Use of Bacterial Interference to Control a Staphylococcal Nursery Outbreak

Deliberate Colonization of All Infants With the 502A Strain of Staphylococcus aureus

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Deliberate colonization of newborn infants with a coagulase-positive Staphylococcus of relatively low pathogenicity (502A) has previously been employed as a means of controlling nursery outbreaks of pathogenic staphylococci. Studies in several university centers have demonstrated the effectiveness and safety of this procedure. The present report demonstrates that the procedure can be utilized equally effectively in a nonuniversity affiliated community hospital.

Material and Methods

Newborn Nursery—Marion, Ind is a community in Northeast Indiana with a population of 48,000 to 50,000. The Marion General Hospital, a privately supported community hospital, serves a total population of 76,000. It has a capacity of 250 beds and 36 bassinets. There are approximately 1,800 deliveries annually. All newborn infants are housed in the newborn unit adjacent to the delivery room and obstetrical department. The average daily census is 20 babies. The newborn unit has a total floor space of 1,500 sq ft. It is divided into four nurseries, each with an area of 250 sq ft. There are two nurseries on each side of a central corridor. Each pair of nurseries is in turn separated by a central area, functioning as a work area and nursing station. Three of the nurseries house normal newborn infants (birthweight > 2,200 gm). These infants are admitted on a modified cohort system, though personnel move freely from nursery to nursery. Infants remain in the nursery one to four days (mean three days). The fourth nursery is an intensive-care unit with seven incubators or bassinets or both. All low-birthweight infants (birthweight < 2,200 gm) and all newborn infants with complications are housed in this unit. Low-birthweight infants remain in the nursery until they weigh 2,200 gm.

Nursing personnel employed on the nursery and obstetrical service are assigned solely to this service. Preemployment nasal cultures are taken and those who carry staphylococci of the potentially virulent phage type are excluded from the department. On entering the nursery, nurses scrub hands and forearms for two minutes with a brush using hexachlorophene (PheroHex) and water. Between handling babies, nurses wash their hands with hexachlorophene and water.

Infants are examined at least once in the nursery by a staff physician. The pediatric care is supplied by the general practitioners and four pediatricians in the community. Procedures for hand care for physicians are similar to those for nurses. In addition physicians don a gown, cap, and mask upon entering the nursery. Gowns are not changed between handling babies.

Routine Newborn Care—In the delivery room 1% silver nitrate is routinely employed as ophthalmic prophylaxis. Plastic clamps are applied to the umbilical cord. Infants are transferred directly from the delivery room to the newborn nursery. Within one hour of admission to the nursery all infants are bathed with undiluted hexachlorophene. The hexachlorophene is
then removed with tap water. Hexachlorophene is not applied to the face and no particular attention is paid to the umbilical site. Infants are then bathed daily using only tap water, however hexachlorophene is used if it is requested by the physician or if the infant appears excessively soiled with stool or meconium. At bath time benzalkonium chloride (Zephiran chloride) is applied to the base of the umbilical cord using a cotton-tipped applicator; circumcision is performed on most male infants during the first two days of life.

Staphylococcal Outbreak.—In October and November 1964, pediatricians in the community noted increasing numbers of purulent lesions in infants following their discharge from the newborn nursery. More than 30 infants with painful skin lesions and four additional infants with breast abscesses were observed. In addition, two infants had staphylococcal pneumonia: one of these infants was a newborn, the other, a sibling of an infant recently discharged from the nursery. The common bacterial pathogen isolated from these lesions was a penicillin-resistant coagulase-positive Staphylococcus of the phage type 80/81.

To determine the source of the outbreak, nasal cultures were taken from all infants in the nursery, from all available physicians associated with the maternity service, and from all nursing personnel employed in the delivery room, newborn nursery, and obstetrical areas. Nasal cultures were obtained on Nov 19, 1964, from 25 newborn infants. Eleven infants (44%) were carriers of penicillin-resistant, coagulase-positive staphylococci of the phage type 80/81. None of these infants had pyogenic lesions at the time of culture. Nasal cultures were obtained from 46 nursing personnel. One carrier of Staphylococcus 80/81 was detected working in the delivery room. Of the 25 physicians cultured, two were nasal carriers of 80/81.

