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Bacteria in a hospital nursery: laboratory and clinical studies

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INTRODUCTION

Of the many investigations for the presence of bacteria and of clinical sepsis in hospital nurseries most have focused attention on the staphylococcus. The publications of Rountree & Barbour (1950); Edmunds, Elias-Jones, Forfar & Balf (1955); Hutchison & Bowman (1957); Gillespie, Simpson & Tozer (1958); Plueckhahn & Banks (1958); Williams (1961), and many others, are testimonies to the interest engendered by this organism. In contrast, published accounts of the ecology of the Gram-negative intestinal bacilli have been more infrequent. Laursen (1963) and Kresky (1964) described the colonization of neonates with Gram-negative bacilli, as also have Shallard & Williams (1965, 1966); and Sarkany & Gaylarde (1967) included these organisms in their account of the skin flora of the immediate newborn. Studies illustrating the epidemiological properties of the Gram-negative intestinal bacilli are being more frequently reported, the most comprehensive being that of Laursen (1962) and Reinarz, Pierce, Mays & Sandford (1965). The increasing importance of the Gram-negative bacilli in hospital nurseries has been summarized. (Editorial. *Am. J. Dis. Child.* 1961.)

The present study took place from August to October 1964, and was a preliminary to a subsequent trial on the effects of antiseptics in two hospital nursery units, of which this unit (Western General Hospital) is one. It was required to know (*a*) the rate of colonization of infants with *Staphylococcus pyogenes* and Gram-negative bacilli under non-epidemic conditions, (*b*) the staff carriage of these bacteria, (*c*) their incidence in the nursery environment, and (*d*) the state of clinical infection due to these organisms. This information was required to establish normal patterns in the ecology of *S. pyogenes* and Gram-negative bacilli in a hospital nursery where no antimicrobial agent was used for infants other than surgical spirit treatment of the umbilical stump and plain soap for washing purposes, plain soap only being used for staff handwashing. This paper also discusses the present position of *S. pyogenes* in a nursery with particular attention to the changes in ecology resulting from the introduction of the penicillinase-resistant penicillins.

MATERIALS AND METHODS

Laboratory investigations

Examination of infants, attending staff and ward environment

Infants. Swabs were taken from the nose, post-nasal space and umbilicus of each infant, up to three times during the first 7 days of life. Swabbing of long-stay infants was continued beyond the seventh day. For nasal and per-nasal sampling small diameter cotton-wool swabs were used. For sampling the umbilicus the usual adult-type throat swab was employed.

Attendant staff. Broth-moistened swabs of the nose, together with hand-impression plates were taken on repeated occasions. The hand impressions were obtained by placing the tips of the digits and the palms of both hands on the surface of solid culture media.

Ward environment. Repeated sampling of ward fixtures and fittings, walls, floors, etc., was done using Sellotape transfers (Thomas, 1961) and broth-moistened swabs. The bacterial content of the air was measured using the 'Casella' slit sampler, sieve sampler (duBuy & Crisp, 1944) and settle plates. Five cu. ft. volumes of air were sampled on each occasion using the slit sampler, and 12·5 cu. ft. volumes with the sieve sampler. Settle plates were placed on the floor for one hour in different parts of the nursery. These examinations coincided with the sampling of infants and staff.

Media

All swabs were inoculated on 5% horse blood agar and MacConkey agar which were incubated for 18 hr. at 37° C. and examined for growth. Each swab was inoculated in a zig-zag manner covering a half plate. The same media were also used for the hand impressions and Sellotape transfers. For air sampling horse blood agar only was used.

Identification of bacterial growth

Staphylococcus pyogenes. A single representative colony from each blood agar plate was subjected to the slide test for coagulase production (Cadness-Graves, Williams, Harper & Miles, 1943) and to the tube method (Cowan, 1938).

Gram-negative bacilli. Identification was by the macroscopic appearance of colonial growth on culture supported by the Gram stain. No attempt was made in this preliminary study to measure the incidence of the different genera, except where clinical infection supervened.

Phage typing of Staphylococcus pyogenes

This was done by the method of Blair & Williams (1961) using phage filtrates at a concentration 100 times the routine test dilution (100 RTD). Where no result was obtained at this dilution undiluted phage filtrate was used and only when this failed to yield lysis was the strain recorded as 'non-typable'. The classification of *S. pyogenes* phage typing patterns into phage groups followed that proposed by Blair & Williams (Table 1).

Sensitivity to antibiotics of Staphylococcus pyogenes

Strains were tested for sensitivity to penicillin (1 unit in disk), streptomycin (10 μg), chloramphenicol (25 μg.), tetracyline (25 μg.), erythromycin (10 μg.) and methicillin (10 μg.) by the filter paper disk method (Gould & Bowie, 1952). The organism was recorded as sensitive when the diameter of the zone of inhibition of growth was 15 mm. or greater, and as resistant when 14 mm. or less, the diameter of the disk being 6·25 mm.

Table 1. *Classification of* Staphylococcus pyogenes *into phage groups (Blair & Williams*, 1961)

Phage group	Lysed by bacteriophages
Group I	29, 52, 52A, 79, 80
Group II	3A, 3B, 3C, 55, 71
Group III	6, 7, 42E, 47, 53, 54, 75, 77, 83A
Group IV	42D
Miscellaneous	81, 187
Mixed	Lysis by phages from more than one group

Note: where phage pattern was 80/81 the strain was placed in group I.

For convenience in handling the different antibiotic-sensitivity patterns encountered, a system of coding was adopted:

Category A. Strains sensitive to chloramphenicol, erythromycin, and methicillin, and resistant to penicillin, streptomycin and tetracycline.

Category B. Strains resistant to penicillin only.

Category C. Strains sensitive to all the antibiotics tested, together with the occasional strain possessing an unusual sensitivity pattern. These latter were mostly encountered in infants, from whom very few multiple-sensitive strains were isolated, and showed resistance to chloramphenicol or to erythromycin, or to tetracycline only.

