Gram(+) anaerobic intestinal cocci in healthy adults and neonates

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Summary: Anaerobic gram(+) cocci were found to be part of the normal indigenous flora of the intestinal tract in healthy adults (19.6%) but they were rarely isolated from healthy neonates (6.1%). A larger variation of species was also present in adults. Their installation in the newborn's intestine seems to be partially inhibited by factors that are not at present very clear.

Key words: Anaerobic cocci; intestine; fecal flora; rectal flora.

INTRODUCTION

Bacterial flora of the rectum presents as extensive and diversified, a spectrum of pathogenic and non-pathogenic organisms.

Disturbance of the normal intestinal balance affords opportunity for these microorganisms to produce infection under appropriate circumstances.

Anaerobic gram (+) cocci constitute the second largest group ([7]), after gram (−) bacilli, of anaerobic bacteria isolated from clinicals specimens. They are important human pathogens and will be isolated most often in mixed culture from intra-abdominal and biliary tract sepsis as well as from infections of the respiratory and female genital tracts ([2, 3]).

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Our study deals with the distribution and frequency of species of gram (+) anaerobic cocci in human rectal flora of newborn and adults by quantitative analysis.

For the present study, we chose Cesarian section newborn, because these infants usually begin life with a bacteriologically clean state, and offer an ideal model for understanding better the installation of gut bacteria in the context of the neonate's hospital.

MATERIAL AND METHODS

Analysed samples comprised two groups (Table 1):

Infant group:

The study was carried out on 27 newborns delivered at term by non-urgent cesarian section. Their birth weight was of 3.500±400 g. The decision for cesarian section was made for fetal macrosomia and dystocia and in case of iterative cesarian section. None of the newborn had a history of abnormal bowel functions or of antibiotic therapy.

Fecal samples of neonates were examined for their viable bacterial counts from birth to 14 days at the following times: at birth (F0), 6h (F6), one (F24), two (F48), four (F4), seven (F7), and fourteen (F14) days.

Table 1. – *Material and Methods.*

Sampling:

Rectal sampling → Adults
Rectal sampling → Newborns (0h, 6h, 24h, 48h, 4d, 7d, 14d)

Culture:

anaerobically in 3 media:
- Columbia blood agar
- Columbia blood agar + nalidixic acid (40 µg/ml)
- Columbia blood agar + neomycin (75 µg/ml)

Adult group:

27 fecal samples from women at term, aged from 18-40 years old were analysed.

Microbial studies:

Fecal samples were collected from the rectum with anaerobic culturette swabs (Marion Scientific), which allowed transport in favourable anaerobic conditions (4).

The mean amount of collected material was 20 mg (range 10 to 30 mg).

Decimal dilutions were performed in 1/4 cysteinated Ringer's solution (5) and spread on three media plates:

- non selective medium glucose (5 g/l), cystein chlorhydrate (0.3 g/l) and horse blood (10%) supplemented Columbia agar,
- neomycin (75 µg/ml) modified blood Columbia agar,
- nalidixic acid (40 µg/ml) modified blood Columbia agar.

The three media were incubated for five days at 37 °C in anaerobic jars (GasPack BBL).

The identification of the isolated anaerobic bacteria was carried out according to Bergey's manual (6).

The detection level was 10^3 CFU/g of feces.

RESULTS

Anaerobic gram (+) cocci were found to be part of the normal indigenous rectal flora in adults. Their frequency constitutes 19.6% of strains isolated (Table 2).

In healthy newborns, anaerobic gram (+) cocci are rarely isolated from the rectal flora and this rate drops to 6.1% of strains isolated (Table 2).

Table 2. – *Frequency of gram (+) anaerobic intestinal cocci.*

	Newborns (14th day)	Adults
Number of samples	27	27
Number of isolates	163	255
Gram (+) anaerobic cocci	10	50
Mean number of species per sample	6.1	9.4
Mean number of gram (+) anaerobic cocci per sample	0.3	1.9
Frequency (%)	6.1	19.6

Comparison of anaerobic gram (+) cocci was made between adults and newborns.

