The bacterial flora of neonates with congenital abnormalities of the gastro-intestinal tract

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SUMMARY

The development of the bacterial flora of neonates with congenital abnormalities of the gastro-intestinal tract was studied in 31 infants during the first 10 days of life. Specimens were collected from the umbilicus, mouth and gastro-intestinal tract on the pre-operative day, at operation and on post-operative days 1, 2, 3, 5, 7 and 10. Bacteria were isolated semi-quantitatively on a variety of plain and selective media and identified by conventional methods.

Staphylococcus albus was the predominant species isolated from the umbilicus; it was recovered from 24 of the 31 babies. The viridans group of streptococci and *Streptococcus salivarius* were the commonest species isolated from the mouth; there were no differences between the babies with different abnormalities and treatment with antibiotics had no effect on the bacterial flora. Ten babies were colonized by each species on the pre-operative day, and 25 and 19 respectively by the tenth post-operative day. Anaerobic gram-positive cocci were the predominant oral anaerobes. Bacteria were not isolated from the rectal swabs of babies with tracheo-oesophageal fistula (TOF) or small bowel atresia on the pre-operative days. Post-operatively the predominant faecal isolates from babies with TOF were *Str. faecalis*, *Escherichia coli* and *Clostridium perfringens*. About 80 % of the babies with small bowel atresia were colonized by *Str. faecalis* and *Bacteroides vulgatus*, 60 % each by *E. coli*, *Klebsiella aerogenes* and *Str. faecium*. The five babies with necrotizing enterocolitis were colonized by *Str. faecalis*, *E. coli*, *Cl. perfringens* and *Cl. difficile*; *Bacteroides* spp. were not recovered from any of them. The commonest facultative species recovered from babies with large bowel obstruction were *Str. faecalis* and *E. coli*. *B. vulgatus*, *Cl. perfringens* and *Bifidobacterium* spp. were the commonest anaerobes and anaerobes outnumbered aerobes. No significant isolates were recovered from the wound swabs and none of the babies developed post-surgical sepsis.

INTRODUCTION

The paediatric surgical unit (PSU) at the Children's Hospital, Sheffield, undertakes the surgical management of neonates with major congenital abnormalities from a wide geographic area. The total admissions to the PSU from January 1977 to the end of March 1980 was 708, of which 409 were infants with congenital

abnormalities; 160 infants with congenital abnormalities required emergency surgery.

Post-operative sepsis and subsequent complications can be a major problem in this type of surgery. As a result, some of the infants are given prophylactic antibiotics routinely. However, little is known about the association between the acquisition of bacteria by these neonates and the development of post-surgical sepsis. To establish if there is any relationship between colonization by bacteria during the stay in hospital and the development of post-surgical sepsis, the bacterial flora of a sample of these neonates was studied from the time of admission to the unit until the tenth post-operative day. The effect of operative procedure on type, site and rate of colonization and the effect of antimicrobial agents used for the prophylaxis or treatment of post-operative sepsis were also observed.

MATERIALS AND METHODS

Subjects

Thirty-one neonates with various congenital abnormalities (Table 1) were studied from the time of admission, usually on the first day of life, until the tenth post-operative day. The neonates selected for the study were not more than 48 h old when admitted and had abnormalities of the gastro-intestinal tract. The study period extended for 18 months from September 1978 to March 1980. The babies were fed with Cow and Gate premium milk or breast milk with supplementary feeds of premium milk orally or through a nasogastric or gastrostomy tube.

Specimens

The flora of the umbilical stump, mouth and gastro-intestinal tract was sampled with a set of three albumen coated cotton wool swabs (Exogen, Dumbarton Road, Glasgow) taken from the umbilicus, mouth and rectum. Gastro-intestinal contents were taken during surgery into sterile universal bottles. Swabs were also used to sample colonic or small bowel contents from colostomies and ileostomies. A set of 3 swabs was collected on the pre-operative day and a set of 4 swabs (mouth, umbilicus, rectum and wound) on 6 post-operative days (days 1, 2, 3, 5, 7 and 10). The oral swab was taken from the buccal mucosa, the surface of the tongue and the saliva. Other swabs taken at operation were from (i) the wound before opening the peritoneum; (ii) peritoneal fluid on opening the peritoneum; (iii) peritoneal fluid at the end of operation and (iv) the wound at the end of operation. The swabs were placed in Amies transport medium (Amies & Douglas, 1965), and transferred immediately to the laboratory, where they were processed within a few minutes of arrival.

