Bacterial Colonization and Infection in the Neonate

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The vast majority of healthy term neonates tolerate their abrupt introduction to the bacterial world with little risk of infection. However, infants who require a longer hospital stay are at greater risk of having an infection, particularly when intensive care is needed. In one study, 15.3 percent of the infants in an intensive care unit acquired a nosocomial infection. Gram-negative bacilli have emerged as the principle cause of nosocomial infection. Nosocomial infection due to gram-negative bacilli usually occurs in neonates already colonized with gram-negative bacilli in the pharynx or intestine, and the risk of colonization with hospital strains of gram-negative bacilli (which are often resistant to multiple antibiotics) increases dramatically the longer a baby stays in intensive care. The factors which predispose individual neonates to colonization and infection with gram-negative bacilli require further study, but gram-negative bacilli are most often transmitted among neonates on the hands of personnel. Neonates in whom intestinal colonization with gram-negative bacilli develops are a particularly important reservoir of gram-negative bacilli in the nursery; once colonized, infants may harbour antibiotic-resistant hospital strains of gram-negative bacilli in their stool for more than a year. Pharyngeal colonization with α streptococci appears to protect neonates from pharyngeal colonization with gram-negative bacilli, and bacterial interference may play an important role in regulating intestinal colonization as well. Investigation of the mechanisms of bacterial interference may lead to better understanding of the colonization process and development of alternatives to classic infection control methods.

The pediatrician who embarks upon a study of nosocomial colonization and infection in the neonate has an advantage over his internist colleagues. Whereas the adult brings a fully developed, complex bacterial flora to the hospital, the neonate usually begins life with a bacteriologic clean slate. The careful observer therefore has an excellent opportunity to evaluate the development of bacterial flora in the context of the neonate's hospital experience and to study the microbiologic, host and environmental factors which contribute to the development of nosocomial infection. In the decade since the last International Conference on Nosocomial Infections, numerous descriptive studies have advanced our understanding of the epidemiology of neonatal colonization and infection. However, as this review will make clear, innovative pathophysiologic studies have been few.

The vast majority of term neonates weather their abrupt exposure to the diverse microbial world of the birth canal without incident. With a few exceptions, such as K1 Escherichia coli and group B streptococci, most of the organisms that the neonate is likely to encounter generally do no harm. Within a few days the neonate begins to develop a "nor-

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mal" bacterial flora derived principally from contact with mother and the immediate environment [1,2]. Alpha streptococci predominate in the throat and Staphylococcus epidermidis colonizes the nose and umbilicus. The stool flora is more complex. In the breast-fed baby a predominance of Bifidobacteria (anaerobic lactobacilli) quickly develops; these are present in very high numbers ($10^7 - 10^{10}$/g stool). Other anaerobes may be present in much smaller numbers. Escherichia coli is the most frequently isolated gram-negative bacillus but is present in much lower numbers than the Bifidobacteria, and some cultures of neonatal stools do not reveal any Esch. coli in the first week of life. It has been hypothesized that lactose fermentation by Bifidobacteria and other gut organisms lowers the pH of stool to about 4 in the presence of breast milk, which is a poor buffer, and that Esch. coli cannot proliferate in such an acid environment [3]. As expected, when Esch. coli are present, they have the same serotypes as strains colonizing the mother. Breast-fed babies are rarely colonized with significant numbers of other gram-negative bacilli. In formula-fed neonates a stool flora develops which is similar to that of adults, although it is less varied. Compared to breast-fed babies, there are fewer Bifidobacteria and relatively more Esch. coli; Bacteroides and other anaerobes are present in much higher numbers. Klebsiella and other aerobic gram-negative bacilli are occasionally isolated (as they are in normal adults) [4,5], but they are generally few in number and virtually never displace Esch. coli.

Regardless of whether the healthy newborn is fed by bottle or breast, the short encounter with the well-baby nursery provides little opportunity to acquire nosocomial pathogens. Infection is rare, although the risk is increased when the baby stays in a nursery which is in the throes of an outbreak. In recent years, Staph. aureus has continued to be the major nosocomial threat to the well baby, particularly since discontinuation of routine hexachlorophene bathing in 1971 [6].

