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A comparative study of gram-negative aerobic bacilli in the faeces of babies born in hospital and at home

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SUMMARY

Examination of the faeces of 50 babies born at home showed that bottle-fed babies carried significantly more *Klebsiella*, *Proteus* and *Pseudomonas* spp. and antibiotic-resistant *Escherichia coli* than did breast-fed babies. Bottle-fed babies born in hospital had a less mixed faecal flora than bottle-fed babies born at home. The possibility that bacterial contamination of home-prepared feeds may account for these differences requires investigation.

INTRODUCTION

The faecal flora of newborn infants is of interest because it is the probable source of many of the severe infections of infancy, such as urinary and wound infection, meningitis, gastro-enteritis and septicaemia. Reports suggest that these infections are less common amongst the breast fed (Winberg & Wessner, 1971; Hanson & Winberg, 1972; Goldman & Smith, 1973). However, most studies on the faecal flora of infants involve either newborn infants in hospital (Michael, Ringenback & Hottenstein, 1971; Willis *et al.* 1973; Bettelheim *et al.* 1974; Noy, Ayliffe & Linton, 1974; Gothefors, Olling & Winberg, 1975; Hewitt & Rigby, 1976) or older children at home (Moorhouse, 1969; Mata & Urrutia, 1971; Linton *et al.* 1972; Ellis-Pagler, Crabtree & Lambert, 1975). Although the work of Bullen & Willis (1971) was on babies that were delivered at home and in hospital, only the effects of breast and bottle feeding on the faecal flora were considered. In this investigation we compare the faecal flora of babies in both environments taking into account the type of feed given.

MATERIALS AND METHODS

Faeces

Specimens were obtained from 50 babies born at home and 50 babies born in hospital. All the births were spontaneous vertex deliveries and none of the babies received antibiotics. We aimed to obtain specimens on the fifth day of life but specimens were taken on days 4–7. Most specimens were examined within 4 h of being passed. Occasional specimens were stored at 4 °C for periods up to 36 h. For each baby the type of feed was noted.

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Table 1. *Faecal carriage of gram-negative bacilli amongst bottle- and breast-fed babies born at home or in hospital*

	Home			Hospital		
Aerobic gram-negative bacilli	Total babies positive	Breast-fed babies positive	Bottle-fed babies positive	Total babies positive	Breast-fed babies positive	Bottle-fed babies positive
No. coliforms present	3/50	2/25	1/25	6/50	3/30	3/20
1 species only	36/50	23/25	13/25	33/50	17/30	16/20
2 or more species	11/50	0/25	11/25	11/50	10/30	1/20
E. coli	37/50	18/25	19/25	37/50	23/30	14/20
Klebsiella spp.	17/50	5/25	12/35	14/50	11/30	3/20
Proteus spp.	2/50	0/25	2/25	6/50	5/30	1/20
Pseudomonas spp.	2/50	0/25	2/25	0/50	0/30	0/20
Klebsiella, *Proteus* and *Pseudomonas* spp. alone or in combination	20/50	5/25	15/25	18/50	14/30	4/20

Bacteriology

Counts of the number of coliforms present per gram of faeces were obtained by making serial dilutions in 1/4 strength Ringer's solution and plating to MacConkey agar. Antibiotic-resistant organisms were examined for by a disk diffusion method and by the incorporation of antibiotics into culture media (Cooke *et al.* 1971). The antibiotics used were ampicillin, chloramphenicol, nalidixic acid, neomycin, nitrofurantoin, streptomycin, sulphonamide and tetracycline. Swarming strains of *Proteus* spp. were examined for by inoculation of a small area of a blood agar plate and *Pseudomonas* and *Klebsiella* spp. examined for and identified as previously (Shooter *et al.* 1969; Cooke *et al.* 1979). Coliforms isolated were identified by the methods of Cowan & Steel (1974).

RESULTS

Coliform counts

There were no significant differences in the coliform counts in the four groups of babies. Six of the hospital specimens and three of the home specimens contained no coliforms.