Laboratory examination

All seeding of culture media, sub-cultures and other manipulations were carried out on the open laboratory bench and inoculation carried out by a standard plating method (Gillies & Dodds, 1976) that enables the numbers of the individual bacterial species isolated to be scored semi-quantitatively (Rotimi & Duerden, 1981).

Table 1. *Congenital abnormalities and their management in 31 neonates*

Groups of anomalies	No. of babies	Surgical management	Antibiotic prophylaxis*
(A) Oesophageal atresia with tracheo-oesophageal fistula	9	Ligation of TOF; end-to-end anastomosis; gastrotomy	Amp & Gent Clox & Amp Cephadrine
(B) Small intestinal obstruction:	5		
Duodenal atresia	1	Resection: duodenal-ileal anastomosis;	Amp & Gent
Jejunal atresia	2	Resection: end-to-end anastomosis;	Amp & Gent
Ileal atresia	2	Resection: end-to-end anastomosis;	Amp & Gent
(C) Large bowel obstruction:	15		
(a) Necrotizing enterocolitis	5	Resection and colostomy	Amp & Gent
(b) Hirschsprung's disease	4	Colostomy	None
Imperforate anus	5	Colostomy	None
Meconium ileus	1	Colostomy	None
(D) Micellaneous	2		
Diaphragmatic hernia	1	Repair	Amp & Gent
Exomphalos	1	Repair	None

* Amp, ampicillin. Gent, gentamicin. Clox, cloxacillin. TOF, Tracheo-oesophageal fistula.

Media

The solid media used for isolation of the various organisms from each specimen were: Blood Agar (Oxoid Columbia Agar Base, CM 331, with Defibrinated Horse Blood (Wellcome) 7.5 %); Chocolate Agar (Oxoid Nutrient Agar Base with 10 % Horse Blood (Wellcome) heated in a water bath at 80 °C for 4–5 min); Sabouraud's Agar (Oxoid, CM 41a); Rogosa Agar (Oxoid, pH 5.8), Neomycin Blood Agar (Oxoid Blood Agar Base No. 2 with 7.5 % Defibrinated Horse Blood (Wellcome) and neomycin 75 µg/ml); BM-Kanamycin Agar (Holbrook, Ogston & Ross, 1978); Mitis-Salivarius Agar (Oxoid); Reinforced Clostridial Medium (Oxoid) with 0.7 % New Zealand Agar, and 0.1 % Cotton Blue at pH 5.0 (Willis *et al.* 1973).

Incubation

One Blood agar, MacConkey agar, Mitis-Salivarius agar and Sabouraud's agar were incubated aerobically at 37 °C and the Chocolate agar incubated in air plus 10 % CO₂ at 37 °C. The other Blood agar, the Rogosa agar, BM-Kanamycin agar, Neomycin Blood agar and Reinforced Clostridial Cotton Blue agar were incubated anaerobically. Plates incubated aerobically were inspected after 24 and 48 h, while those incubated anaerobically were inspected after a minimum period of 48 h and inoculation was then continued to 96 h.

Method of scoring

This was a previously described (Rotimi & Duerden, 1981); growth of bacteria on the inoculum well was scored 1+, growth on inoculum well plus the first set of streaks was scored 2+, and so on to 5+.

Identification of isolates

Representative colonies of each colony-type were subcultured for identification. The aerobes and facultative species were identified according to the methods of Cowan (1974). Particular consideration was given to the identification of anaerobes; they were identified by methods described previously (Duerden *et al.* 1980; Hafiz & Oakley, 1976; Rotimi, Faulkner & Duerden, 1980; Willis, 1977).