A far different fate awaits the baby who is sick enough to require care in a neonatal intensive care unit (ICU), in which the risk of infection has been reported to be as high as 15.3 percent [7]. The hazards of the ICU have been confirmed in our institution. From 1974 to 1976, infection developed in 6.0 percent of the neonates admitted to the ICU although the rate fell to 1.8 percent in the following two years concomittant with a move to a new facility. In this five year period, 58.6 percent of the nosocomial infections were due to gram-negative bacilli. This trend towards gram-negative infections, which are often resistant to multiple antibiotics, has been noted elsewhere [8]. In addition, numerous reports of outbreaks of gram-negative infection have appeared in the past 10 years, with Klebsiella being the prime offender [9–11].

Some insight into the pathogenesis of these infections may be gained by studying bacterial colonization of the babies in the ICU. The bacterial flora of these patients is radically different from that of healthy breast- or bottle-fed neonates. The development of this abnormal flora was dramatically demonstrated in a study of 63 neonates admitted to our ICU [12]. Semiquantitative cultures of specimens from the nose, throat, umbilicus and stool were obtained every three days. Anaerobic cultures were not performed, but other studies have suggested that neonates and bottle-fed babies in the ICU have a similar anaerobic flora [1]. We found delayed colonization in patients in the ICU. Even after three days, almost one-third had no growth from most sites, and cultures of many other sites grew only Staphylococcus epidermidis. In vaginally delivered babies flora developed no more quickly than in babies with cesarean birth. It is possible that delayed colonization was caused by the barage of antibiotics faced by these babies; 81 percent received antibiotics, and all but two received multiple agents. It is also possible that separation from normal maternal contact and reduced oral intake played a role. In any event, colonization did eventually occur (**Table I**). In most of the babies elements of normal flora developed such as Staph. epidermidis in the nose and umbilicus, and α streptococci in the throat. Group B streptococci and Staph. aureus were noted infrequently. Gram-negative bacilli were recovered from all sites in a high percentage of neonates. As expected, gram-negative colonization of stool was nearly universal, but unlike healthy babies, neonates in the ICU were colonized with Esch. coli less frequently than with other gram-negative bacilli. Klebsiella, Enterobacter and Citrobacter (KEC), which were the only Enterobacteriaceae other than Esch. coli isolated in the six months of the study, were recovered from 52 percent of the babies, whereas Esch. coli was isolated from only 48 percent. Stool was the major reservoir of these bacteria; other sites were never colonized with KEC unless stool

TABLE I Percent of Neonates Colonized with Bacteria at Any Time During Study*

	Nose	Throat	Umbilicus	Stool
Gram-negative bacteria				
Esch. coli	16	25	25	48
Klebsiella	6	8	14	38
Enterobacter	14	14	11	22
Citrobacter	8	8	10	13
KEC†	22	22	24	52
Pseudomonas	6	5	2	5
Acinetobacter	3	5	2	8
Neisseria	11	25	0	0
Gram-positive bacteria				
Staph. aureus	17	17	21	10
S. epidermidis	89	84	90	86
Alpha streptococcus	51	89	13	3
Enterococcus	6	11	30	41
Group B streptococcus	5	3	5	5

* Most frequently isolated organisms; number of infants cultured = 63.
† Klebsiella, Enterobacter or Citrobacter.

