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THE NATURAL HISTORY OF BACTERIAL COLONIZATION OF THE NEWBORN IN A MATERNITY HOSPITAL
(Part II)

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Summary. *The object of this study was to determine the sources from which the newborn infant derives his bacterial flora during the first 5 days after birth. Detailed bacteriological data was obtained from 193 mothers antenatally and during labour, and from their infants on the day of birth and on Day 5. Organisms were typed by appropriate methods and the 193 mother-baby pairs declared to be a 'match' or a 'non-match' according as to whether or not identical bacteria were isolated from both members of the pair. Weekly swabs from hands and noses of staff were taken throughout the 12 months of the study. Swabs were also taken from the environment and air samples from different areas in the hospital.*
In approximately one third of infants the colonizing bacteria are derived from their mothers, in over 70 per cent of instances from the mother's rectum. The mother's birth canal is not a common source of bacteria and there is no direct relationship with the noses or hands of staff. Artificial feeds are not a source of the colonizing bacteria. The overall distribution of the bacterial species in the infants differed from that found in the hospital environment but this does, none the less, constitute a reservoir of bacteria which is being continually replenished from human sources. Pathogens such as Staph. aureus *and* Strep. pyogenes *are no longer commonly found in newborn infants in the modern maternity hospital, but the need for continual vigilance and an efficient bacteriological service in all maternity units has been in no way diminished.*

THE first stage of this study on 1103 infants answered 2 questions (McAllister *et al.*, 1974);

(i) At what age are newborn infants colonized by bacteria?

(ii) With what organisms are they colonized?

We were surprised by the high level of colonization of all sites (nose, throat, umbilical cord, groin and rectum) by Day 3 (48 hours), and by the fact that bacteria could be grown from 23.4 per cent of gastric aspirates taken within an hour of birth. On the other hand, staphylococcal colonization was rare and this was attributed to the intensive anti-infective precautions in the hospital. It was considered that the results from the gastric aspirates indicated that some of the colonizing bacteria had a maternal origin, but the other sources from which the newborn acquires his bacterial flora are far from obvious. The bacteriological data described in our previous paper was then linked by computer to a series of purely clinical observations which had been recorded for every infant in the study. These included maturity, time of rupture of membranes, number of vaginal examinations, type of delivery, type of feeding, evidence to suggest infection, and use of antibiotics. It was hoped to define any relationship between bacterial colonization by various species of bacteria and clinical observations or evidence of infection. In fact, few significant correlations came out of this computer exercise. This may have been related to the paucity of infections—83 infants out of 1103. Of the 36 infants who died evidence of bacterial infection was found in only 5. We concluded, therefore, that there is little evidence of a relationship between the bacterial colonization of the infants (of all weights and maturities) in a modern maternity hospital and the prevalence of infection. This is in marked contrast to the well documented relationship between staphylococcal colonization and staphylococcal infection in the

maternity units of the 1950's (Hurst, 1957; Gillespie *et al.*, 1958). Indeed, the first stage of our study revealed that if organisms customarily classified as 'non-pathogens' (Micrococcus sp. and *Strep. viridans*) are disregarded, the Gram-negative colonizers greatly outnumber the Gram-positives.

In an attempt to determine the sources of the organisms which colonize the newborn infant a second stage of the study was started on 1st July 1972 and continued for 12 months. Adequate bacteriological data was obtained from 193 mothers and their infants.

Methods and material

One of us (J. MacV.) selected patients for the study in his antenatal clinic at the 38th week of pregnancy, thus avoiding the inclusion of pre-term infants who might require a prolonged hospital stay. The following cultures were obtained from the consenting and informed expectant mothers: high vaginal swab (HVS) at 38 weeks; nasal, rectal and HV swabs on admission to hospital before or during labour; HVS and *liquor amnii* at the time of induction of labour when this procedure was being done; HVS at each vaginal examination during labour; swab of the fourchette at the time of delivery.

The infants were studied as follows: culture of gastric aspirate, and nasal, throat, groin and rectal swabs within one hour of birth (Day 0); culture of umbilical cord on Day 3; nasal, throat, groin and rectal swabs on Day 5, at which age bacterial colonization had been shown to be maximal in the first stage of our study (McAllister *et al.*, 1974).