RESULTS

The congenital abnormalities of the 31 neonates studied are listed in Table 1 which also shows the category of babies given antibiotic prophylaxis. Group A included babies with oesophageal atresia and tracheo-oesophageal fistula (TOF). All had antibiotic prophylaxis pre- and post-operatively and had a gastrotomy tube inserted for milk feeds. Group B comprised babies with congenital small intestinal atresia, two of whom had an intra-uterine perforation proximal to the atretic portion. Groups A and B were admitted within 24 h of birth.

Group C were those babies with large bowel abnormalities; five cases of severe necrotizing enterocolitis (NEC), that required either subtotal colectomy or more limited resection were included in this group. The NEC babies had prophylactic antibiotics. All the other members of this group were congenital abnormalities that resulted in large bowel obstruction; all required colostomy and did not receive

Table 2. *Umbilical bacterial flora in 31 babies with congenital abnormalities*

Organisms	Number of babies colonized by the given bacterial species						
	Pre-operatively	Post-operative days:					
		1	2	3	5	7	10
<i>E. coli</i>	4	4	5	5	8	9	9
<i>K. aerogenes</i>	0	3	3	3	3	3	4
<i>Str. faecalis</i>	5	7	11	13	14	16	12
<i>Staph. albus</i>	5	7	13	15	19	19	24
<i>Staph. aureus</i>	4	6	7	6	6	6	4
Viridans streptococci	1	2	7	10	10	5	3
Anaerobic diphtheroids	0	0	0	0	1	1	0

antibiotic prophylaxis. The time of admission varied from a few hours to 48 h after birth. Group D was a miscellaneous group of conditions in which the bowel was not opened at operation.

Umbilical flora

Table 2 shows the number of babies colonized on the given days by the various bacterial species; there were no differences between the babies in the four groups. Pre-operatively four babies were colonized by *E. coli*, five by *Str. faecalis*, five by *Staph. albus*, four by *Staph. aureus* and one by viridans streptococci. There was no significant change in the rate of colonization by these bacterial species on the first post-operative day, but there was a marked increase in the number of babies colonized by *Str. faecalis*, *Staph. albus* and viridans streptococci between the second and the fifth days; the number of babies colonized by *Str. faecalis* doubled from seven to 14 on the fifth day, whereas colonization by *Staph. albus* increased from seven to 19 in the same period. *Staph. albus* was isolated from 24 babies by the tenth day, whereas the numbers of babies colonized by *Str. faecalis*, *Staph. aureus* and viridans streptococci decreased to 12, three and five respectively by the tenth day. The semi-quantitative assessment of growth showed that *Staph. albus* was the most numerous species with a mean score of 4+.

Oral flora

There was no significant difference between the oral flora of the babies given antibiotic prophylaxis and those not given antibiotics and there were no differences between the babies in the four groups. The commonest species isolated from the mouth were the viridans streptococci (Table 3). Ten of the 31 babies were colonized by these organisms on the pre-operative day. The next commonest species was *Str. salivarius* which was recovered from ten babies on the pre-operative day and 19 on the tenth day. Exposure to antibiotics did not appear to affect the rate of isolation of these species. *Staph. albus* and *Neisseria* spp. were isolated from ten and 12 babies each by the end of the study period.

The isolation of *Haemophilus influenzae*, *E. coli* and *K. aerogenes* was sparse on the pre-operative day and for the first post-operative day and they were found in only a minority of babies by the tenth day. *Candida albicans* was also isolated from

Table 3. *Bacterial flora of the mouth of 31 babies*

Organisms	Number of babies, colonized by the given species							
	Pre-operative	Post-operative days						
		1	2	3	5	7	10	
<i>E. coli</i>	1	2	2	1	4	4	7	
<i>K. aerogenes</i>	1	0	1	1	2	3	4	
<i>Str. faecalis</i>	2	5	3	2	3	4	1	
<i>Str. salivarius</i>	10	14	14	14	18	19	19	
Viridans streptococci	10	20	20	21	22	23	25	
<i>Staph. albus</i>	4	9	11	11	11	12	10	
<i>H. influenzae</i>	0	2	2	2	2	4	5	
<i>Neisseria</i> spp.	2	6	9	9	9	11	12	
Anaerobic cocci								
gram + ve	1	3	7	7	9	10	9	
gram - ve (veillonellae)	0	0	2	2	2	2	4	
<i>C. albicans</i>	0	0	0	0	2	3	4	
Micrococci	0	0	0	0	0	2	4	