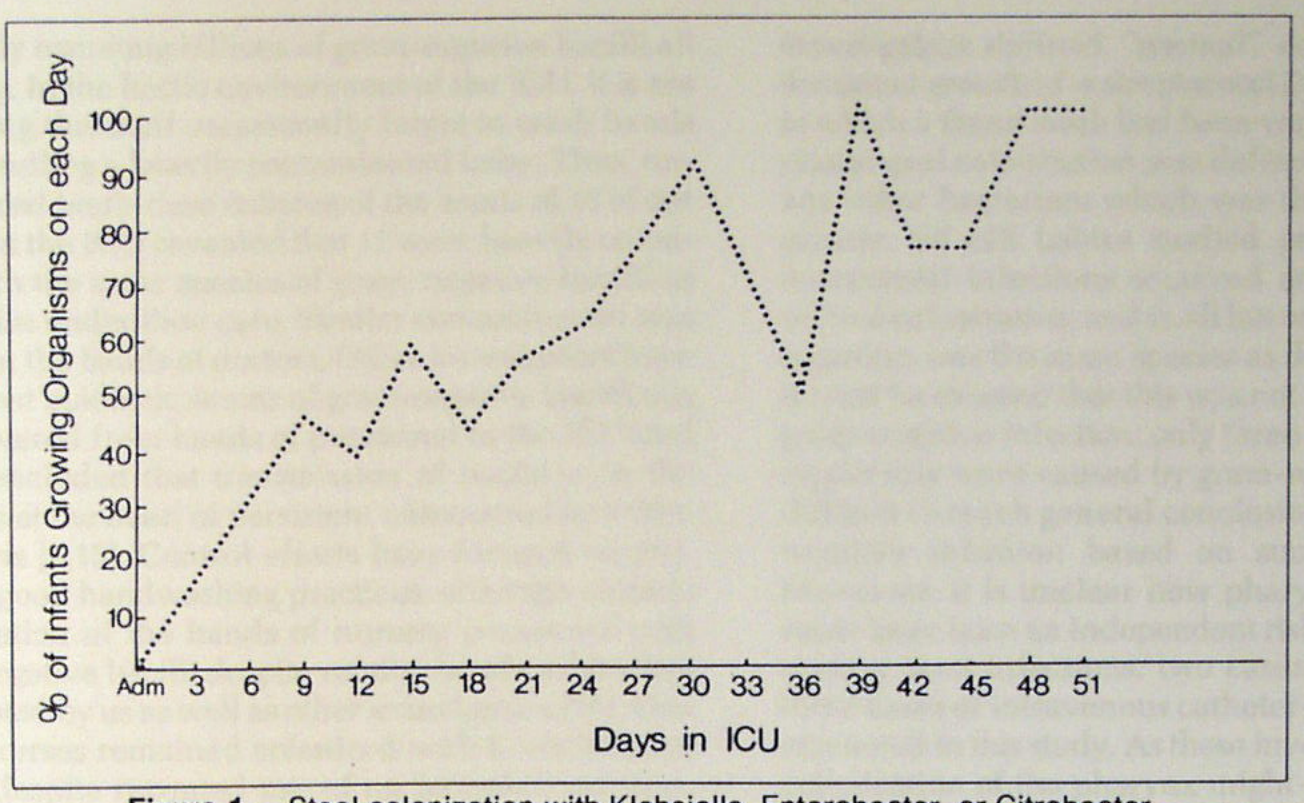

Figure 1. Stool colonization with Klebsiella, Enterobacter, or Citrobacter.

was also, and stool was colonized twice as frequently as any other site.

The types of bacteria found in neonatal stool, the limited number of biotypes noted throughout the study and the tendency of the strains to be antibiotic-resistant strongly suggest that babies were colonized with hospital strains of bacteria. Most importantly, the risk of acquiring KEC strongly correlated with duration of stay in the ICU (**Figure 1**); KEC were isolated from stool of 2 percent of the babies on admission, from 60 percent at 15 days and from 91 percent remaining in the ICU for 30 days. These findings are consistent with results of at least two other studies which have systematically examined acquisition of stool flora in nonepidemic settings [1,13]. Although KEC were the principle bacteria colonized in neonates in our study, different organisms may predominate in other nurseries. For example, in 80 percent of the babies hospitalized for at least one month in the Brown University ICU had stool colonization with K1 Esch. coli which were presumably acquired nosocomially in most cases [13]. In epidemic settings, colonization with a single strain may be particularly impressive. Rates of colonization in the stool of more than 90 percent have been reported, with a smaller percentage of babies having the epidemic organism in the throat or other body sites [9,11].

