

BACTERIA IN COLOSTRUM AND MILK OF GUATEMALAN INDIAN WOMEN*†

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Little attention has been given to the bacteriology of human colostrum and milk in developing countries. Human milk is thought of as being essentially free of contaminating bacteria under normal circumstances. Bacteriological studies of human milk, therefore, are done only when there is mastitis or when tuberculosis, typhoid fever and other diseases are suspected or present in the mother.

SCHERER (1951) reported that milk from mothers residing in an industrial country with adequate level of hygiene may contain as much as 500,000 bacteria per ml. In another study, when an aseptic technique was observed in the collection of milk samples, counts ranged from 0 to 2,500 per ml; the usual bacteria demonstrated were micrococci, non-hemolytic streptococci, diptheroid bacilli and anaerobic lactobacilli (WILSON and MILES, 1966).

In a community with a low level of personal hygiene and environmental sanitation and where infectious disease is highly prevalent, it is expected that the skin of the areola and nipple and perhaps the mammary ducts could be more frequently contaminated and shed harmful microorganisms into maternal milk. In order to examine this aspect, a qualitative and quantitative study of bacteria was made in freshly obtained breast milk of Indian women representing one of the lower socio-economic groups of Guatemala.

METHODS

The area of study was the semi-isolated village of Santa María Cauqué with 1,300 inhabitants located in the highlands of Guatemala. Established rapport of INCAP workers engaged in longitudinal studies during the past 6 years (MATA et al., 1967) was invaluable in obtaining the cooperation of the mothers.

Fifty-one samples of colostrum and milk from thirty-one mothers were collected. All mothers had been lactating for periods ranging from two days to 201 weeks. An attempt was made to obtain at least 2 ml. on each occasion. At the beginning of lactation, samples were collected with a sterile breast pump; later the mothers were able to provide the specimens by expressing the breast manually. Specimens obtained in this manner were only in contact with the nipple surface. No cleaning or treatment of the breast was made; the aim was to determine the characteristics of milk as it is suckled by the babies. The mothers were instructed not to touch the nipple during collection of samples.

Specimens were processed in the field laboratory within one hour after collection. Calibrated loopfuls (0.01 ml.) of undiluted colostrum or milk and 10^{-1} and 10^{-2} dilutions of these materials, were inoculated onto agar plates, Table I.

These media enabled cultivation of anaerobic, aerobic and microaerophilic bacteria; T 7 T was used to grow enterobacteriaceae. Bacteria growing in BC-1, L and BA agars were identified on the basis of: 1) capacity to grow aerobically or anerobically, 2) colonial morphology, and 3) microscopic characteristics in Gram-stained preparations. The criteria used are described elsewhere (MATA et al., 1969). Colonies on T7T were transferred to TSI (BBL) agar slants and identified as outlined by EDWARDS and EWING (1962).

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RESULTS

All specimens cultured contained bacteria in concentrations (total) ranging from 10^3 to 10^6 per ml. and averaging 3×10^4 per ml. No difference was seen in the mean total concentration and range of concentration of bacteria between mothers lactating for long or short periods of time. Also, there was no trend to increasing or decreasing numbers of Gram-negative bacilli or lactobacilli with respect to time of lactation.

Table II lists the bacterial groups identified in order of decreasing frequency. Micrococci were the only species isolated from all specimens cultured; streptococci were next in frequency and were found in 46 of 51 cases.

TABLE I. Culture media and conditions of incubation of colostrum and milk.

Medium	Number of plates	Incubation at 37 C. for 48 hours.
Schaedler agar base* (BC-I)	1	Anaerobic (Gaspak)†
Schaedler agar base (BC-I)	1	Aerobic
Lactobacilli agar* (L)	1	Candle jar
Blood agar (BA)	1	Candle jar
Tergitol 7 (BBL)* (T7T)	1	Aerobic

*Schaedler, R. W., Dubos, R., and Costello, R. (1965). *J. Exper. Med.*, **122**, 59; Mata, L. J., Carrillo, C. and Villatoro, E. (1969). *Appl. Microbiol.*, **17**, 596.

†Brewer, J. H. and Allgeier, D. L. (1966). *Appl. Microbiol.*, **14**, 985.

Lactobacilli were isolated from 17 specimens and Gram-negative aerobic bacilli from 16. In 10 of the 16 samples with Gram-negative aerobic bacilli, one single species was isolated; in the remaining six samples, two or three different species were cultured. The concentration of enterobacteriaceae reached up to 10^6 per ml.

Five of the 51 samples were collected in duplicate consecutively from the same breast and were cultured as outlined above. No significant difference in total concentration of bacteria or bacterial groups was noted.

DISCUSSION

The bacteria found in the colostrum and milk could represent an interchanging flora passing between the mother's breast and infant's mouth. The bacterial groups isolated from human milk in this study are similar to those isolated from normal skin and infant's mouth as listed by ROSEBURY (1962), with the exception of the enterobacteriaceae. The latter have been isolated from normal skin, but they were present as a result of auto-contamination. ROSEBURY (1962) has noted undifferentiated coliforms on the "saliva-tooth surfaces" in 65 per cent of instances, and with some frequency in preeruptive infants. *Escherichia coli*, however, was recovered from the mouth in only 4.2 per cent of cases, and *Alkaligenes* was not demonstrated.

E. coli was found in 18 per cent and *Alkaligenes* in 14 per cent of the mothers' milk samples tested during the course of this investigation. Coliforms were not isolated with regularity from the skin of individuals living in countries with better hygiene. Although no studies of the bacterial flora of the skin of women in this area have been done, the isolation of *E. coli* from milk rather frequently suggests that these bacteria are probably often present in the skin, and that autocontamination may be the source of these organisms in milk from village mothers.

TABLE II. Bacteria in 51 samples of colostrum and milk from Indian women as it is suckled by the baby Santa María Cauqué, Guatemala. 1968.

Bacterial group	Number of cases Group demonstrated	Mean concentration and range when found (log 10)
Micrococci	51	4.3 (3-6)
Streptococci	46	3.9 (1-5)
Corynebacteria	37	3.5 (1-5)
Gram-negative diplococci	20	3.1 (1-5)
Lactobacilli	17	3.6 (1-5)
<i>Enterobacteriaceae</i>	16	2.9 (1-6)
<i>Esch. coli</i>	9	3.3 (1-6)
<i>Alkaligenes</i>	7	2.3 (1-3)
<i>Klebsiella-Aerobacter</i>	5	3.8 (1-6)
Providence	2	4.0 (1-6)
Bacilli (Gram +)	15	3.1 (1-4)

The presence of enterobacteriaceae in the samples cultured is of importance regardless of their source. In the process of suckling, a considerable proportion of neonates receive large numbers of enterobacteriaceae which may play a role in intestinal colonization. The presence of enterobacteriaceae in human colostrum and milk reflects the low levels of personal hygiene and environmental sanitation in the population studied.

SUMMARY

Bacteria in freshly obtained colostrum and milk from 51 Guatemalan Indian women were studied qualitatively and quantitatively. Samples contained an average of 3×10^4 bacteria per ml. The commonest bacterial groups identified were micrococci, streptococci, and corynebacteria. Sixteen of the 51

samples contained enterobacteriaceae in concentrations ranging from 10^1 to 10^6 per ml. In addition to reflecting a low level of personal hygiene, these organisms may be important in the process of intestinal colonization of infants.

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