Consultation was obtained from the Indiana State Board of Health. On the recommendation of the Board of Health staphylococcal control measures were instituted on Dec 2, 1964. Hexachlorophene bathing was performed on all infants within one hour of admission to the nursery and then repeated at daily intervals. Neomycin nasal ointment was applied three times daily to the nasal mucosa of all infants, mothers, physicians, and nursing personnel. Ointment was applied with a cotton-tipped applicator introduced approximately 1 cm into the external nares, a separate applicator being used for each of the nares. Nasal cultures were obtained from all nursery personnel. All known carriers of 80/81 staphylococci were removed from the service. Fomites were cultured, looking for sources of contamination. Visitors to the obstetrical nurseries were limited, and fathers were prohibited from visiting the labor room. The procedures were unsuccessful as will be shown later. Utilization of 502A colonization was considered.

Procedure of Colonization With 502A.—Administration.—The lack of success of the control measures was discussed by the members of the Nursery Committee. Consideration was given to a program of purposeful colonization of all infants in the newborn nursery with Staphylococcus aureus of relatively low pathogenicity (502A). This proposal was discussed with the Infection Committee. After much discussion the recommendations of these committees were presented to the Executive Committee. It was decided that all infants newly admitted to the newborn nursery be colonized with the 502A strain.

Preparation of the Inoculum.—The organism utilized for colonization was the previously isolated coagulase-positive, penicillin-sensitive Staphylococcus of relatively low pathogenicity designated 502A. As tested by disc sensitivity methods it has an easily recognizable characteristic antibiotic sensitivity pattern. It is sensitive to discs labeled 2 units of penicillin, 25 μg of tetracycline, 25 μg of gentamicin, and 5 μg of novobiocin. The sensitivity to tetracycline discs of different concentrations is particularly useful. The organism is resistant to the 5 μg disc. The organism, however, is sensitive to discs labeled 1 μg of tetracycline.

The organism was stored in its lyophilized state and was reconstituted from its lyophilized state by adding 1 ml of sterile distilled water to the 502A vial. The identity of the organism was confirmed by mannitol-fermentation, coagulate production, pigment production, antibiotic sensitivity pattern, and phage typing. A loopful of the reconstituted media was inoculated into 5 ml of sterile trypticase soy broth which was then incubated at 37°C for 18 hours. The 18-hour broth (without mixing) contained 10⁹ organisms/ml. In the early part of the study 1 ml of this broth was diluted in 9 ml of trypticase soy broth to produce a 1:10 dilution of the 18-hour broth containing 10⁸ organisms/ml. In the latter part of the study 0.25 ml of the 18-hour broth was diluted in 9.75 ml of trypticase soy broth. Further dilution was effected by adding 2.5 ml of the broth to 250 ml of trypticase soy broth. The final result was a 1:40,000 dilution of the original 18-hour broth, estimated to contain 2.5 × 10⁶ organisms per ml.

One milliliter aliquots of the diluted broth in screw-top sterile test tubes were stored at 0°C. Colony counts of the prepared broth before freezing and on tubes of thawed broth at week 1, 2, 4, and 6 are shown in Table 1. In many laboratories the 18-hour count is 10⁸ or unmixed cultures of this organism.
STAPHYLOCCAL NURSERY OUTBREAK—LIGHT ET AL

Penicillin-resistant, mannitol-positive, coagulase-positive staphylococci lysed by phages 80 and 81 were designated 80/81. Infants from whom one or more colonies of 80/81 staphylococci were isolated from either the nasal or umbilical site were considered carriers of 80/81.

Mannitol-positive, coagulase-positive staphylococci showing the characteristic antibiotic sensitivity pattern and lysed by the appropriate phages were identified as 502A. On occasion, organisms showing the characteristic 502A antibiotic sensitivity were not lysed by any of the standard phages. These were designated 502A.

A program of deliberate colonization of all infants newly admitted to the newborn nursery was instituted. The first period of colonization began Feb 8, 1965, and extended through April 21, 1965. From April 24, to July 5, purposeful colonization with the 502A strain was discontinued. The second period of colonization began on July 6 and was continued until Oct 5. The colonization procedure was performed by the nurses (Appendix). From previous experience it was estimated that this procedure delivered 0.01 ml to each inoculation site.

Bacteriologic Procedures—All cultures were taken by the nursing personnel. Bacteriologic procedures were performed by the hospital bacteriologist. Phage typing was done by the Indiana Board of Health.