The factors which predispose newborns to colonization with nosocomial pathogens have been inadequately investigated. The influence of breast milk on colonization is uncertain. Birth weight did not effect colonization in our studies. We could not attribute colonization of the throat with gram-negative bacilli to respirator care, an observation which confirms studies in adults and probably reflects proper disinfection of equipment. We did find that colonization of the stool with KEC and of the throat with gram-negative bacilli was more likely to occur if antibiotics were given for more than three days. However, because of the small number of babies in our study, we could not rule out the possible influence of confounding variables on these results. Indeed, babies who received the most antibiotics tended to be those who stayed in the nursery longest and thus had a greater chance of encountering noscomial gram-negative bacilli. Although others have also suggested that antibiotics predispose to colonization with nosocomial pathogens, no studies of sufficient size to apply appropriate multivariate analytic techniques have appeared. However, it is known that in sick babies who do not receive antibiotics colonization can still occur given sufficient exposure to hospital bacteria. In one outbreak of kanamycin-resistant gram-negative infection, 82 percent of the babies not treated with antibiotics acquired the epidemic strains [9]. Clearly, further study is needed to define the relative importance of host, therapeutic, environmental and microbiologic factors in colonization of sick babies.

It is important to realize that colonization of the neonate's bowel is not trivial. We were consistently able to recover from 10^6 to $> 10^8$ pathogens/g of stool. Most babies remained colonized with these staggering numbers of bacteria even in the face of treatment with antibiotics to which their bacteria were susceptible in vitro. In fact, colonization with nosocomial gram-negative bacilli may persist for more than a year, even after the baby has left the hospital [14]. If such patients are readmitted to a hospital at a later time, they may provide an unsuspected vehicle for spread of nursery bacterial strains to infant wards.

Given these data concerning colonization of the stool, it is easy to understand how neonates newly admitted to the ICU become colonized with nosocomial strains so quickly. Here you have a room full of neonates who

are busily excreting billions of gram-negative bacilli all day long. In the hectic environment of the ICU, it is not surprising that staff occasionally forget to wash hands after handling a heavily contaminated baby. Thus, unannounced broth-rinse cultures of the hands of 16 of our nurses in the ICU revealed that 11 were heavily colonized with the same species of gram-negative bacilli as the babies under their care. Similar contamination was found on the hands of doctors. Other investigators have found that epidemic strains of gram-negative bacilli can be recovered from hands of personnel in the ICU and have concluded that transmission of bacteria on the hands is at the heart of persistent nosocomial infection problems [9,15]. Control efforts have focused on promoting good handwashing practices, although chronic colonization of the hands of nursery personnel with gram-negative bacilli despite routine handwashing has been noted by us as well as other investigators [15]. One of our nurses remained colonized with Klebsiella for weeks despite repeated use of an iodophor solution. Thus, routine handwashing may not always be sufficient to prevent spread of gram-negative bacilli. Moreover, there are, of couse, other ways in which nosocomial pathogens can be transmitted in the nursery. The list of outbreaks of infection caused by contaminated medical devices, solutions, medications and oral feedings is too extensive to be reviewed here. Very rarely, infections are transmitted by a member of the ICU staff who harbours pathogenic bacteria in the vagina or rectum. However, most babies undoubtedly become colonized with nosocomial gram-negative bacilli because someone has contaminated his or her hands with stool bacteria and has failed to stop at the sink.

It has generally been assumed that the patient who is colonized by hospital-acquired gram-negative bacilli is at increased risk for the development of nosocomial infection. This has been amply demonstrated in adult ICU's; colonization of stool with Klebsiella predisposes to Klebsiella infection, and patients with gram-negative bacilli in the pharnyx tend to have gram-negative pneumonia. Certainly, colonization is a prerequisite for infection in the sick baby as well. However, since so many neonates are colonized with gram-negative bacilli, the critical issue is how to determine in which colonized infant infection is most likely to develop. Host factors are undoubtedly important, but to date only low birth weight has been correlated with an increased risk of infection [7], and the independence of this risk factor from other host and therapeutic variables has not been confirmed by appropriate statistical analysis. With few exceptions, the bacteria themselves have not been examined for their relative invasiveness or virulence.