a few babies particularly those on antibiotic prophylaxis. Anaerobic gram-positive cocci were isolated from nine babies and veillonellae from only four. The semi-quantitative assessment of individual species gave the viridans streptococci and the non-haemolytic *Str. salivarius* a mean score of 4+ in the post-operative period. No β -haemolytic streptococci were isolated.

The gastro-intestinal flora

Group A (Table 4). This group (see Table 1) comprised the nine babies with TOF. Rectal swabs taken on the day of admission, before surgery, did not yield any bacteria. Samples of oesophageal and gastric contents taken at operation were also sterile. On the first post-operative day, however, *Str. faecalis*, *Staph. albus* and *Cl. difficile* were isolated in small numbers (score 1+) from the rectal swabs of one baby each. Assessment of the sequential development of the gastro-intestinal flora in this group showed that six of the babies (c. 67%) were colonized by *Str. faecalis* by the fifth post-operative day and seven (c. 78%) by the tenth day. *Staph. albus* was isolated from most of the babies in the post-operative period. *E. coli*, *K. aerogenes* and *Proteus mirabilis* were each isolated from only a few babies on days 3 to 7, but from five, six and three babies on the tenth post-operative day. No anaerobes were isolated pre- or per-operatively. *Cl. perfringens* was the commonest anaerobe isolated from the babies in this group; seven babies were colonized by the tenth day. Other *Clostridium* spp., e.g. *Cl. cochlearium*, *Cl. sporogenes* and *Cl. tertium*, were isolated from relatively few babies and *Cl. difficile* was isolated from three babies. *B. vulgatus* and *B. thetaiotaomicron* were isolated from two babies on the tenth day and one baby was colonized by *B. fragilis*. *Bifidobacterium* spp. were isolated from a larger number of babies; three babies were colonized on the third and fifth post-operative days, and four on days 7 and 10. *Eubacterium* spp. and anaerobic gram-positive cocci were each isolated from three babies by the tenth day. When bifidobacteria, bacteroides and eubacteria were present these

Table 4. *The gastro-intestinal flora of nine babies with oesophageal atresia*

Species	Number of babies colonized by the given species							
	*Pre-op	†Per-op	Post-operative days					
			1	2	3	5	7	10
<i>E. coli</i>	—	—	—	1	2	2	3	5
<i>K. aerogenes</i>	—	—	—	1	1	2	2	6
<i>P. mirabilis</i>	—	—	—	1	1	1	3	3
<i>Citrobacter</i> spp.	—	—	—	—	—	1	1	1
<i>Str. faecalis</i>	—	—	1	4	4	6	7	7
<i>Str. faecium</i>	—	—	—	1	2	2	4	5
Viridans streptococci	—	—	—	1	1	1	1	1
<i>Staph. albus</i>	—	—	1	3	5	7	7	5
Micrococci	—	—	—	—	—	1	2	2
<i>C. albicans</i>	—	—	—	—	—	—	1	1
<i>B. vulgatus</i>	—	—	—	—	1	1	2	2
<i>B. thetaiotaomicron</i>	—	—	—	—	—	2	2	2
<i>B. fragilis</i>	—	—	—	—	—	1	1	1
<i>Cl. perfringens</i>	—	—	—	1	4	6	7	7
<i>Cl. difficile</i>	—	—	1	1	1	1	3	3
<i>Cl. cochlearium</i>	—	—	—	—	—	1	1	1
<i>Cl. sporogenes</i>	—	—	—	—	1	1	1	1
<i>Cl. tertium</i>	—	—	—	—	—	1	2	2
Anaerobic gram + ve cocci	—	—	—	1	1	2	3	3
<i>Bifidobacterium</i> spp.	—	—	—	1	3	3	4	4
<i>Eubacterium</i> spp.	—	—	—	2	2	2	3	3

* Pre-op, pre-operative.