Nasal and hand swabs were obtained approximately once per week from all medical, nursing and other staff on duty in the Labour Suite and Special Care Neonatal Department. Frequent cultures were taken from 'contact' sites in the labour suite, neonatal special care unit and the 'night nurseries' in the wards; these included incubators (cradles and water baths), operating tables, walls, floors and sink traps.

Air samples using a Casella Slit-Sampler were taken at various times of day in the labour suite, corridors, wards and the neonatal special care unit. Air samples exposed revolving 140 mm. blood-agar plates to 5.2 c.ft. of air, and all tests were performed in triplicate. The plates were incubated for 18 hours and a total bacterial count was made. A search was then made for pathogens such as *Staph. aureus* and *E. coli* which were identified as described below.

It is a routine practice in the hospital to submit samples of reconstituted dried milk for culture every week. These include some freshly prepared in the milk kitchen, and some after refrigeration for 24 hours.

Data collection

The swabs were used dry or moistened in sterile broth according to the site sampled. Samples from skin and nose were plated on blood-agar and incubated aerobically and anaerobically. Swabs from *liquor amnii*, gastric aspirate and HVS were inoculated on blood-agar (aerobically and anaerobically), MacConkey and Sabouraud media. Rectal and groin swabs were similarly incubated on blood-agar and MacConkey medium. Moist swabs from inanimate objects were plated on blood-agar and MacConkey medium.

The bacteria were identified by the methods of Cowan and Steel (1974) and Gram-negatives further checked using the API method. Up to 10 Gram-negative bacilli per swab were identified by this technique and further classified by the resistogram method (Elek & Higney, 1970) whereby it is possible to define a stable profile for each single strain. *Candida* species were screened for *albicans* by the Germ-tube test and those with negative results sent to medical mycology for further identification. *Candida albicans* were tested against nystatin and amphotericin B. All *Staph. aureus* were phage typed.

A proforma was completed for each mother and her baby and when the bacteriological results became available a punched card was instituted to facilitate manual analysis. Each 'mother-baby pair' was then declared to be either a 'match' or a 'non-match'. A pair was considered a 'match' when both mother and her baby yielded (i) *Candida*, or (ii) *Staph. aureus* of the same phage type, or (iii) identical *E. coli* according to their resistograms, or (iv) other Gram-negative organisms which matched by resistogram typing. The environmental data was also tabulated and analysed.

Mothers

The basic clinical information for the matched and non-matched mother-baby pairs is shown in Table I. There would not appear to be any relevant differences between the two groups with regard to obstetric or neonatal factors. Sixty-three pairs (32.6%) matched in respect of bacteria cultured from both mother and baby. In 3 pairs there was a double 'matching' in that both mother and baby grew more than one identical species. The matching organisms isolated were *E. coli* on 52 occasions; *Staph. aureus* 5; *Candida albicans* 5; *Enterobacter cloacae* 1; *beta-haemolytic Streptococci* (not group A) 1; *Klebsiella aerogenes* 1; and *Proteus mirabilis* 1. In addition 19 mothers and 19 infants grew *Staph. aureus* while the other member of the pair remained free of the organism. In another 2 mother-baby pairs both members grew *Staph. aureus* but of different phage types. Eleven mothers yielded *Candida albicans* but without spread to their infants.

The culture results of swabs taken from the 193 mothers at the antenatal clinic and as in-patients are shown in Table II. It will be seen that under both circumstances there is a higher proportion of positive cultures and a higher rate of pathogens per swab in the mothers of the 'matched' group than in those of the 'non-matched'. The rate of pathogens isolated per swab is, however, comfortably low. In Table III, the organisms generally regarded as pathogenic which were isolated from the maternal swabs are recorded. In the

Table I. Analysis of clinical findings in 193 mother/baby pairs.

	Matched	Non-matched
Total number	63	130
Primigravida	26	66
Parous	37	64
ARM (before or during labour)	48	94
Method of delivery	47	78
SVD	1	11
Caesarean section	14	40
Forceps	1	1
Other	0	14
Pyrexia (maternal)	0	2
Membranes ruptured <24 hr	63	129
Live births	0	1
Stillbirths	0	0
Neonatal deaths	31	65
Males	32	65
Females	9	36
Baby in special care unit	41	71
Bottle-fed		

Table II. Analysis of swab results from 193 mothers in the study.