Can the site of colonization or the concentration of bacteria recovered from clinical specimens suggest who is at greatest risk? Dr. Katherine Sprunt and her colleagues at Babies Hospital in New York [16] have performed interesting studies which relate to this question. Using semiquantitative cultures of the pharynx, these investigators defined "normal" colonization as predominant growth of α streptococci ($>10^4$ cfu/ml of broth in which a throat swab had been vortexed). "Abnormal" pharyngeal colonization was defined as $>10^4$ cfu/ml of any other bacterium which was the predominant organism. Of 223 babies studied prospectively, all 18 nosocomial infections occurred among 115 with abnormal colonization, and in all but one case the infecting organism was the same species as the colonizing strain. It must be stressed that this was not primarily a study of gram-negative infection; only three of the nine cases of septicemia were caused by gram-negative bacilli. It is difficult to reach general conclusions regarding gram-negative infection based on such small numbers. Moreover, it is unclear how pharyngeal colonization could have been an independent risk factor for the three urinary tract infections, two cases of omphalitis and three cases of intravenous catheter-associated septicemia noted in this study. As these investigators remarked, colonization of the pharynx might merely reflect total body colonization with a single organism or colonization of a potentially more significant site, such as the gut. Nonetheless, this study is the best effort to date to determine the relationship between colonization and infection. The Babies Hospital group has now extended their observations to 321 babies with similar results.

More recently Dr. Sprunt and her colleagues have investigated the possibility of colonizing babies with normal throat flora, specifically α streptococci, in an effort to prevent or eliminate pharyngeal colonization with nosocomial pathogens. This approach, which is

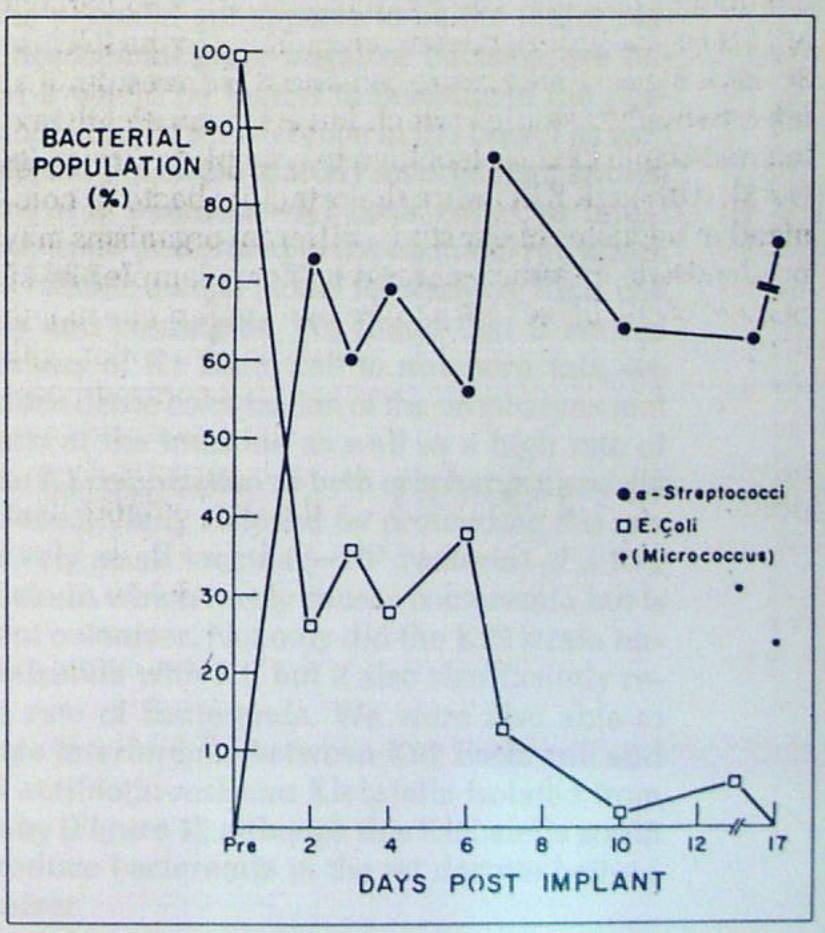

Figure 2. Effect of implantation with α streptococci on pharyngeal colonization with Esch. coli (published with permission of Dr. Katherine Sprunt).