† Per-op, per-operative, i.e. from specimens taken during operations.

organisms formed the predominant species quantitatively in the faecal flora; each had a mean score of 4+. The enterobacteria had a score of 3+ and the enterococci, candida and clostridia each had a score of 2+.

Group B (Table 5). Eighty percent each of the babies in this group were colonized by *Str. faecalis* and *B. vulgatus* by the end of the tenth post-operative day, 60% each by *E. coli*, *K. aerogenes* and *Str. faecium*, 40% each by *C. albicans*, *B. thetaiotaomicron*, *Cl. sporogenes*, *Eubacterium* spp., anaerobic gram-positive cocci and veilloneallae, and only 20% were colonized by *P. mirabilis*, *Staph. albus*, micrococci, *B. fragilis*, *Cl. perfringens*, *Cl. difficile* and *Cl. cochlearium*. The sequential acquisition of the flora showed that *Str. faecalis*, *Staph. albus*, *Str. faecium* and *E. coli* were present in the faecal flora of some of the infants on the pre-operative day and were found with increasing frequency from the first post-operative day. *B. vulgatus* was isolated from the per-operative specimen from one baby and remained the commonest anaerobe throughout. The other anaerobes were isolated from a relatively small number of babies. The bacteroides, eubacteria and bifidobacteria predominated quantitatively, each with a mean score of 4+.

Group C (a). Although necrotizing enterocolitis was not considered a congenital abnormality, babies with this condition were included when surgical intervention was required within 72 h of birth. Five babies with severe NEC were seen. Table 6 shows the distribution of the species isolated from these babies. By the tenth

Table 5. *The intestinal flora of the five neonates with congenital abnormalities of the small intestine*

Species	Number of babies colonized by the given species							
	*Pre-op	†Per-op	Post-operative days					
			1	2	3	5	7	10
<i>E. coli</i>	1	1	1	2	2	3	3	3
<i>K. aerogenes</i>	—	1	—	3	3	3	3	3
<i>P. mirabilis</i>	—	—	—	—	—	1	1	1
<i>Str. faecalis</i>	3	3	1	3	3	3	4	4
<i>Str. faecium</i>	2	2	2	2	2	2	2	3
Viridans streptococci	—	—	—	—	—	—	—	—
<i>Staph. albus</i>	3	1	1	1	1	1	1	1
Micrococci	—	—	—	—	—	1	1	1
<i>C. albicans</i>	—	—	—	—	1	2	2	2
<i>B. vulgatus</i>	—	1	1	2	2	3	3	4
<i>B. thetaiotaomicron</i>	—	—	—	—	—	1	1	2
<i>B. fragilis</i>	—	1	1	1	1	1	1	1
<i>Cl. perfringens</i>	—	—	—	—	—	1	1	1
<i>Cl. difficile</i>	—	—	—	—	—	—	—	1
<i>Cl. cochlearium</i>	—	—	—	—	—	—	—	1
<i>Cl. sporogenes</i>	—	1	1	1	2	2	2	2
<i>Cl. tertium</i>	—	—	—	—	—	—	—	—
<i>Bifidobacterium</i> spp.	—	1	1	1	2	2	1	—
<i>Eubacterium</i> spp.	—	2	—	—	—	1	2	2
Anaerobic gram +ve cocci	—	—	—	—	—	2	2	2
Veillonellae	—	—	—	1	1	2	2	2
<i>Pseudomonas</i> spp.	1	—	1	1	—	—	—	—

* Pre-op, pre-operative.

† Per-op, per-operative, i.e. from specimens taken during operations.