Distribution of bacterial species

This is shown in Table 1. The faeces of both bottle-fed home babies and breast-fed hospital babies had a more mixed flora than the breast-fed home babies and bottle-fed hospital babies ($P = 0{\cdot}0002$). The distribution of *Klebsiella* spp. follows the same pattern being commoner in the first two groups. Bottle-fed home babies more commonly carried faecal *Klebsiella*, *Proteus* and *Pseudomonas* spp. than home breast-fed ($P = 0{\cdot}01$) and hospital bottle-fed babies ($P = 0{\cdot}018$).

Table 2. *Antibiotic resistance of faecal aerobic gram-negative bacilli isolated from breast- and bottle-fed babies at home and in hospital*

	Home			Hospital		
No. of antibiotics to which resistant	Total babies positive	Breast-fed babies positive	Bottle-fed babies positive	Total babies positive	Breast-fed babies positive	Bottle-fed babies positive
0	17 (18)	13 (13)	4 (5)	13 (16)	10 (13)	3 (3)
1	13 (8)	7 (3)	6 (5)	5 (3)	3 (2)	2 (1)
2	5 (4)	2 (1)	3 (3)	12 (7)	6 (2)	6 (5)
3	5 (5)	1 (1)	4 (4)	3 (3)	1 (2)	2 (1)
4	1 (2)	0 (0)	1 (2)	4 (5)	1 (2)	3 (3)
5	1 (0)	0 (0)	1 (0)	6 (3)	5 (2)	1 (1)
6	3 (0)	0 (0)	3 (0)	1 (0)	1 (0)	0 (0)
7	2 (0)	0 (0)	2 (0)	0 (0)	0 (0)	0 (0)
Total	47/50	23/25	24/25	44/50	27/30	17/20

Main figures refer to the most resistant strain of the total aerobic gram-negative flora; the figures in parentheses refer to *E. coli*. Nine babies had no aerobic gram-negative bacilli in the faeces.

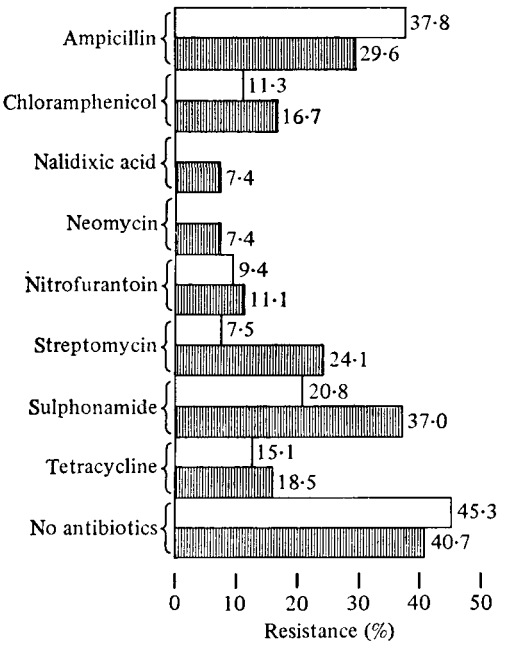

Fig. 1. Resistance of *E. coli* strains to individual antibiotics. □, *E. coli* strains from 50 babies delivered at home (53 strains). ▥, *E. coli* strains from 50 babies delivered in hospital (54 strains).

Antibiotic resistance

The sensitivity of the faecal aerobic gram-negative bacilli is shown in Table 2. Two of the home bottle-fed babies had a faecal flora which was only sensitive to one of the eight antibiotics against which it was tested whereas of the home breast-fed babies the total flora was always sensitive to at least five of the antibiotics tested.

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The antibiotic resistance of the strains of *E. coli* isolated is also shown in Table 2. Sensitive faecal *E. coli* occurred mainly in the breast-fed babies ($P = 0{\cdot}002$). At home, the bottle-fed babies carried more resistant *E. coli* ($P = 0{\cdot}01$). Multiply-resistant *E. coli* were least common in home breast-fed babies, only two of 25 babies carrying such organisms.

Resistance to individual antibiotics

The resistance of strains of *E. coli* to individual antibiotics is shown in Fig. 1. Ampicillin resistance was slightly more common in 'home' *E. coli* and resistance to other antibiotics was more common in hospital strains. Resistance to ampicillin, chloramphenicol, tetracycline and streptomycin was greater among the *Klebsiella* spp. isolated in the home.