generally termed "bacterial interference," is not new to the nursery. Shinefield et al. [17] demonstrated that Staph. aureus phage type 502A interferes with colonization by more virulent staphylococci when implanted in the nose and umbilicus. Light and his co-workers [18] found that Staph. aureus in the umbilicus interferes with colonization by such gram-negative bacilli as Pseudomonas, and the abolition of gram-positive flora by hexachlorophene permits overgrowth by gram-negative bacilli. Dr. Sprunt's interest in bacterial interference began a decade ago when she noticed that cardiac surgery patients with colonization in the pharynx with α streptococci rarely had colonization or infection with hospital strains of gram-negative bacilli. In vitro experiments demonstrated that many α streptococci inhibit the growth of a wide variety of gram-negative bacilli, as well as group A streptococci, pneumococci and other pathogens, although the relationship, if any, between these observations and the apparent interference seen in patients has not been established. Encouraged by her observations in neonates, to which I have already alluded, Dr. Sprunt decided to evaluate pharyngeal implantation of an interfering strain of α streptococci in babies with "abnormal" colonization of the pharynx. A strain with moderate penicillin resistance was selected so that it would not be easily eradicated by the antibiotics neonates in the ICU often receive. Gratifying results have been noted in very preliminary studies. Colonization with nosocomial pathogens has been dramatically reduced in some babies coincident with implantation of α streptococci (**Figure 2**). Moreover, during an outbreak of aminoglycoside-resistant gram-negative infection at the Children's Hospital of Louisville, α streptococci were implanted in three colonized neonates; the gram-negative bacilli were eradicated in all three, although other control measures were instituted at the same time [19].