Interpretation of cultures

The presence of small numbers of pathogenic bacteria recovered from the nose, and from the skin and fomites, can not necessarily be considered of epidemiological significance (Wallace & Duguid, 1952; White, 1961; Gonzaga, Mortimer, Wolinsky & Rammelkamp, 1964). For this reason a system of scoring the numbers of colonies yielded from the samples was employed in this survey. Growth on blood agar and MacConkey agar was scored as follows:

Swabs from infants and attendant staff. More than 100 colonies of *S. pyogenes* or Gram-negative bacilli constituted a heavy growth and was scored + + +, 30 to 99 colonies a moderate growth and scored + +, and 10 to 29 colonies a scanty growth, scored +. Less than 10 colonies, and a + growth in the presence of other bacteria was not considered of epidemiological significance and has not been included in the results.

Hand impressions, Sellotape transfers and swabs of ward surfaces. More than eight colonies of either *S. pyogenes* or Gram-negative bacilli constituted a heavy growth

and was scored + + +, 5 to 8 colonies a moderate growth scored + +, and 2 to 4 colonies a scanty growth scored +. Less than 2 colonies was not considered significant and has not been included in the results. A + growth was considered significant even when other organisms were present.

Slit and sieve sampler, and settle plates. The total bacterial count was recorded, and all *S. pyogenes* isolated were considered of epidemiological significance.

Clinical investigation

Independently of the laboratory investigations, bacteriological examinations were arranged on any infant showing clinical evidence suggesting infection. A careful record was kept of suspected infections and any treatment given.

Description of nursery

Two main receiving wards each of up to twenty cots, together with a premature baby unit of three to six cots or incubators, a wash room, isolation ward, and a corridor separating the two receiving wards constituted the nursery unit. These areas were all examined bacteriologically.

Routine infant care and ward hygiene

Infants were bathed with soap and water at delivery and every fourth day thereafter. Sponging with water only was done each day. The umbilical stump was treated with spirit after each napkin change and was left without any occlusive dressing. Ward floors were vacuum-cleaned daily and washed weekly with 'Savlon'. Furniture, cots, window ledges, etc., were 'damp-dusted' with 'Savlon' daily. No special treatment was employed for cot sheets and blankets; baths were cleaned with 'Savlon' after each bathing.

RESULTS

Incidence of bacteria

Staphylococcus pyogenes

Infants. Two hundred and twenty-two infants were examined, from whom a total of 1753 swabs were taken. There was a progressive increase in the isolation rate of staphylococci from the three sites sampled (Fig. 1), reaching a peak on the sixth day in normal infants (i.e. those which were discharged on the seventh day of life). The umbilicus was the most frequently colonized, 73·1% of swabs examined on the sixth day yielding these organisms.

The low rate of isolation from the nose during the first 3 days of life is in marked contrast to the results of other workers (Cunliffe, 1949; Gillespie *et al.* 1958) who obtained 44–55% carriage rate even on the second day. Our finding that 73·1% of umbilical swabs yielded staphylococci on the sixth day is significantly greater than that of Edmunds *et al.* (1955), where only 40·1% of umbilical samples were positive for this organism on the eighth day. However, Hutchison & Bowman (1957) obtained a 77·2% prevalence on the third day. That the umbilicus was

colonized before the nose agrees with the findings of Gillespie *et al.* (1958) and Hurst (1960*a*).

The slow acquisition of nasal carriage observed by us may be attributable to the use of penicillinase-resistant penicillins, introduced in 1960. Traces of these penicillins in the ward air may be inspired, thus delaying nasal colonization. That the umbilical carriage rates were unaffected may be due to the smaller quantity of air impinging on the umbilicus, and hence much less exposure to antibiotic.

Attendant staff. Nine of 51 subjects (18%) were nasal carriers, and 27 (53%) yielded staphylococci from their hands (Table 2). Nasal carriage is lower than has

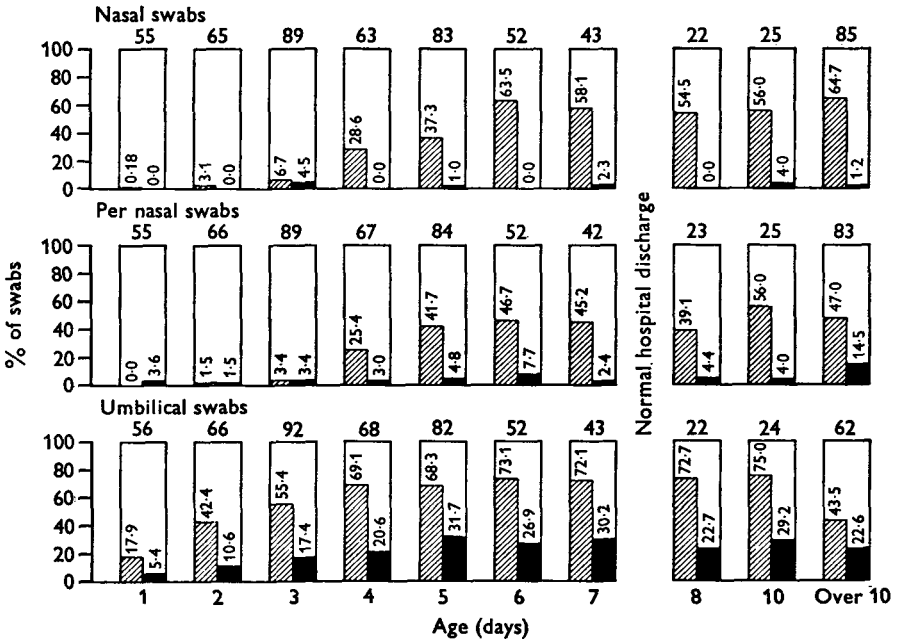

Fig. 1. Infants. Percentage isolations of *Staphylococcus pyogenes* and Gram-negative bacilli. Figures at head of columns denote the number of swabs taken. ▨ *Staphylococcus pyogenes*; ■ Gram-negative bacilli.

Table 2. *Carriage by staff of* Staphylococcus pyogenes *and Gram-negative bacilli*

	Total number	Number positive for *Staph. pyogenes*	Number positive for Gram-negative bacilli
Staff investigated	51	28 (55)*	8 (16)
Nasal swabs taken	116	18 (16)	0
Hand swabs taken	116	35 (30)	10 (9)
Nasal carriers		9 (18)	0
Hand carriers		27 (53)	8 (16)

* Figures in parentheses indicate percentages.