	High vaginal swabs taken at ante-natal clinic		Swabs from vagina, liquor and fourchette on admission and in labour	
	Matched	Non-matched	Matched	Non-matched
Number of mothers	63	130	63	130
No growth	13	41	112	273
Not done	2	8	0	0
Pathogens	15	20	45	80
Commensals	52	104	117	163
Total bacteria	67	124	162	243
Number of swabs	61	122	210	450
Pathogens per swab	0.25	0.16	0.21	0.18
% Swabs with no growth	21.3	33.6	53.3	60.7

Table III. Pathogens isolated from 193 mothers studied.

	High vaginal swabs taken at ante-natal clinic		Swabs from vagina, liquor and fourchette on admission and in labour	
	Matched	Non-matched	Matched	Non-matched
Candida albicans	63	130	63	130
Torulopsis glabrata	9	8	16	33
Strep. faecalis	2	1	3	13
Enterococci	3	4	3	20
Staph. aureus	0	2	1	3
E. Coli	0	1		2
Pr. mirabilis	0	4	11	6
Alcaligenes faecalis	1	0	3	2
	0	0	0	1

antenatal clinic 85.7 per cent of these bacterial species were Gram-positive and after admission to the hospital 81.6 per cent were Gram-positive. These results would suggest that the mother's birth canal is not an important source of the Gram-negative organisms which were shown to colonize the healthy newborn in the first stage of our study. The higher incidence of positive cultures, both with pathogenic and non-pathogenic bacteria, in the mothers of the 'matched' group did not relate to any obvious obstetric factor. It may have reflected a lower general standard of personal hygiene among these women.

The nasal and rectal swabs taken from the mothers on admission to hospital showed no significant differences in the incidence of positive cultures nor in the distribution of the various bacterial species between the 'matched' and the 'non-matched' groups. As might be expected the rectal swabs yielded predominantly Gram-negative bacteria whereas those cultured from the nasal swabs were predominanty Gram-positive.

Infants

In Table IV the pattern of colonization is shown in the infants of both the 'matched' and the 'non-matched' groups. The gastric aspirates taken within one hour of birth show a higher rate of pathogens per culture in the 'matched' than in the 'non-matched' group. This is a less marked tendency in the umbilical cord cultures taken on Day 3. There are no differences between the 2 groups for nasal, throat, groin or rectal cultures on the day of birth (Day 0), but there is a higher rate of pathogens per culture in the throat and groin swabs from the 'matched' group on Day 5. The relevance of these findings is doubtful.

Table IV. Comparison of colonization at 6 sites in matched and non-matched infants.

Site		Matched		Non-matched	
		Day 0	Day 5	Day 0	Day 5
NOSE	No growth	56	11	115	37
	Total bacteria	1	71	7	108
	commensals	0	62	3	98
	pathogens	1	9	4	10
	Swabs	57	58	120	103
	Pathogens per swab	0.02	0.16	0.03	0.10
THROAT	No growth	53	1	112	10
	Total bacteria	5	144	4	203
	commensals	2	128	1	190
	pathogens	3	16	3	13
	Swabs	56	58	119	102
	Pathogens per swab	0.05	0.28	0.03	0.13
GROIN	No growth	45	1	102	5
	Total bacteria	16	144	20	234
	commensals	11	58	14	109
	pathogens	5	86	6	125
	Swabs	57	58	118	102
	Pathogens per swab	0.09	1.48	0.05	1.23
RECTUM	No growth	50	1	114	1
	Total bacteria	11	156	5	259
	commensals	4	50	5	85
	pathogens	7	106	0	174
	Swabs	57	58	120	103
	Pathogens per swab	0.12	1.83	0	1.69
GASTRIC ASPIRATE	No growth	24		95	
	Total bacteria	21		6	
	commensals	6		5	
	pathogens	15		1	
	Cultures	43		10	
	Pathogens per culture	0.35		0.01	
CORD	No growth	1 (Day 3)		3 (Day 3)	
	Total bacteria	92		176	
	commensals	33		86	
	pathogens	59		90	
	Cultures	49		93	
	Pathogens per culture	1.21		0.97	