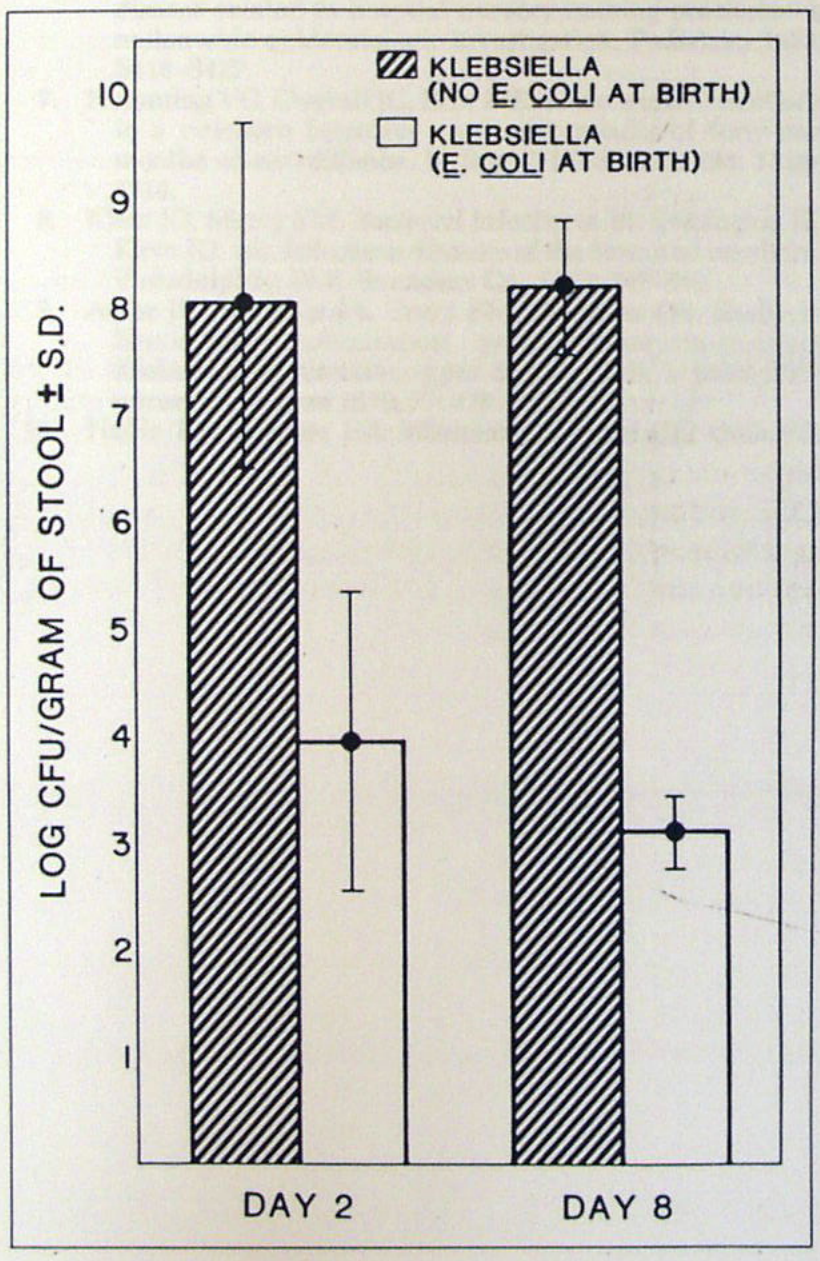

Figure 3. Effect of pre-feeding with Esch. coli on stool colonization with Klebsiella.

This work is of great interest, but it should be noted that in our studies pharyngeal colonization with α streptococci did not impede acquisition of gram-negative bacilli [12]. Fourteen of the 36 patients (39 percent) in whom moderate to abundant α streptococci had previously grown from at least two sequential cultures eventually became colonized with gram-negative bacilli; six of 20 patients (30 percent) in whom α streptococci had not grown became colonized with gram-negative bacilli ($x^2 = 0.4$). In 13 (65 percent) of the 20 patients in whom colonization developed with gram-negative bacilli after admission, moderate to abundant α streptococci grew on both the first culture growing gram-negative bacilli and on the previous culture. Of course, our study was not primarily designed to examine bacterial interference, and our definitions of significant colonization were different from those of Dr. Sprunt.

Since the neonatal gut appears to be the major reservoir of nosocomial gram-negative bacteria, we believed that it would be logical to investigate the phenomenon of bacterial interference in the bowel as well as the pharynx. We chose to study an organism known to be invasive in neonates—K1 Esch. coli. Our initial experiments were performed in the neonatal rat, which has been a reliable animal model for studying Esch. coli bacteremia and meningitis. We found that if we fed small numbers of K1 Esch. coli to newborn rats, we could produce dense colonization of the oropharynx and all segments of the intestine as well as a high rate of bacteremia. K1 colonization of both oropharynx and gut could be substantially reduced by prefeeding the rats with relatively small inocula ($\sim 10^3$ bacteria) of a K92 Esch. coli strain which rarely causes bacteremia but is an excellent colonizer. Not only did the K92 strain impede colonization with K1, but it also significantly reduced the rate of bacteremia. We were also able to demonstrate interference between K92 Esch. coli and a strain of antibiotic-resistant Klebsiella isolated from a septic baby (**Figure 3**), although this Klebsiella strain did not produce bacteremia in the rat despite being a good colonizer.

The principle purpose of these experiments is not to evaluate K92 or other enteric bacteria for possible in vivo use, but to elucidate the specific interaction be-

tween gram-negative bacilli and the gut and oropharynx. We are currently studying the mechanism of the interference we have observed with particular emphasis on the possibility that K92 Esch. coli is competing with K1 Esch. coli and Klebsiella for binding sites on pharyngeal and gut epithelium. Such mucosal adherence, which may be mediated by bacterial pili, appears to be important in the pathogenesis of gonorrhea, streptococcal pharyngitis, enterotoxigenic Esch. coli diarrhea, urinary tract infection and other infections. We believe that investigation into the basis of colonization, adherence and interference may lead to inovative approaches to preventing not only these diseases but nosocomial infections as well.

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