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been previously recorded (Ludlam, 1953; Edmunds *et al.* 1955; Hutchison & Bowman, 1957; Monro & Markham, 1958; Poole, 1960), where the findings ranged from 21 to 58%. This low nasal carriage may be due to exposure to the new penicillins as postulated in the case of infants. Reports of the incidence of hand carriage are infrequent, though Love *et al.* (1963) found 11·8% of nurses' hands yielded staphylococci after handling a colonized infant, and Plueckhahn & Banks (1958) found 28 out of 210 nurses' hands (13·4%) positive for staphylococci.

Table 3. *Ward environment examinations. Bacterial contamination of ward fixtures and fittings, and bacterial content of air*

	Number of samples	Number positive for: *Staph. pyogenes*	Number positive for: Gram-negative bacilli	Total counts: Max.	Total counts: Min.	Total counts: Aver.
Sellotape transfers	131	41 (31)*	7 (5)			
Broth-moistened swabs	103	6 (6)	4 (4)			
Air						
Slit sampler	30	16 (53)	NR	320†	6†	77†
Sieve sampler	77	51 (66)	NR	320‡	11‡	112‡
Settle plates	67	27 (40)	NR	176	3	61

* Figures in parentheses indicate percentages.
† Counts per 5 ft.^{3}.
‡ Counts per 12·5 ft.^{3}.
NR = not recorded.

Ward environment. Table 3 shows the results of sampling ward surfaces and ward air. Sellotape was more effective than swabs in surface sampling, yielding a 31% isolation rate. The three air examination methods (slit and sieve samplers, settle plates) yielded mean isolation rates as follows:

0·11 *S. pyogenes*-carrying particles per cu. ft. (slit),

0·05 *S. pyogenes*-carrying particles per cu. ft. (sieve),

0·40 *S. pyogenes*-carrying particles per one hour (settle).

Staphylococci constituted 0·7% of the total bacterial count (slit), 0·6% (sieve) and 0·7% (settle). These results are in close agreement with those reported in an earlier study of staphylococcal air contamination in a hospital nursery (Wallace & Duguid, 1952).

Gram-negative bacilli

Infants. Colonization was much less evident than with staphylococci (Fig. 1). The highest incidence of carriage of Gram-negative bacilli was observed in the umbilicus on the fifth day (31·7%). Laursen (1963) found Gram-negative bacilli in 15·6% of umbilical swabs taken after the separation of the cord, which presumably was about the eighth day of life. As in our study, Laursen did not use any occlusive dressings, but she did apply a topical antibiotic powder to the umbilicus

in all cases. Shallard & Williams (1966) found 58% of umbilical swabs taken on the first day of life to contain Gram-negative bacilli, a much more rapid acquisition than observed in this series. The same authors obtained a 36% carriage rate from the noses of infants less than 24 hr. old, which compares markedly with our inability to recover Gram-negative bacilli from the infants' noses during the first 48 hr. of life. The high prevalence of Gram-negative bacilli reported by Shallard & Williams was possibly due to their daily use of chlorhexidine in infant care, this antiseptic being especially active against Gram-positive flora. They did not state whether the umbilicus was covered or not.

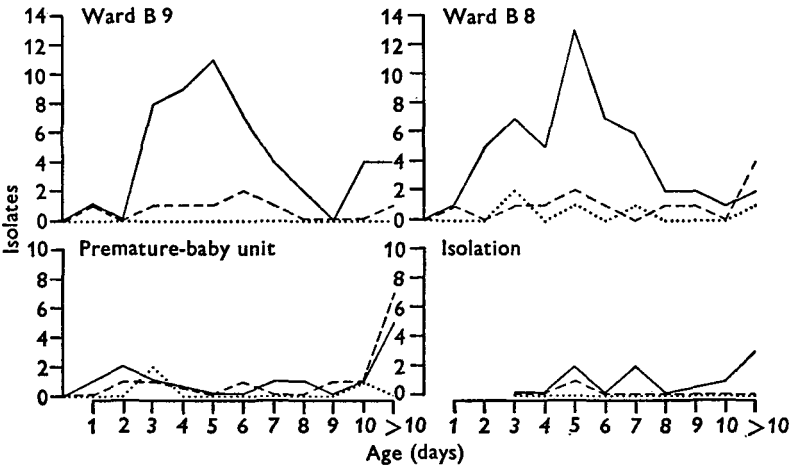

Fig. 2. Gram-negative strains from component wards of nursery (infants).
... Nose; ---- post-nasal area; —— umbilicus.

The component units of the nursery were studied, and an increased frequency of isolation of Gram-negative bacilli from those infants in the two main receiving wards was noted (Fig. 2). The frequency of isolation in the two wards (premature baby unit and isolation ward) where special nursing precautions were enforced was negligible.

Attendant staff. No Gram-negative bacilli were isolated from the noses of the staff, but 16% showed transient hand carriage (Table 2). This is in close agreement with Shallard & Williams (1965), who obtained these organisms from 10% of hands of staff, and who stated that the noses of the staff 'seldom contained Gram-negative bacilli'.

Ward environment. Only 5% of surface samples yielded Gram-negative bacilli (Table 3), which contrasts with the 35% yield-rate from ward surfaces reported by Shallard & Williams (1965). The reason for this marked difference is not apparent. Certainly no antibiotics were used in treating infections caused by Gram-negative bacilli during our study, and this may well have been a factor in the low surface contamination observed.

The incidence of these organisms in the ward air was not measured in this parti-

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cular investigation. Unpublished observations in the same nursery (Winton, 1965) have shown that the mean isolation rates were:

0·005 Gram-negative bacilli carrying particles per cu. ft. (sieve),

0·20 Gram-negative bacilli carrying particles per one hour (settle).

Gram-negative bacilli constituted 0·09% of the total air-borne flora (sieve) and 0·15% (settle). It may thus be stated that Gram-negative bacilli at the time of this study were present in the air in about one-half to one-tenth the concentration of *S. pyogenes*.

The ratio of recovery of *S. pyogenes* to Gram-negative species from surfaces was 4·3 to 1. This is similar to the proportions of these bacterial species in the air, which was 4·7 to 1 (settle plates).