Concerning newborns, comparison was made on the 14th day of life; on this day, stabilisation of their fecal flora had occurred (7).

The species specific distribution was different for each group of study. *Peptococcus sp.* were mainly found in newborns (95.9% of isolated strains); the most frequent species being *P. magnus* and *P. prevotii* (Table 3).

In healthy adults, their incidence was lower (76%) but there was a larger va-

Table 3. – *Percentage distribution of species of gram (+) anaerobic cocci in rectal samples.*

Species	Newborns (14th day)	Adults
Peptococcus	95.9	76
P. magnus	16.7	38
P. prevotii	79.2	26
P. asaccharolyticus	0	6
P. saccharolyticus	0	6
P. morbillorum	0	0
P. constellatus	0	0
Peptostreptococcus	4.1	24
P. parvulus	0	0
P. productus	0	0.6
P. anaerobius	4.1	10
P. micros	0	8
P. intermedius	0	8

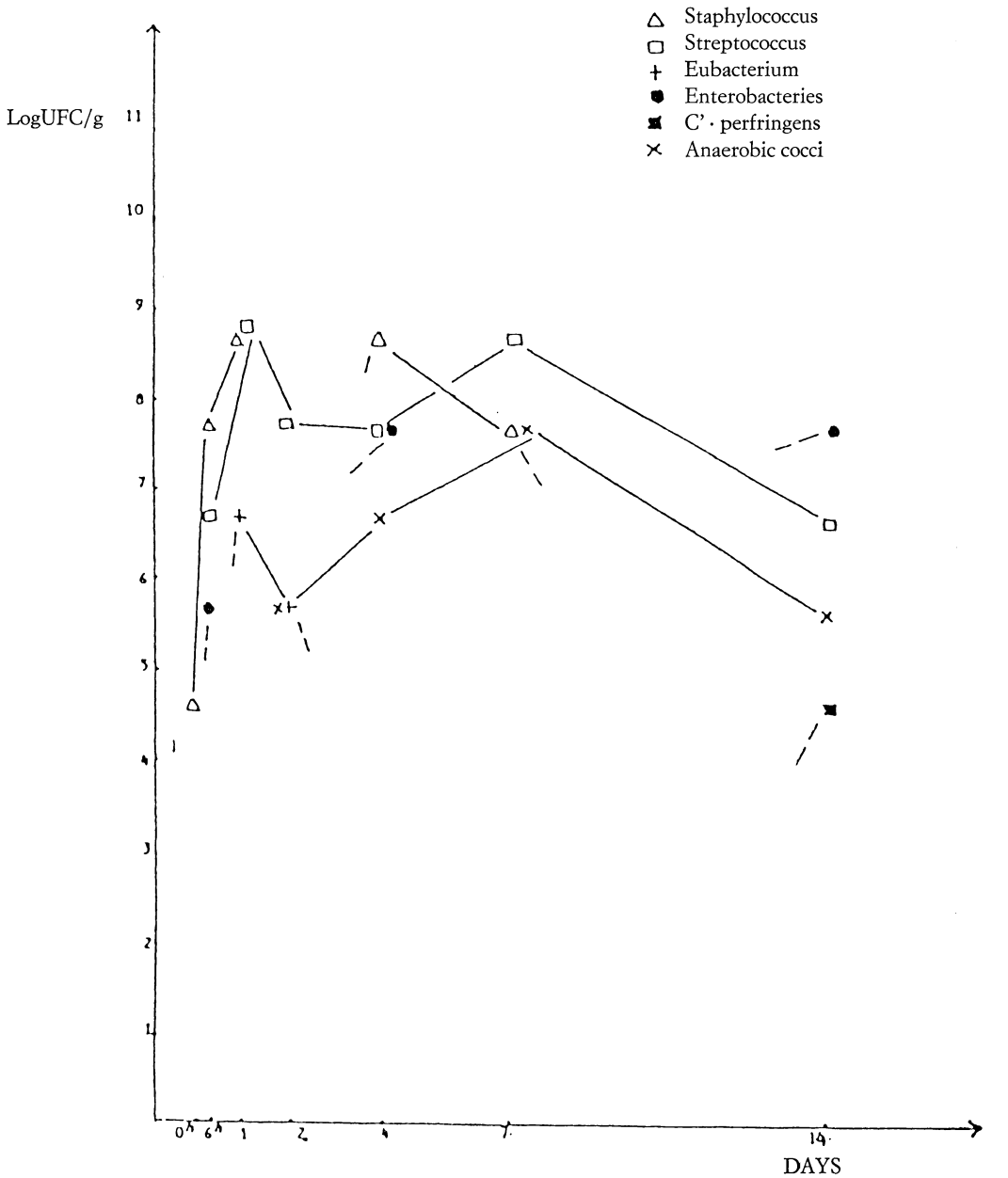

Fig. 1. – Fecal counts in the newborn after cesarian section during the first two weeks of life.

riation of isolated strains. *P. magnus, P. prevotii, P. asaccharolyticus* and *P. saccharolyticus* were encountered (Table 3).

With reference to *Peptostreptococcus,* in healthy adults, their rate amounted to 24% of strains isolated.

P. micros, P. anaerobius and *P. intermedius* were the most frequently isolated (Table 3).

P. anaerobius was the predominant anaerobic coccus (4.1% of isolates) in the normal intestinal flora of neonates.

Afterwards, our interest was focussed on the colonization of the newborn's intestine by anaerobic gram (+) cocci (Fig. 1).

CONCLUSIONS

Although in recent years, there have been major advances in the study of intestinal bacteriology, our knowledge of the intestinal flora is still far from adequate.

Actually, it is known that the intestinal bacterial flora appears to live in happy symbiosis with the host, although under certain conditions this balance is disturbed and intestinal flora has been shown to cause clinical disorders ([8, 9, 10]).

Anaerobic cocci are becoming increasingly recognised as potential pathogens associated with infection in hospital patients ([2]).

In the course of our investigations in newborns we observed great fluctuations in the population of anerobic gram (+) cocci.

We report the prevalence of *P. magnus, P. prevotii* and *P. anaerobius* in the stools of infants.

Additionally, neonates present a lower frequency (6.1%) compared to healthy adults (19.6%).

In the fecal flora of adults a larger distribution of species was found.