Table 6. *The intestinal flora of five babies with severe necrotizing enterocolitis*

Species	Number of babies colonized by the given species							
	*Pre-op	†Per-op	Post-operative days					
			1	2	3	5	7	10
<i>E. coli</i>	2	3	4	4	4	4	4	4
<i>K. aerogenes</i>	1	1	1	1	1	1	1	1
<i>P. mirabilis</i>	—	—	—	—	1	2	2	2
<i>Str. faecalis</i>	4	4	4	4	5	5	5	5
<i>Str. faecium</i>	1	1	3	3	3	3	3	3
<i>Staph. albus</i>	1	—	—	2	1	1	1	—
<i>Staph. aureus</i>	2	2	2	2	2	2	2	2
<i>Cl. perfringens</i>	1	1	1	4	4	4	4	4
<i>Cl. difficile</i>	2	2	3	5	5	5	5	5
<i>Cl. tertium</i>	—	—	1	1	1	1	1	1
Anaerobic gram +ve cocci	—	—	—	1	1	1	1	1
<i>Bifidobacterium</i> spp.	—	—	—	—	2	2	2	2

* Pre-op, pre-operative.

† Per-op, per-operative, i.e. from specimens taken during operations.

Table 7. *The intestinal flora of ten babies with congenital abnormalities of the large bowel*

Species	Number of babies colonized by the given species							
	*Pre-op	†Per-op	Post-operative days					
			1	2	3	5	7	10
<i>E. coli</i>	—	4	4	7	7	7	7	7
<i>P. mirabilis</i>	—	—	—	1	2	3	3	3
<i>K. aerogenes</i>	—	2	2	3	3	4	4	4
<i>Serratia marcescens</i>	—	—	—	1	1	2	2	2
<i>Str. faecalis</i>	—	3	5	9	9	10	10	10
<i>Str. faecium</i>	—	2	4	4	5	5	5	5
Viridans streptococci	—	—	—	—	2	2	1	1
<i>Staph. albus</i>	—	1	6	6	6	7	6	6
<i>C. albicans</i>	—	—	1	2	2	2	2	2
<i>Ps. aeruginosa</i>	—	—	1	1	1	1	1	1
Lactobacilli	—	—	3	4	4	4	4	4
<i>B. vulgatus</i>	—	1	2	4	4	5	5	6
<i>B. thetaiotaomicron</i>	—	—	—	2	2	3	3	3
<i>B. fragilis</i>	—	—	—	—	—	2	2	2
<i>B. distasonis</i>	—	—	—	—	1	2	2	2
<i>Cl. perfringens</i>	—	1	2	3	3	7	7	7
<i>Cl. difficile</i>	—	—	—	2	2	2	2	2
<i>Cl. bifermentans</i>	—	—	1	1	1	2	2	2
Anaerobic gram + ve cocci	—	1	1	2	2	2	2	2
Veillonellae	—	—	—	—	1	2	3	3
<i>Bifidobacterium</i> spp.	—	1	2	5	5	6	6	6
<i>Eubacterium</i> spp.	—	—	—	—	1	2	3	3
Micrococci	—	—	—	—	—	1	1	1
<i>Citrobacter</i> spp.	—	—	—	—	1	1	1	1

* Pre-op, pre-operative.

† Per-op, per-operative, i.e. from specimens taken during operations.

post-operative day, *Str. faecalis* and *Cl. difficile* had been isolated from the colostomy specimens of all the babies. Three babies were colonized by *Str. faecium* and two babies each by *P. mirabilis*, *Staph. aureus* and *Bifidobacterium* spp. When the sequential development of the flora was examined, only *Cl. tertium*, anaerobic gram-positive cocci, *P. mirabilis* and bifidobacterium were not isolated pre-operatively. *Str. faecalis* was the commonest isolate on the pre-operative day and its isolation remained consistently high throughout the post-operative period. *Cl. difficile* was isolated from all the babies from the second post-operative day onwards. *Cl. perfringens* and *E. coli* were each isolated from four of the babies early in the post-operative period and the rate of isolation remained constant throughout the period. No *Bacteroides* spp. were isolated.