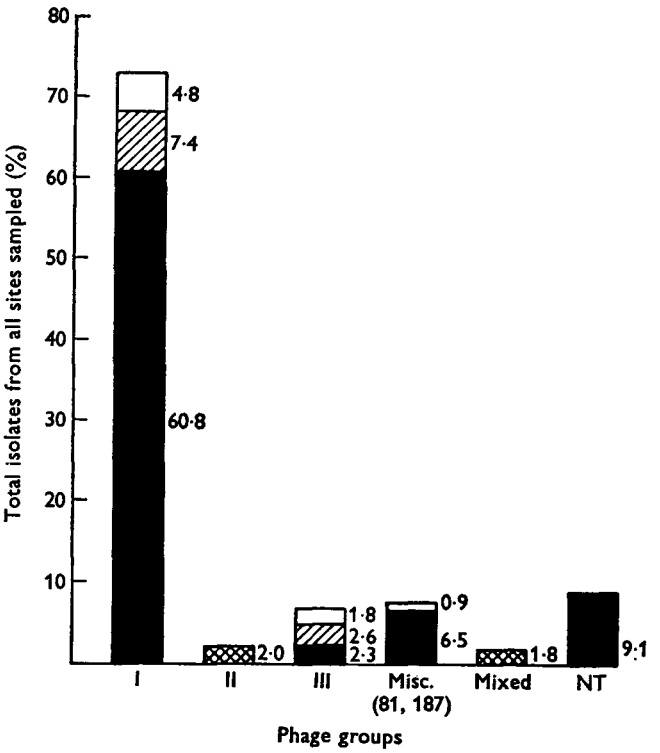

Fig. 3. Infants. Phage groups and antibiotic sensitivity patterns of *Staphylococcus pyogenes* strains. ■ Category A, strains sensitive to chloramphenicol, erythromycin and methicillin, and resistant to penicillin, streptomycin and tetracycline; ▨ category B, strains sensitive to all antibiotics except penicillin; □ category C, strains possessing other sensitivity patterns (majority multiple-sensitive); ▩ mixture of foregoing sensitivity patterns.

Phage typing of Staphylococcus pyogenes

Infants

A total of 567 strains from 182 infants were typed (171 strains from nose, 129 from post-nasal area, 267 from umbilicus). All three sites yielded almost the same incidence of the different phage groups, Fig. 3 representing the observations

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obtained from each site. Phage group I predominated, constituting 73·0% of all strains. Other investigators have found group III pre-eminent (Wallmark, 1953; Baldwin, Rheins, Sylvester & Shaffer, 1957; Gillespie *et al.* 1958; Hurst, 1960*a*). Hutchison & Bowman (1957) reported group III strains to account for 81·0% in a similar non-epidemic study.

Gould (1958) observed that when penicillin was in common use group III was the predominant phage group in ward air, and when penicillin was excluded groups I and II were most often encountered. Thus it may be that the use of the penicillinase-resistant penicillins together with other antimicrobial agents, i.e. the newer disinfectants, at the expense of penicillin, has resulted in the change to phage group I observed by us.

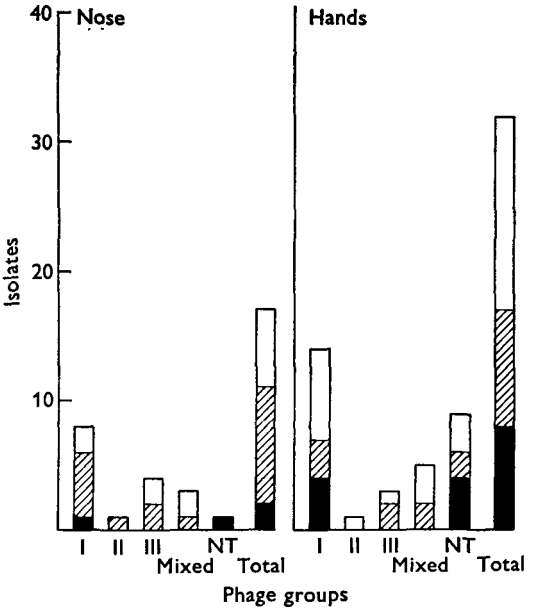

Fig. 4. Attending staff. Phage groups and antibiotic sensitivity patterns of *Staphylococcus pyogenes* strains. ■ Category A, strains sensitive to chloramphenicol, erythromycin and methicillin, and resistant to penicillin, streptomycin and tetracycline; ▨ category B, strains sensitive to all antibiotics except penicillin; □ category C, strains possessing other sensitivity patterns (majority multiple-sensitive).

Attendant staff

Forty-nine strains were typed, seventeen from the nose and thirty-two from the hands (Fig. 4). Group I composed most of the strains from the nose (47·0%) and the hands (43·7%). Baldwin *et al.* (1957) found group III to be the predominant nursery staff phage group (54·0%). However, our findings are supported by Poole (1960) and Barber (1961), who stated that strains obtained from nurses were usually of group I phage pattern.

Ward environment

Surfaces. Groups I and III were predominant, composing 37·5 and 31·2% respectively of typed strains (Fig. 5). This finding differs from that of Poole (1960), who reported no predominant phage group in forty samples from fomites. Yet other reports have shown group III pre-eminent in floor dust (Alder, Gillespie & Thompson, 1955; Hutchison & Bowman, 1957).

Air. Groups I and III were again the commonest found, constituting 43·1 and 33·8% respectively. Rountree & Barbour (1950) found group III most often in the air of a hospital nursery, as did Hurst (1960*a*).

Both surface and air strains appear to be showing a change in phage group, from group III of previous reports to approximately equal incidence of groups I and III in this study. This 'swing' towards group I parallels the results obtained with infants' strains, and the same interpretation is applicable, namely, that the introduction of the new penicillins is bringing about the disappearance of group III strains with their replacement by group I.

Antibiotic sensitivities of Staphylococcus pyogenes

Infants

All strains phage typed were tested for their sensitivity to antibiotics, and were placed into one of the three sensitivity categories already described. Fig. 3 illustrates the results obtained, and also shows the incidence of the three sensitivity categories within each of the phage groups. Group II and 'mixed' groups contained too few strains to justify separation into their component sensitivity categories. Separate analysis of the three sites sampled with regard to antibiotic patterns does not warrant description, Fig. 3 reproducing closely the results obtained from each of the sites.