DISCUSSION

In this study we demonstrated no significant difference in the faecal coliform counts of breast- and bottle-fed babies. This contrasts with the results obtained by other workers (Michael *et al.* 1971; Willis *et al.* 1973). However, Mata & Urrutia (1971) showed that in breast-fed babies it is towards the end of the first week of life that the *E. coli* count begins to fall relative to the *Lactobacillus bifidus* count and possibly this study took place too early in the neonatal period to show a difference in the faecal coliform counts between breast- and bottle-fed babies. Moreover, many of the hospital breast-fed babies received a bottle feed during the night and there is evidence (Bullen & Willis, 1971; Bullen, Tearle & Stewart, 1977; Bullen, Tearle & Willis, 1976) that such babies have a faecal flora resembling that of bottle-fed babies. The home breast-fed babies who received no supplements had slightly but not significantly lower coliform counts than the other groups.

The study did, however, demonstrate a difference in the distribution of bacterial species according to place of delivery and type of feed. The faeces of bottle-fed home babies and breast-fed hospital babies more commonly contained aerobic gram-negative bacilli other than *E. coli*, such as *Proteus*, *Pseudomonas* and *Klebsiella* spp. than did the other two groups.

In addition, multiply-antibiotic resistant *E. coli* were rare in home breast-fed babies whereas home bottle-fed babies more commonly carried gram-negative bacilli that were resistant to several antibiotics.

It is difficult to judge whether these differences are due to the beneficial effects of breast milk (Michael *et al.* 1971; Willis *et al.* 1973; Adinolfi *et al.* 1966; Bullen, Rogers & Leigh, 1972), or to the contamination of bottle feeds with bacteria (Ayliffe, Collins & Pettit, 1970). The beneficial effects of breast milk in this respect were not demonstrated in hospital babies who received bottle feed supplements. Serious mistakes in home infant feed preparation are common (Jones & Belsey, 1978) and errors in sterilization may similarly occur.

The possible reasons for the higher carriage rate by hospital breast-fed babies of *Klebsiella* and *Proteus* species and antibiotic resistant *E. coli* compared with home breast-fed babies are less clear but in the case of *Klebsiella* spp. skin carriage

may be important (Casewell & Phillips, 1977). The higher carriage rate of this organism in hospital breast-fed compared with hospital bottle-fed babies has been reported elsewhere (Shinebaum, Cooke & Brayson, 1979).

Of the babies in this study 61 % carried antibiotic resistant organisms. This is comparable with the results obtained by Linton *et al.* (1972) but rather lower than the figure obtained by Moorhouse (1969). Antibiotic resistant organisms were not evenly distributed between the four groups of babies and were most common in home bottle-fed babies. The transfer of resistance to strains of *E. coli* from *Proteus*, *Klebsiella* and *Pseudomonas* spp. also more commonly carried by these babies may account for the significantly higher antibiotic resistance of their faecal *E. coli*. The acquisition of antibiotic resistant *E. coli* from the kitchen environment due to the inadequate sterilization of bottles or feeds is also a possibility.

The results of this study indicate that the faecal flora of home delivered bottle-fed babies may be more antibiotic resistant than that of home delivered breast-fed babies. An investigation of the bacterial contamination of home produced bottle feeds would be of interest as these may be the source of antibiotic resistant organisms, not only in home delivered babies but also in hospital delivered babies after they leave the hospital environment.

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REFERENCES

Adinolfi, M., Glynn, A. A., Lindsay, M. & Milne, C. M. (1966). Serological properties of γA antibodies to *Escherichia coli* present in human colostrum. *Immunology* **10**, 517.

Ayliffe, G. A., Collins, B. J. & Pettit, F. (1970). Contamination of infant feeds in a Milton milk kitchen. *Lancet* i, 559.

Bettelheim, K. A., Breadon, A., Faiers, M. C., O'Farrell, S. M. & Shooter, R. A. (1974). The origin of O serotypes of *Escherichia coli* in babies after normal delivery. *Journal of Hygiene* **72**, 67.

Bullen, C. L. & Willis, A. T. (1971). Resistance of the breast-fed infant to gastroenteritis *British Medical Journal* iii, 338.

Bullen, J. J., Rogers, H. J. & Leigh, L. (1972). Iron-binding proteins in milk and resistance to *Escherichia coli* infection in infants. *British Medical Journal* i, 69.

