC 19 d)
3	17 ^W	NT 17 ^W	NT 20 ^C			
4	NT ^X 11 22	11		11	11	
5	NT 25	11 20 ^C 29 32 ^C 33 ^W	11 30	11		
6	NT	11 40 ^C	34	34	39	
7	41 42	11 41 45 ^W	41	11 44	NT 44	
8	46 47	46 48 50 ^C 51 ^W	3 46 50 ^C	3		3 (BC+T 2 m), 46 (T 16 d), 49 (BC 4d), 50 ^C (T×2, 2 m) 54 (T 17 d), 55 (BC 10 d)
9	54	11 55 56 59	55	11 55	11 55	
10	11	11		11	11 57	

All isolates were *S. epidermidis* except ^c=*S. capitis*, ^w=*S. warneri*, ^h=*S. haemolyticus*, ^x=*S. xylosum*. NT=not typable (no plasmids seen).

and *Malassezia furfur*, which have slower growth rates and might require greater maturation of skin structures than coagulase negative staphylococci. The difference between patterns of skin colonisation by staphylococci and the other major skin residents is also noted in adults.³² The limbs carried relatively few micro-organisms, and numbers fluctuated considerably on a daily basis, as noted at several sites in previous studies.¹⁶⁻¹⁸ Regular contamination by bacteria originating from sites harbouring large reservoirs may explain this instability. The observation that different sites on the same baby are much more likely to share the same types of coagulase negative staphylococci than are skin sites of different babies is consistent with this hypothesis. It would also be expected that there would be a certain degree of correlation between counts found at 'source' and 'destination' sites. This was the case to a certain extent when comparing candidate skin reservoirs with the leg and forearm, the most notable correlations being between the axilla and these sites (table 3). These data must be interpreted with caution because positive correlations may well be the result of phenomena other than contamination from one site to another, such as maturation of both sites simultaneously or response to systemic antibiotics (although no relation was noted between microbial density on the skin and antibiotic treatment).

Table 3 Correlation between staphylococcal counts at sites used for catheter insertion (forearm and leg) and sites with large staphylococcal populations

	Ear, anterior	Ear, posterior	Axilla	Nares	Faeces
Leg	NS NS	NS NS	<i>r</i> =0.35; <i>P</i> <0.001 <i>r</i> =0.25; <i>P</i> =0.034	NS NS	<i>r</i> =-0.41; <i>P</i> =0.001 <i>r</i> =-0.43; <i>P</i> =0.001
Forearm	<i>r</i> =0.22; <i>P</i> =0.037 NS	NS NS	<i>r</i> =0.32; <i>P</i> =0.002 <i>r</i> =0.41; <i>P</i> <0.001	<i>r</i> =0.26; <i>P</i> =0.014 NS	NS NS

Individual data points were used rather than means. Data in italics are for samples taken while patients were hospitalised.

Figure 2 Mean propionibacteria counts at various sites during the first six months of life.

The bowel may be a particularly important reservoir of coagulase negative staphylococci. Faeces contained large numbers, particularly during the first month when babies are at greatest risk of acquiring coagulase negative staphylococcal infection. During routine care of babies, particularly the changing of soiled nappies, the hands of parents and unit nursing staff are likely to become faecally contaminated. This contamination will be spread to multiple skin sites on the baby. Staphylococci transferred to the skin of the infant by this process may persist for prolonged periods because of the resistance of staphylococci to desiccation. Furthermore, the large numbers of staphylococci present in faeces, in high concentrations of organic matter, make it very unlikely that contaminated hands could be effectively disinfected by hand washing. This will result in a high rate of cross contamination between babies, ensuring that antibiotic resistant strains of coagulase negative staphylococci strains endemic in many special care baby units can readily be transferred to newborn patients in whom they are likely to establish new foci of infection under the selective pressure of intensive antibiotic use. Selection of resistant isolates is more likely to occur in the bowel, into which many antibiotics penetrate well via excretion in the bile, than on the skin, where only low concentrations of hydrophilic antibiotics such as aminoglycosides and β -lactams are found following systemic use. Although the only significant correlation between population densities in faeces and on the limbs was negative (faeces *vs* leg), contamination of the skin by bowel contents may only

Figure 3 Mean *Malassezia furfur* counts at various sites during the first six months of life.

occur to an appreciable extent after defecation, which can be infrequent in this patient group. Resultant 'spikes' in numbers of coagulase negative staphylococci at contaminated skin sites would be difficult to detect without serial sampling before and after changing soiled nappies. At other times other coagulase negative staphylococci reservoirs would probably contribute more bacteria to sparsely populated sites than would the bowel.

Understanding the spread of coagulase negative staphylococci between babies and from site to site on the same baby may facilitate the design of effective interventions for reducing the incidence of systemic coagulase negative staphylococcal infections in these patients. Emphasis is frequently placed on the importance of preventing the spread of microorganisms between patients. However, such measures are clearly inadequate in preventing colonisation of infants by antibiotic resistant coagulase negative staphylococci endemic to a unit.^{17 33 34} In this study plasmid analysis suggested that a coagulase negative staphylococcal clone was carried by seven of 10 babies, and that most of the other isolates were multiply antibiotic resistant, implying that they may well also be endemic to the unit.

If coagulase negative staphylococci causing infection frequently gain access at catheter insertion sites, and there is no stable resident bacterial population on surrounding skin, it may be possible to reduce infection rates by reducing spread of organisms to these sites from numerically important reservoirs of coagulase negative staphylococci on the same baby during routine handling. Interventions which might be considered include improved aseptic handling protocols for neonates, relating particularly to 'high risk' procedures such as nappy changing, or by antisepsis directed at the reservoir sites. Alternatively, as transient populations are superficially located, it might be possible to eradicate most bacteria at the site of catheter implantation using antiseptics,⁶ and prevent ingress of coagulase negative

staphylococci by a combination of occlusive dressings and careful handling of catheters and insertion sites with gloved hands.

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