Category A constituted 79·0% of all strains, this being the prevailing sensitivity throughout the hospital. Hutchison & Bowman (1957) found that 67·5% of their strains gave antibiotic-sensitivity patterns which correspond to our category C, only 14·5% belonging to our category A. Thus, most of their strains were penicillin-sensitive. Only 10·1% of our strains showed sensitivity to penicillin (this figure includes strains from group II and 'mixed' phage group), so we agree with Barber & Burston (1955), who found 11·0% of their strains sensitive.

When note is taken of the sensitivity pattern within each phage group category A was found to compose 83·3% of group I strains (Fig. 3). Group I strains possessing category A sensitivity pattern were, therefore, the colonizers of infants. It is of interest that this sensitivity category was found more often among group III strains by Barber & Burston, though Hurst (1960*b*) found group I to contain strains with this sensitivity pattern. Categories A and B, i.e. the penicillin-resistant staphylococci, compose 93·4% of group I strains and 73·1% of group III strains. These figures for penicillin resistance differ markedly from the results of Anderson & Williams (1956), who found 35% of group III strains and 17% of group I resistant to penicillin. However, our observation that penicillin resistance was commoner among group I strains is supported by Barber & Burston (1955).

Attendant staff

The most noticeable feature was the infrequency of Category A strains, only two of seventeen nasal strains and eight of thirty-two hand strains possessing this pattern (Fig. 4). Penicillin-resistant strains (categories A and B) constituted 64·7% of nose staphylococci and 53·1% of hand staphylococci; these results agreeing closely with previous reports (Ludlam, 1953; Barber & Burston, 1955; Hutchison & Bowman, 1957; Monro & Markham, 1958; Poole, 1960).

Among group I strains categories B and C were more frequently seen, which contrasts with the almost total predominance of category A in infants' group I strains.

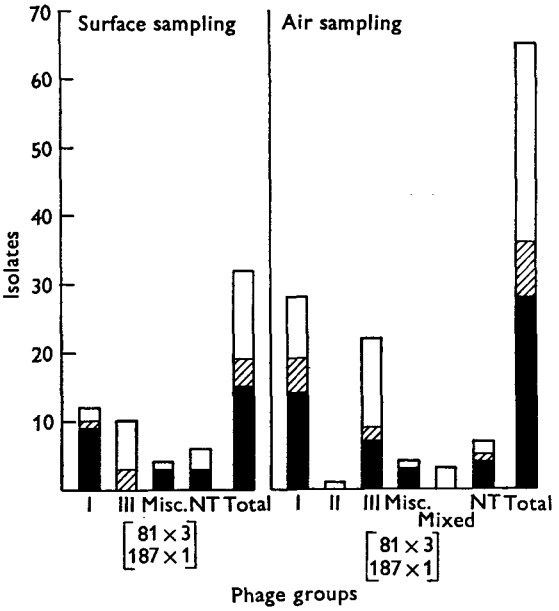

Fig. 5. Environment. Phage groups and antibiotic sensitivity patterns of *Staphylococcus pyogenes* strains. ■ Category A, strains sensitive to chloramphenicol, erythromycin and methicillin, and resistant to penicillin, streptomycin and tetracycline; ▨ category B, strains sensitive to all antibiotics except penicillin; □ category C, strains possessing other sensitivity patterns (majority multiple-sensitive).

Ward environment

Surfaces. Almost half the strains were of category A (46·9%). (See Fig. 5.) Penicillin-resistant staphylococci accounted for 59·4% of all strains, this being significantly less than the 87·5% penicillin resistance quoted by Ludlam (1953) from forty-eight dust strains. This is probably due to the reduced use of benzylpenicillin since the introduction of the new penicillins.

Category A pattern was more often seen in group I strains, and category C in group III.

Air. The sensitivity patterns were very similar to those found among surface strains, category A making up 43·1% and penicillin-resistant strains 55·4% (Fig. 5).

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Within the phage groups category A sensitivity pattern was linked with group I, and category C with groups I and III.

Clinical infection

During the period of this investigation, 247 infants were admitted to the nurseries. Of these, 5 died and 16 were discharged home or transferred to other units within 48 hr. of delivery and were excluded from the study. Of the remaining 226 infants 40 showed sufficient abnormality to justify bacteriological investigation involving 54 specimens (Table 4). Swabs of conjunctival discharge were taken from 18 infants and local antibiotic treatment was given to 16 of these. Specimens were of stool in 14 cases and no pathogenic organisms were isolated from 12 of these. From the remaining 22 specimens, organisms were cultured from 20. The organisms isolated from all specimens are shown in Table 4. Of the 36 organisms isolated, 16 were *S. pyogenes* and these came from 15 infants. The antibiotic sensitivity pattern showed that 14 belonged to category A, 1 to category B and 1 to category C. Phage typing was not carried out routinely on these strains. Seven of the thirty-six organisms isolated were Gram-negative bacilli and were isolated from the eye (2), urine (2), umbilicus (1), throat (1), skin (1).

Table 4. *Organisms isolated from cases of clinical infection*

Organism	Total	Eye	Skin	Umbilicus	Mouth	Nose and throat	Urine	Stool	Gastric washing
Staphylococcus pyogenes	16	8	4	2	—	—	—	2	—
Staphylococcus albus	5	4	—	—	—	1	—	—	—
E. coli	2	—	1	—	—	—	1	—	—
Proteus spp.	1	—	—	—	—	—	1	—	—
Ps. pyocyanea	1	—	—	1	—	—	—	—	—
Paracolon bacilli	3	2	—	—	—	1	—	—	—
Streptococcus pyogenes	3	—	—	2	—	1	—	—	—
Pneumococcus	1	1	—	—	—	—	—	—	—
Candida spp.	4	—	—	—	3	—	—	—	1
None	18	3	—	1	—	1	1	12	—
Total	54	18	5	6	3	4	3	14	1

Systemic antibiotic therapy was given to five infants. In two there was no bacteriological support to the clinical impression of infection. One of premature twins died at 18 days of age from bilateral adrenal haemorrhage, probably associated with septicaemia. Blood cultures were not carried out before death. *Staph. pyogenes* had been cultured from skin lesions and conjunctival discharge. This organism was also cultured from the twin whose blood culture was negative. She was also treated, and survived. The fifth infant was treated successfully for a respiratory infection, *Streptococcus pyogenes* having been cultured from the throat swab.