Bullen, C. L., Tearle, P. V. & Willis, A. T. (1976). Bifidobacteria in the intestinal tract of infants: An *in vivo* study. *Journal of Medical Microbiology* **9**, 325.

Bullen, C. L., Tearle, P. V. & Stewart, M. G. (1977). The effect of 'humanised' milks and supplemented breast feeding on the faecal flora of infants. *Journal of Medical Microbiology* **10**, 403.

CASEWELL, M. & PHILLIPS, I. (1977). Hands as route of transmission for *Klebsiella* species. *British Medical Journal* ii, 1315.

COOKE, E. M., BREADON, A. L., SHOOTER, R. A. & O'FARRELL, S. M. (1971). Antibiotic sensitivity of *Escherichia coli* isolated from animals, food, hospital patients and normal people. *Lancet* ii, 8.

COOKE, E. M., BRAYSON, J. C., EDMONDSON, A. S. & HALL, D. (1979). An investigation into the incidence and sources of Klebsiella infection in hospital patients. *Journal of Hygiene* **82**, 473.

COWAN, S. T. & STEEL, K. J. (1974). *Manual for the Identification of Medical Bacteria*, 2nd edition. Cambridge University Press.

ELLIS-PAGLER, R. B., CRABTREE, C. & LAMBERT, H. P. (1975). The faecal flora of children in the United Kingdom. *Journal of Hygiene* **75**, 135.

GOLDMAN, A. S. & SMITH, C. W. (1973). Host resistance factors in human milk. *Journal of Pediatrics* **82**, 1082.

GOTHEFORS, L., OLLING, S. & WINBERG, J. (1975). Breast feeding and biological properties of faecal *E. coli* strains. *Acta Paediatrica Scandinavica* **64**, 807.

HANSON, L. A. & WINBERG, J. (1972). Breast milk and defence against infection in the newborn. *Archives of Disease in Childhood* **47**, 845.

HEWITT, J. H. & RIGBY, J. (1976). Effect of various milk feeds on numbers of *Escherichia coli* and Bifidobacterium in the stools of newborn infants. *Journal of Hygiene* **77**, 129.

JONES, R. A. K. & BELSEY, E. M. (1978). Common mistakes in infant feeding: Survey from a London Borough. *British Medical Journal* ii, 112.

LINTON, K. B., LEE, P. A., RICHMOND, M. H. & GILLESPIE, W. A. (1972). Antibiotic resistance and transmissible R-factors in the intestinal coliform flora of healthy adults and children in an urban and a rural community. *Journal of Hygiene* **70**, 99.

MATA, L. J. & URRUTIA, J. J. (1971). Intestinal colonization of breast-fed children in a rural area of low socio-economic level. *Annals of the New York Academy of Science* **176**, 93.

MICHAEL, J. G., RINGENBACK, R. & HOTTENSTEIN, S. (1971). The antimicrobial activity of human colostral antibody in the newborn. *Journal of Infectious Diseases* **124**, 445.

MOORHOUSE, E. C. (1969). Transferable drug resistance in Enterobacteria isolated from urban infants. *British Medical Journal* ii, 405.

NOY, J. H., AYLIFFE, G. A. J. & LINTON, K. B. (1974). Antibiotic-resistant gram-negative bacilli in the faeces of neonates. *Journal of Medical Microbiology* **7**, 509.

SHINEBAUM, R., COOKE, E. M. & BRAYSON, J. C. (1979). The acquisition of *Klebsiella aerogenes* by neonates. *Journal of Medical Microbiology* **12**, 201.

SHOOTER, R. A., COOKE, E. M., GAYA, H., KUMAR, P., PATEL, N., PARKER, M. T., THOM, B. T. & FRANCE, D. R. (1969). Food and medicaments as possible sources of hospital strains of *Pseudomonas aeruginosa*. *Lancet* i, 1227.

WILLIS, A. T., BULLEN, C. L., WILLIAM, K., FAGG, C. G., BOURNE, A. & VIGNON, M. (1973). Breast milk substitute: A bacteriological study. *British Medical Journal* iv, 67.

WINBERG, J. & WESSNER, G. (1971). Does breast milk protect against septicaemia in the newborn? *Lancet* i, 1091.