In Table V we show the individual species of matching bacteria which were cultured on separate occasions from 63 mothers and their infants who 'matched'. In 46 of 52 pairs growing *E. coli* the matching organisms were isolated from rectum of both mother and baby. Five of these mothers also grew *E. coli* of the same resistogram profile from the HVS taken on admission to hospital. In 2 cases the matching *E. coli* were cultured from the mothers' fourchette and rectum and from the gastric aspirates of their infants; in 2 cases the matching *E. coli* came from the HVS and the infants' rectum; in 1 case the organism was grown from the *liquor amnii* and the infant's rectum; and in another case the *E. coli* was found in the mother's rectal swab and her baby's gastric aspirate. These findings can leave little doubt that *some* of the bacteria which colonize newborn infants have been acquired from their mothers.

Hospital staff

During the year of the study 1545 nasal and 1543 hand swabs were cultured from staff members in the special care unit and the labour suite (Table VI). The results of the hand swabs reflect the scrupulous hand-washing discipline throughout the hospital. Separate analysis of the results from these 2 areas of the hospital revealed an identical pattern. The pathogenic organisms isolated from noses and hands are detailed in Table VII. and the great preponderance of Gram-positive organisms will be noted. This makes it improbable that the medical or nursing staff are an important direct source of the organisms which colonize all newborn infants in a modern maternity hospital, although the bacteria in the hospital environment must arise in part from the people who work there.

Equipment

Two hundred and seventy three swabs were taken from incubators, walls and floors. Only 49 pathogens were cultured from these swabs of which 44 (90%) were Gram-negative. The Gram-negative bacteria were distributed as follows; *E. coli* 9, *Alcaligenes faecalis* 21, *Acinetobacter lwoffii* 10, *Klebsiella aerogenes* 1, *Pseudomonas aeruginosa* 3. Of the 5 Gram-positive bacteria, 3 were *Staph. aureus* and 2 *Strep. faecalis*.

Table V. Micro-organisms isolated on separate occasions from 63 matched mother/baby pairs. (The same species was sometimes grown from mother and baby on several occasions and from several sites).

Organisms	Mothers		Infants	
Staph. aureus	Nose	4	Nose	1
	Rectum	2	Throat	2
			Cord	2
			Rectum	1
Candida albicans	HVS	9	Gastric aspirate	4
	Liquor amnii	2	Oral thrush	1
E. coli	Nose	1	Gastric aspirate	7
	HVS	7	Rectum	51
	Rectum	47		
	Fourchette	3		
	Liquor amnii	1		
Enterobacter cloacae	HVS	1	Gastric aspirate	1
			Nose	1
			Throat	2
			Groin	2
			Rectum	1
			Cord	1
Beta-haemolytic streptococci (not group A)	Rectum	1	Rectum	1
			Cord	1
			Groin	1
Proteus mirabilis	HVS	1	Rectum	1
	Rectum	1		
	Fourchette	1		
Klebsiella aerogenes	Rectum	1	Gastric aspirate	1

Table VI. Analysis of nasal and hand swabs from staff in special care unit and labour suite.

	Nose	Hand
Total bacteria	2185	535
commensals	1851	500
pathogens	336	33
Swabs	1545	1543
Pathogens per swab	0.22	0.02

Table VII. Pathogens isolated from staff in special care unit and labour suite.

Bacterial species	Nose	Hand
Staph. aureus	302	26
Strep. pneumoniae	3	0
Strep. faecalis	1	1
Total Gram-positives	306	27
Proteus mirabilis	18	2
Klebsiella aerogenes	7	1
E. coli	4	1
Enterobacter cloacae	1	0
Acinetobacter lwoffii	0	2
Total Gram-negatives	30	6

The air

The results of air sampling in various parts of the hospital are shown in Table VIII. Tests were initially performed when routine activity appropriate to the area was going on, and subsequently further sampling was done in certain areas under conditions of high activity, for example, bathing and napkin-changing rounds. The latter exercise was undertaken in one of the wards and its associated 'night nursery' with different numbers of infants in residence.