Group C (b) (Table 7). Ten babies were studied in this group. At the end of the study period, the most common species recovered was *Str. faecalis* which was isolated from colostomy specimens of all ten babies. *E. coli* and *Cl. perfringens* were also common; they were each recovered from seven babies. *B. vulgatus*, *Bifidobacterium* spp. and *Staph. albus* were each isolated from six babies. *Str.*

faecium was recovered from half of the babies, and *K. aerogenes* and lactobacilli from four babies each; the other species were recovered from only a few babies. *E. coli*, *K. aerogenes*, *Str. faecalis*, *Str. faecium*, *Staph. albus*, *B. vulgatus* and *Cl. perfringens*, anaerobic gram-positive cocci, and bifidobacteria were all recovered from the gastro-intestinal contents at operation. By the fifth post-operative day all the given species (Table 7) were established. *Str. faecalis* was isolated from 90–100 % of the babies from the second post-operative day onwards, and *E. coli*, *Staph. albus*, *B. vulgatus*, *Cl. perfringens* and bifidobacteria, were all isolated from most babies post-operatively. *Cl. difficile* was recovered from two babies from the second post-operative day onwards.

Group D. Two babies were seen with conditions that could not be allocated to the above groups (see Table 1); one had exomphalos and the other a diaphragmatic hernia. Both babies had essentially identical faecal flora; there were no bacteria isolated on the pre-operative day, and both were colonized on the second post-operative day by *E. coli*, *Str. faecalis*, *B. vulgatus*, *B. thetaiotaomicron* and *Cl. perfringens*. On the fifth day *Cl. difficile* was recovered from the baby with exomphalos. The faecal flora of the two babies did not change for the remainder of the study period.

In general, the semi-quantitative analysis showed that when the *Bacteroides* spp., *Bifidobacterium* spp. and *Eubacterium* spp. were isolated from the faecal or colostomy specimens, they outnumbered the aerobes with scores of 4–5+. *E. coli*, *Klebsiella* spp., *Proteus* spp. and the anaerobic cocci had average scores of 3+. The other species were present in smaller numbers (1–2+).

Wound swabs

Swabs were taken from the wounds of all the babies at the end of the operation and on each post-operative test day. No significant isolates were recovered and none of the babies developed post-surgical sepsis. Bacterial colonization of the umbilical stump was taken as an index of skin colonization in these infants (Mortimer *et al.* 1966).

DISCUSSION

The commonest isolate quantitatively (density of growth) and qualitatively (number of babies colonized) from the umbilicus of the 31 neonates was *Staph. albus*; it was isolated from 77.4 % of the babies by the end of the study period. *Staph. aureus* was isolated from only 12.9 %. This is in sharp contrast to the isolation rates of these species in a parallel study of healthy normal neonates (Rotimi & Duerden, 1981) where *Staph. aureus* was the predominant isolate. In adults, most skin staphylococci are coagulase-negative whereas *Staph. aureus* is found frequently on the skin of infants, usually without signs of disease (Woodroffe & Shaw, 1974). The finding of an adult type flora on the majority of these babies may reflect the contact between the nursing staff and the babies, and nursing procedures in the PSU such as compulsory wearing of masks over the mouth and nose, frequent hand washing, etc. may prevent contamination of the babies with *Staph. aureus*. The isolation of enterococci from over half of the babies by the seventh day and of *E. coli* from 38.7 % was expected in a moist area such as the umbilicus and particularly where

there was contamination from colostomies. There was no obvious effect of antibiotics on the development of the umbilical flora in these neonates and surgery did not alter the isolation rates.

The viridans streptococci and the non-haemolytic *Str. salivarius* were the predominant isolates from the mouths of all groups of babies. This accords with the findings of Rotimi & Duerden (1981) in the normal healthy neonates. The use of antibiotics in already compromised babies may have favoured the emergence of *Candida* spp. in the mouths of the group given antibiotic prophylaxis. However, isolation of *C. albicans* from a high proportion of normal healthy neonates has been reported (Lay & Russell, 1972). From the results obtained, there is no evidence to show any adverse effect of antibiotics or surgery on the development of the oral flora in these neonates.