Thus, 21 of the 226 infants (9·3%) showed clinical or bacteriological evidence of infection requiring local or systemic antibiotic therapy.

Ante-natal and post-natal wards

The patients in these wards were nose-swabbed on one occasion only. One isolation of *Staph. pyogenes* in significant numbers was made from eighteen ante-natal patients. This strain was non-typable and possessed category C pattern. This patient's infant subsequently yielded *S. pyogenes* from all three sites; these strains were all group I and of category A pattern.

Of twenty-six mothers who were examined after delivery two yielded *S. pyogenes* in significant numbers. One of these strains was of phage group I and category B sensitivity: her infant was not examined. The other mother yielded a group III strain of category B, but her infant was not a carrier.

No firm conclusions can be drawn from these findings in view of the small survey. Perhaps all that may be said is that there did not appear to be any transference of staphylococci between the mother and her infant.

No Gram-negative bacilli were isolated from any of these patients.

DISCUSSION

This combined laboratory and clinical study was undertaken to provide an indication of the behaviour of *Staphylococcus pyogenes* and Gram-negative bacilli in a hospital nursery not employing any specific antiseptic or aseptic methods (other than those described). Although the role of the staphylococcus in a nursery has been investigated extensively (mostly between 1942 and 1960), comparatively little attention has been devoted to the presence of the Gram-negative bacilli. We only know of one similar investigation which has examined the rate of colonization of infants with these bacteria, and which has also included the measure of their presence in the ward staff and in the ward environment (Shallard & Williams, 1965, 1966). However, these workers only swabbed infants at weekly intervals and were thus unable to show the day-by-day gradual acquisition of bacteria at the usual sites of carriage. Also no measure was given of separate nose and hand carriage of Gram-negative organisms by the staff. With the continuing use of antiseptics in the nursery, almost all of which are active primarily against the Gram-positive flora, the analysis of the Gram-negative bacilli becomes increasingly important.

Clinical sepsis

The pattern of clinical sepsis was that commonly found at the time of the trial. Surface infection of the eyes, skin and umbilicus accounts for the majority of the lesions, with *S. pyogenes* the predominant organism. The antibiotic sensitivity pattern, with fourteen of sixteen staphylococci belonging to category A, is in keeping with the organisms grown from the sampling of all infants but differs from the pattern found in the hands and noses of the attendant staff. Satisfactory bacteriological proof of infection as the cause of death of the premature infant who

died from adrenal haemorrhage is lacking but with this probable exception there were no serious infections. Gram-negative organisms did not give rise to any infections of sufficient severity to justify systemic antibiotic therapy. This contrasts with the present position in the same nursery where Gram-negative infections are now the chief indication for systemic antibiotic therapy (Keay, Syme & Barnes, 1967).

Routes of spread of Staphylococcus pyogenes

Figure 6 depicts the possible routes by which we believe infection is transmitted in a hospital nursery. For each of the five main aspects of the nursery (air, ward surfaces, staff hands, staff noses, infants' carriage sites) we have formulated a pattern combining the predominant phage group isolated with the most fre-

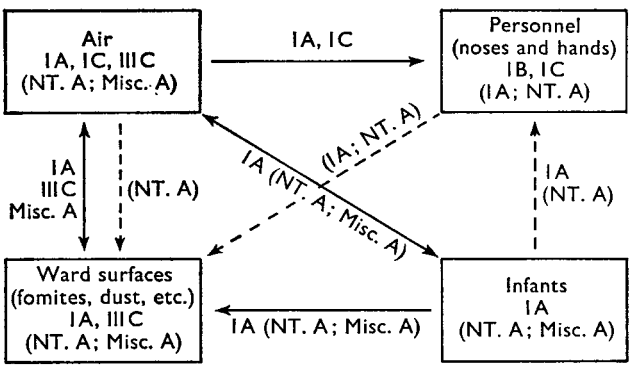

Fig. 6. Routes of transmission of *Staphylococcus pyogenes*. IA, IIIC, etc., denote strains of phage group I, sensitivity category A; phage group III, category C, etc. NT. A = non-typable strain, category A. Misc. = miscellaneous phage group. Strains in parentheses are regarded as secondary colonizers. Continuous line = probable route of transmission; broken line = possible but not proven route.

quently occurring antibiotic sensitivity category. This pattern is presented as IA, IIIC, etc. Less frequently encountered patterns are shown in parentheses. The ward air, for example, contained staphylococci of IA, IC and IIIC patterns predominantly, and less frequently NT.A (non-typable category A), and miscellaneous A.

We believe the ward air to be the primary *depot* of nursery staphylococci. Transmission of aerial staphylococci occurred to both infants and the noses of attending personnel. The infants became colonized primarily with IA strains (Fig. 6). The aerial staphylococci which colonized the nose of the attending personnel possessed different patterns, IA strains being very infrequent. In summary, the infants and attending staff 'abstract' different staphylococci from the air as their dominant colonizing strains. There is no transfer of staphylococci from the noses of the attending staff to infants.

The air is also a *depot* for direct transmission of staphylococci to ward surfaces. Both IA and IIIC patterns were deposited from the air on to the floors and other ward objects including bedclothing.

The infants, once colonized, become a sub-depot for transmission of *some* of their strains (I A, NT.A) to the hands of the attending staff and thence to ward objects. It was observed that *S. pyogenes* of the miscellanous phage group were only isolated from the air, infants and ward surfaces (Fig. 6) and not at all from the attending staff. Similarly III A strains were only isolated from the air and the infants. The hands of the nurses cannot, therefore, be implicated as an important route of spread of staphylococci from infant to infant.