The bacterial counts ranged from 4 to 34 per cu. ft. in the delivery rooms; from 11 to 48 per cu. ft. in the operating theatres; from 11 to 75 per cu. ft. in the incubator room of the special care unit; from 13 to 132 per cu. ft. in the special care unit corrider; and from 29 to 90 per cu. ft. in the wing nursery. It should be remembered, however, that a quiet operating theatre should have a total count of 0 to 2 bacteria per cu. ft. and during activity this rises to 2 to 5 per cu. ft. Our results, therefore, reflect a high level of human activity in all areas. On the other hand, only a very small proportion of the high bacterial counts were pathogens—usually less than 5 per cu. ft.—and settlement of these would represent a very small inoculum for each infant. The low ratio of pathogens per test can be seen in Table VIII, but the rising ratio during periods of high activity and its correlation with the number of infants in the area should be noted. The low ratio of pathogens per test in the incubator room both during routine activity and high activity is not surprising in view of the fact that the infants are largely (but not entirely) isolated within their incubators.

Infant feeds

Weekly cultures were made of freshly prepared reconstituted dried milk and of samples after storage for 24 hours in a refrigerator. No pathogens were grown from the freshly prepared samples, and pathogens were isolated from the stored samples on only 3 occasions; *Strep. faecalis* in September 1972, *Staph. aureus* in November 1972, *Pseudomonas aeruginosa* in April 1973. Clearly the infants did not acquire their bacterial flora from the artificial feeds.

Table VIII. Results of airborne bacterial sampling in clinical areas during periods of routine and high activity.

Clinical area	Routine activity periods					High activity periods				
	No. of tests	patho-gens	Gram — ve	Gram — ve	Pathogens per test	No. of tests	Patho-gens	Gram — ve	Gram — ve	Pathogens per test
Incubator room	18	6	1	5	0.33	12	8	4	4	0.67
Special care corridor	21	12	9	3	0.57	—	—	—	—	—
Special care cubicles	—	—	—	—	—	12	19	10	9	1.58
Operating theatres	18	1	0	1	0.06	—	—	—	—	—
Delivery rooms	18	2	2	0	0.11	—	—	—	—	—
Ward and its 'night nursery'	17	10	6	4	0.59					
No. of infants 4						3	6	4	2	2.0
in residence 5						6	8	5	3	1.33
6						3	11	5	6	3.67
10						3	15	8	7	5.0

DISCUSSION

This study set out in an attempt to define the sources from which newborn infants derive their bacterial flora. The vast majority of these bacteria are human parasites unable to survive and reproduce to any degree outside the body or its fluids. We have previously shown that high levels of bacterial colonization are reached by Day 3 and this reaches a maximum by Day 5. How is this achieved? In approximately one third (32.7%) of infants, the colonizing bacteria are derived from their mothers, and in them the source is most often (74.6%) the mother's rectum. On the other hand, the maternal birth canal is not an important source of bacteria under normal labour conditions. Our findings would seem also to exculpate a direct relationship with the noses or hands of the hospital staff, or with artificial feeds.

Although the overall distribution of the bacterial species derived from equipment and from air samplings was quite different from that previously found in the infants (McAllister *et al.*, 1974), these constitute a reservoir of bacteria capable of airborne transmission. Furthermore, this reservoir is continually replenished from human sources, both patients and staff. It is, however, hard to envisage the small proportion of pathogens isolated in our swabs from equipment and in the air samples as a major source of bacteria unless the very small inoculum which must reach each infant is capable of extremely rapid multiplication. This may indeed be the situation in the modern maternity unit, but provided we can avoid the entry into these units of large numbers of certain pathogens which once wrought such havoc among the newborn such as *Streptococcus pyogenes* and *Staphylococcus aureus* or of enteropathogenic *E. coli*, the epidemics of former years should not return. It is difficult to see how the occurrence of an occasional Gram-negative infection in the newborn infant could be entirely prevented (Forfar *et al.*, 1968; Conn, 1969) but the need for continual vigilance and an efficient bacteriological service within all maternity units has been in no way diminished.

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