As expected rectal swabs from infants with oesophageal atresia were sterile in the pre-operative period when there was no continuity of the gastro-intestinal tract. Bacteria began to appear on the second post-operative day and by the fifth day all groups of the faecal bacteria were represented. The commonest isolates were *Str. faecalis* and *Cl. perfringens*. However, *Bifidobacterium* and *Bacteroides* spp. that are frequently isolated from normal healthy neonates were less frequently isolated from this group. Continuity of the gastro-intestinal tract may be essential for the early establishment of *Bacteroides* spp. Most of the babies were given artificial feeds and only a few had expressed breast milk through a gastrotomy tube; this may explain the relative scarcity of bifidobacteria in the faeces of this group of babies. *Cl. difficile* was isolated from this group more frequently than from the normal neonates; none had any evidence of colitis and this supports the findings of Larson *et al.* (1978) and Smith *et al.* (1980) that this species is a member of the normal faecal flora in neonates, despite its association with pseudomembranous colitis in adults. The isolation of enterobacteria was similar to the findings with normal healthy neonates, but there was a higher proportion of *Klebsiella* spp. and *Proteus* spp; *K. aerogenes* appears to replace *E. coli* in some babies. The isolation rate of 33 % for the anaerobic gram-positive cocci indicates that these organisms are a common part of the faecal flora when sought (Drasar & Hill, 1974). Parenteral antibiotic prophylaxis did not suppress the development of the faecal flora in this group of newborn infants.

In infants with small bowel abnormalities *B. vulgatus* and *Str. faecalis* were the predominant faecal species. *E. coli* and *K. aerogenes* were also established by the second post-operative day but these species also showed the mutual displacement seen in previous group. Generally the range of bacteria isolated from these babies with ileostomies resembles that of the faecal flora of normal neonates, except for a lower isolation rate of *Clostridium* spp. (particularly *Cl. perfringens*). The prominence of bacteroides, streptococci and enterobacteria in this group of babies is similar to that found in the normal bacterial population of the terminal small intestine in adults (Drasar & Hill, 1974; Kalser *et al.* 1966). The anaerobes were isolated less frequently than aerobes in these babies and this agrees with the results of previous studies of ileostomy contents in adults (Finegold *et al.* 1970; Gorbach *et al.* 1967).

The colostomy flora of the babies with NEC differed from the faecal flora of healthy normal babies in the absence of bacteroides. *Cl. difficile* was recovered from

all the babies in this group but a high isolation rate in healthy normal neonates has been observed (Larson *et al.* 1978; Smith *et al.* 1980). The high isolation rates of *Cl. perfringens* and *E. coli* may be significant because these organisms are among those that may be associated with intramural gas production in the lower intestine of NEC babies (Aszodi & Soper, 1978). *Cl. perfringens* has been found in the peritoneal fluid of four of 12 infants with NEC (Kosoloske, Ulrich & Hoffman, 1978) and in the present study four of five infants with NEC were colonized by *Cl. perfringens*. A possible association between NEC and *Cl. perfringens* appears worthy of further investigations. *Cl. butyricum* was not isolated from any of these babies which contradicts the claim of Howard *et al.* (1977) that it is the cause of NEC and is in agreement with recent reports (Smith *et al.* 1980). All neonates with NEC were given antibiotics. The type of surgical procedure did not have any effect on the development of the gastro-intestinal flora. The colostomy flora of the babies with large bowel obstruction resembled the faecal flora in normal healthy neonates (Rotimi & Duerden, 1981). The commonest species isolated from all of them was *Str. faecalis*; *Cl. perfringens*, *B. vulgatus* and *Bifidobacterium* spp. were the commonest anaerobes. Semi-quantitative analysis showed that *Bifidobacterium* spp. and *Bacteroides* spp. were the predominant species. None of these babies was given antibiotics and surgery appeared to have no effect on the development of the flora.

Generally, *Str. faecalis*, *Bacteroides* spp., *Clostridium* spp. and *E. coli* appear to be permanent members of the resident flora of the gastro-intestinal tract in normal and abnormal neonates as long as there is continuity of the tract. In this study the administration of prophylactic antibiotic conferred no advantage over those not given as regards development of post-surgical sepsis.

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