Ward surfaces and infants are, therefore, contaminated primarily from the air. The question thus arises: from what *source* do the aerial staphylococci come? We believe that there are two sources, one being one or more of the infants in the ward, those infants being active 'dispersers', and the other source being the ward surfaces. Those infants who are active 'dispersers' may be infants normally colonized in the nose but who have been converted into 'dispersers' or 'spreaders' following therapy with tetracycline, kanamycin or the penicillinase-resistant penicillins (Ehrenkranz, 1965). The staphylococci are dispersed into the air around the infant, and also to the cot bedclothing. Thus the second source becomes obvious, namely, the ward surfaces, which include the blankets and the floor. There develops a constant interchange of bacteria between the ward surfaces and the air, this being readily brought about by such routine ward activities as cot-making and the movement of cots and other objects during ward cleaning. The importance of the presence of the ward personnel in disturbing floor dust cannot be underestimated. However, the personnel play no part in *direct* transmission of staphylococci, they are merely 'onlookers at the cycle, infant → ward surfaces → air → infant', as depicted in Fig. 6.

Efforts designed to reduce the load of aerial bacteria should result in a lowering of infant colonization rates and ward surface contamination, and this has been shown to occur following the use of methicillin aerosols in wards (Elek & Fleming, 1960). An alternative method of reducing the aerial staphylococci is to treat all the infants with an antiseptic effective against these bacteria and so minimize the consequences of infant 'spreaders' in the population. This latter method has been the subject of many trials, with varying degrees of success (Baldwin *et al.* 1957; Gillespie *et al.* 1958; Gluck & Wood, 1961).

Ecology of the Gram-negative bacilli

Nasal carriage of these bacteria was minimal, despite their not inconsiderable presence in the ward air. Such bacteria are, therefore, poor colonizers of the healthy infant's, and adult's, upper respiratory tract.

Gram-negative organisms colonizing the umbilical area of the infants had, almost certainly, resulted from spread from the infants' peri-anal area. We did not carry out a detailed examination of the umbilical flora, but it would be interesting to know the extent of the relationship which exists between the umbilical flora of the infant and its faecal flora. Perhaps comparative serotyping of *Esch. coli* strains from both the infant's umbilicus and its faeces would prove of value.

We propose that the transmission of Gram-negative bacteria in the hospital

nursery parallels that of *S. pyogenes*, except that, (*a*) little, or no, nasal colonization by aerial Gram-negative bacilli occurs, and, (*b*) the infants' Gram-negative umbilical flora is of endogenous and not aerial origin. The air is thus the *depot* and the infants the *source* of these organisms. The nurses' hands convey some of the infants' Gram-negative flora to ward surfaces, but cannot be held responsible for infant to infant spread. The ward surfaces are further contaminated with bacteria sedimenting from the air. This latter mode of transmission also operates in reverse, there being a constant interchange between ward surfaces and the air, as we have already described for staphylococci.

SUMMARY

Two hundred and twenty-two infants and fifty-one staff were examined for carriage of *Staphylococcus pyogenes* and Gram-negative bacilli, and the presence of these bacteria in the ward environment was also investigated. Staphylococci were phage-typed and tested for sensitivity to antibiotics. Assessment of clinical infection caused by these bacteria was made.

Infant carriage of *Staphylococcus pyogenes* was maximal on the sixth day, 73·1% of umbilical swabs yielding significant growth. Carriage of Gram-negative bacilli was maximal on the fifth day, 31·7% of umbilical swabs being positive. Where special nursing was enforced, i.e. premature baby unit, isolation of Gram-negative bacilli was very infrequent.

The low nasal carriage of staphylococci seen in infants and nursing staff is attributed to traces of penicillinase-resistant penicillins in the ward air.

Staphylococcus pyogenes was recovered from 23% of ward surfaces, and constituted 0·7% of the air-borne flora. In contrast, Gram-negative bacilli were recovered from 4·7% of surfaces, and composed only 0·15% of the air-borne flora.

Phage group I constituted 73·0, 44·9 and 41·2% of strains from infants, staff and ward environment respectively.

Antibiograms were grouped into three categories, A, B and C. Category A (sensitive to chloramphenicol, erythromycin and methicillin but penicillin-resistant) composed 79·0% of infants' strains, being found mostly in group I, but was rare among staff strains.

Of 54 specimens taken from 40 infants showing evidence of infection, *Staphylococcus pyogenes* was isolated from 16, of which 14 were category A. Gram-negative bacilli were isolated 7 times. Only 21 infants required antibiotic therapy (9·3%) and none were infected with Gram-negative bacilli—this contrasts with the frequency of these bacilli in nursery infection today.

Ward air is considered to be the main *depot* of *Staphylococcus pyogenes*. Infant 'dispersers' are probably the principal *source* of the air-borne staphylococci. Infants and staff abstract from the air those staphylococci which become their colonizing strains. No transfer is thought to occur from the staff to the infants. Transmission of Gram-negative bacilli is similar in that the infants are the principal source and the air the main *depot* of these bacteria. Nasal colonization by Gram-negative bacilli is low, this is possibly due to poor colonizing properties. Infant umbilical carriage almost certainly results from endogenous faecal spread.

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REFERENCES

ALDER, V. G., GILLESPIE, W. A. & THOMPSON, M. E. M. (1955). Virulence and phage patterns of antibiotic-resistant staphylococci in a hospital. *J. Path. Bact.* **70**, 503.

ANDERSON, E. S. & WILLIAMS, R. E. O. (1956). Bacteriophage typing of enteric pathogens and staphylococci and its use in epidemiology. *J. clin. Path.* **9**, 94.

BALDWIN, J. N., RHEINS, M. S., SYLVESTER, R. F. & SHAFFER, T. E. (1957). Staphylococcal infections in newborn infants. III. Colonization of newborn infants by *Staphylococcus pyogenes*. *Am. J. Dis. Child.* **94**, 107.

BARBER, M. (1961). Hospital infection yesterday and today. *J. clin. Path.* **14**, 2.

BARBER, M. & BURSTON, J. (1955). Antibiotic-resistant staphylococcal infection. A study of antibiotic sensitivity in relation to bacteriophage types. *Lancet* ii, 578.

BLAIR, J. E. & WILLIAMS, R. E. O. (1961). Phage typing of staphylococci. *Bull. Wld Hlth Org.* **24**, 771.