The species mainly found in adult fecal flora was: *P. magnus, P. prevotii, P. asaccharolyticus, P. saccharolyticus, P. productus, P. anaerobius* and *P. intermedius.*

In newborns delivered by vaginal delivery, colonization by anaerobic gram (+) cocci are usually of endogenous origin derived from the maternal floras, vaginal and rectal ([10]).

Newborn delivered by cesarian section without premature rupture of the membranes, is considered sterile. Thus, it constitutes an ideal model for colonization by bacteria, which probably do not originate from the mother, but either from the environment ([7]) or from the hospital staff ([5]).

Independent of origin, the mean kinetic curve of CFU per gram of fecal flora during fourteen days allowed us to perceive fluctuations in the population of anaerobic gram (+) cocci.

On the first day of life, anaerobic gram (+) cocci constitute an important component of the newborn's intestinal flora (Figure 1).

A regression in their level is observed on the second day. Finally, reappearance of anaerobic cocci and stabilisation occurs on the 14th day.

Possible explanations for these results are the following:

– Anaerobic cocci of maternal origin constitute a transient load for the newborn's intestine; newborns intestinal cocci substitute this transient load.

– Environmental anaerobic cocci colonizing cesarian section newborns do not easily adapt to the human environment and present fluctuations before their stabilisation.

– Presence of local host defence factors in the newborn which prevent colonization. These factors may be of maternal origin transferred to the fetus via the placenta.

– All the above factors may be responsible.

The controlling mechanisms that limit the growth of enteric micro-organisms to such definable and reproductible limits are extremely complex and not fully understood.

They play an important role in determining both the total numbers and the relative frequency of various species in the gut.

Experiments with germ-free animals will permit conclusions on the interactions of defined species. But the importance of these specific interactions in vivo remains obscure in the control of a complex flora ([11]).

Investigation of bacterial interference in the newborn intestine ([3]), may lead to better understanding of the colonization process and the development of alternative to classic infection control methods.

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