Infants.—A survey of the daily staphylococcal flora of infants in the nursery was compiled (Fig 1). In constructing this chart it was assumed that the infant harbored from birth the organism isolated on the day of discharge. If infants remained in the nursery longer than one week it was assumed that the organism isolated from the weekly or discharge culture had been present throughout the interval following the preceding culture.

First Outbreak.—With the implementation of staphylococcal control measures which included daily hexachlorophene bathing and neomycin nasal ointment, the frequency of infants found to be harboring 80/81 staphylococci initially decreased from 15% to 6%. However, despite continuation of these measures, this frequency of 80/81 carriers subsequently increased rapidly to a maximum of 50%. On Feb 1, 1965, Staphylococcus 80/81 was isolated from 40% of the infants (eight) in the newborn nursery.
Fig 1.—Daily staphylococcal flora in infants in the newborn nursery during the first outbreak, and during the first period of purposeful colonization.

Fig 2.—Staphylococcal flora in infants obtained at weekly intervals during the second outbreak, and during the second period of purposeful colonization.

*Amer J Dis Child—Vol 113, March 1967*
On Feb 8, 1965, *Staphylococcus* 80/81 was isolated from 10% of infants in the nursery (two infants). Colonization with the 502A strain was started on this day. Initially the concentrated broth was administered to the first 85 infants admitted to the nursery. During the latter part of this colonization period the dilute broth was administered to 218 infants. Following the onset of purposeful colonization all *staphylococci* other than 502A rapidly disappeared from the nursery, and virtually all infants were found to be carriers of the 502A strain. The first period of colonization was terminated on April 23, 1965.

**Table 1.**—Mixed *Staphylococcal* Flora

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<tr>
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<th>Nose</th>
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<tr>
<td>Only 502A</td>
<td>34</td>
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<td>502A and another</td>
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<td>Total</td>
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Nasal and umbilical cultures obtained on the day of discharge from 44 deliberately colonized infants were plated on media with and without added penicillin.

**Second Outbreak.**—During the seven-week period following cessation of purposeful colonization with the 502A strain the organism was isolated from more than 80% of the infants cultured. There then occurred a change in nursery housekeeping technique. Because of the hospital building program, minor construction work was done in each nursery, following which all nurseries were scrubbed. The following week the 502A *Staphylococcus* was isolated from 67% of the infants cultured. The next week the 80/81 *Staphylococcus* reappeared, and continued to be present in 25% to 40% of infants cultured over the ensuing four weeks (Fig. 2). The 80/81 *Staphylococcus* isolated possessed a different antibiotic sensitivity pattern than that recovered during the first 80/81 outbreak. Colonization with 502A was re instituted on July 6, 1965, using the dilute broth containing $2.5 \times 10^5$ organisms/ml. During the following 13 weeks, 469 infants were purposely colonized with the 502A strain. Follow-up cultures were obtained from 202 infants, 195 of whom (96%) were...
502A carriers. The second period of purposeful colonization was terminated on Oct 5, 1965. During the following 12-week period the 502A strain was isolated from 70% to 100% of infants cultured. Coagulase-positive staphylococci other than 502A were not isolated from any infants during this time.

** Cultures on Penicillin Media.**—Nasal and umbilical cultures from 44 deliberately colonized infants were plated on media with and without added penicillin to detect double colonization (Table 1). Coagulase-positive staphylococci were isolated from 77 of the 88 sites cultured in this manner. Four (5%) of the sites harboring coagulase-positive staphylococci were found to be harboring two strains, one of which was penicillin-resistant. None of these penicillin-resistant organisms were of the phage type 80/81.

**Personnel.**—In early November 1964 at the time of recognition of the initial outbreak, nasal cultures from all nursery, delivery room, and obstetrical personnel revealed one of the delivery room personnel harboring 80/81 staphylococci (Fig 3).