CADNESS-GRAVES, B., WILLIAMS, R., HARPER, G. S. & MILES, A. A. (1943). Slide test for coagulase-positive staphylococci. *Lancet* i, 736.

COWAN, S. T. (1938). The classification of staphylococci by precipitation and biological reactions. *J. Path. Bact.* **46**, 31.

CUNLIFFE, A. C. (1949). Incidence of *Staph. aureus* in the anterior nares of healthy children. *Lancet* ii, 411.

DUBUY, H. G. & CRISP, L. R. (1944). A sieve device for sampling air-borne micro-organisms. *Publ. Hlth Rep., Wash* **59**, 829.

EDITORIAL (1961). Water bugs in the bassinet. *Am. J. Dis. Child.* **101**, 273.

EDMUNDS, P. N., ELIAS-JONES, T. F., FORFAR, J. O. & BALF, C. L. (1955). Pathogenic staphylococci in the environment of the newborn infant. *Br. med. J.* i, 990.

EHRENKRANZ, N. J. (1965). Transmission of *Staphylococcus aureus* in man–epidemiologic and experimental studies. In *Skin Bacteria and Their Role in Infection*, pp. 201–215. Ed. H. I. Maibach and G. Hildick-Smith. New York: McGraw-Hill Book Co.

ELEK, S. D. & FLEMING, P. C. (1960). A new technique for the control of hospital cross-infection. Experience with BRL. 1241 in a maternity unit. *Lancet* ii, 569.

GILLESPIE, W. A., SIMPSON, K. & TOZER, R. C. (1958). Staphylococcal infection in a maternity hospital. Epidemiology and control. *Lancet* ii, 1075.

GLUCK, L. & WOOD, H. F. (1961). Effect of an antiseptic skin care regimen in reducing staphylococcal colonization in newborn infants. *New Engl. J. Med.* **265**, 1177.

GONZAGA, A. J., MORTIMER, E. A., WOLINSKY, E. & RAMMELKAMP, C. H. (1964). Transmission of staphylococci by fomites. *J. Am. med. Ass.* **189**, 711.

GOULD, J. C. (1958). Environmental penicillin and penicillin-resistant *Staphylococcus aureus*. *Lancet* i, 489.

GOULD, J. C. & BOWIE, J. H. (1952). The determination of bacterial sensitivity to antibiotics. *Edin. med. J.* **59**, 178.

HURST, V. (1960*a*). Transmission of hospital staphylococci among newborn infants. I. Observations on the contamination of a new nursery. *Pediatrics, Springfield* **25**, 11.

HURST, V. (1960*b*). Transmission of hospital staphylococci among newborn infants. II. Colonization of the skin and mucous membranes of the infants. *Pediatrics, Springfield* **25**, 204.

HUTCHISON, J. G. P. & BOWMAN, W. D. (1957). Staphylococcal epidemiology in a maternity hospital. *Acta paediat., Stockh.* **46**, 125.

KEAY, A. J., SYME, J. & BARNES, P. M. (1967). Cephaloridine in the treatment and prophylaxis of infection in the newborn. *Postgrad. Med. J.* August, 1967, Suppl. vol. **43**, 105.

KRESKY, B. (1964). Control of Gram-negative bacilli in a hospital nursery. *Am. J. Dis. Child.* **107**, 363.

LAURSEN, H. (1962). Incidence of *Pseudomonas aeruginosa* and other Gram-negative rods in newborn infants. *Acta obstet. gynec. scand.* **41**, 254.

LAURSEN, H. (1963). Bacteriological colonization of infants and mothers in a maternity unit. *Acta obstet. gynec. scand.* **42**, 43.

LOVE, G. J., GEZON, H. M., THOMPSON, D. J., ROGERS, K. D. & HATCH, T. F. (1963). Relation of intensity of staphylococcal infection in newborn infants to contamination of nurses' hands and surrounding environment. *Pediatrics, Springfield* **32**, 956.

LUDLAM, G. B. (1953). Incidence and penicillin sensitivity of *Staphylococcus aureus* in the nose in infants and their mothers. *J. Hyg., Camb.* **51**, 64.

MONRO, J. A. & MARKHAM, N. P. (1958). Staphylococcal infection in mothers and infants. Maternal breast abscesses and antecedent neonatal sepsis. *Lancet* ii, 186.

PLUECKHAHN, V. D. & BANKS, J. (1958). The ubiquitous staphylococcus. *Med. J. Aust.* i, 664.

POOLE, P. M. (1960). The reinvasion of a maternity unit by *Staphylococcus aureus*. *Mon. Bull. Minist. Hlth* **19**, 113.

REINARZ, J. A., PIERCE, A. K., MAYS, B. B. & SANDFORD, J. P. (1965) The potential role of inhalation therapy equipment in nosocomial pulmonary infection. *J. clin. Invest.* **44**, 831.

ROUNTREE, P. M. & BARBOUR, R. G. H. (1950). *Staphylococcus pyogenes* in newborn babies in a maternity hospital. *Med. J. Aust.* i, 525.

SARKANY, I. & GAYLARDE, C. C. (1967). Skin flora of the newborn. *Lancet* i, 589.

SHALLARD, M. A. & WILLIAMS, A. L. (1965). A study of the carriage of Gram-negative bacilli by newborn babies in hospital. *Med. J. Aust.* i, 540.

SHALLARD, M. A. & WILLIAMS, A. L. (1966). Studies on Gram-negative bacilli in a ward for newborn babies. *Med. J. Aust.* ii, 455.

THOMAS, M. (1961). The sticky film method of detecting skin staphylococci. *Mon. Bull. Minist. Hlth* **20**, 37.

WALLACE, A. T. & DUGUID, J. P. (1952). *Staph. aureus* air infection in a maternity hospital. *Edin. med. J.* **59**, 200.

WALLMARK, G. (1953). Bacteriophage types, sensitivity to antibiotics, and penicillinase production of *Staphylococcus aureus* (*pyogenes*). *Acta Soc. Med. upsal.* **59**, Nos. 3–4.

WHITE, A. (1961). Quantitative studies of nasal carriers of staphylococci among hospitalized patients. *J. clin. Invest.* **40**, 23.

WILLIAMS, R. E. O. (1961). Carriage of staphylococci in the newborn. *Lancet* ii, 173.