In early January 1965 during the period of administration of neomycin nasal ointment (to all babies, mothers, and personnel) repeat nasal cultures were obtained from the nursing personnel. The incidence of carriers of 80/81 staphylococci was 20% in the nursery personnel, 31% in the delivery room personnel, and 36% in the obstetrical personnel. Repeat nasal cultures on Feb 1, 1965, just prior to the first period of purposeful colonization with 502A, demonstrated an increase in the incidence of carriers of 80/81 staphylococci to 50% in the nursery personnel. No increase was noted in the delivery room (31%) and obstetrical (18%) personnel. Two weeks after the onset of purposeful colonization of all newborn infants, nasal cultures were obtained from all personnel. *Staphylococcus* 80/81 was not isolated from any of the obstetrical and delivery room personnel. Four (18%) of the personnel in the nursery continued to be carriers of 80/81 staphylococci. These persistent carriers were treated with a course of antibiotic nasal ointment plus therapeutic doses of systemic oxacillin, followed by a course of colonization with the 502A *Staphylococcus.* Repeat nasal cultures were obtained on all personnel in March 1965. *Staphylococcus* of the phage type 80/81 was not isolated from any of the personnel examined. The 502A strain was isolated from eight (35%) of the 22 personnel examined.

**Lesions.**—During the first outbreak of *Staphylococcus* 80/81 the organism was isolated from 83 newborn infants (Table 2). Pustular skin lesions, caused by this organism were noted in 19 of 41 males (46%) from whom the organism was isolated, and in 8 of 32 (25%) females. Skin lesions were noted in additional infants after their discharge from the newborn nursery. Breast abscesses were noted in four infants, and staphylococcal pneumonia in two infants.

In the period between the two periods of colonization 332 infants were admitted to the newborn nursery. Cultures were obtained from 128 of these infants. *Staphylococcus aureus* of the phage type 80/81 was isolated from 18 infants, 12 (66%) of whom had pustular skin lesions. In contrast, skin lesions were noted in 11 (11%) of the 97 infants from whom the 502A strain was isolated.

The 502A strain was purposely administered to a total of 772 infants. While in the hospital, 30 (4.7%) of these infants developed
oped skin lesions from which the 502A strain could be isolated. The majority of these were tiny vesiculo-pustular lesions without surrounding erythema. In two exceptional cases purulent lesions were noted, for which systemic antibiotic therapy was felt to be needed. In both instances gram-negative enteric organisms, in addition to the 502A strain were isolated from the purulent material. In occasional infants a mild conjunctivitis was noted from which the 502A strain was isolated. No severe pyogenic skin lesions or systemic staphylococcal disease was noted in any infants colonized with the 502A strain.

Of 85 infants colonized with the broth containing 10⁷ organisms/ml, 502A pustules were noted in 12 infants (14%). In contrast, 672 infants were colonized with a more dilute broth containing 2.5 × 10⁶ organisms/ml. Twenty-four (3.5%) of these infants were noted to have pustular 502A skin lesions. The higher frequency of skin lesions among infants receiving the concentrated broth is highly significant ($\chi^2 = 19.2, P < 0.01$).

Comment

A nursery staphylococcal epidemic was recognized by physicians in Marion, Ind following an increase in the occurrence of pyogenic lesions. When other measures failed, a program of deliberate colonization with the 502A strain of Staphylococcus was begun. The colonization procedure was performed by the nurses; the procedure was simplified.

Though more than 94% of infants deliberately colonized acquired the 502A strain, the role of the colonization program in controlling the first outbreak of Staphylococcus 80/81 is unclear. The pathogenic Staphylococcus was present in the nursery for at least 12 weeks preceding the first period of deliberate colonization. One week prior to the onset of deliberate colonization the 80/81 Staphylococcus was isolated from 40% of infants in the nursery. On the day of initiating the program the frequency of 80/81 infant carriers was only 10% (two infants), though the frequency of cultures with "no growth" was 38%. Because this spontaneous decrease in carriers of the 80/81 strain may have been spurious, related to changing procedures, it is difficult to evaluate the effectiveness of the first program of purposeful colonization in eliminating the 80/81 strain from the nursery. The effectiveness of the second program of deliberate colonization in terminating the second outbreak of pathogenic staphylococci is apparent. Immediately following reinstitution of purposeful colonization with 502A there was an abrupt termination of the presence of the pathogenic strain.

It has been demonstrated in both endemic and epidemic situations that many adults working in nurseries are transient staphylococcal carriers. These individuals lose the organisms when they have no contact with infants harboring organisms. There are occasional permanent carriers who continue to harbor pathogenic staphylococci even after cessation of contact with infants harboring organisms. In the present study, during the first outbreak of 80/81 staphylococci in the infants, the frequency of nasal carriers among nursing personnel was as great as 50%. Termination of the outbreak by the successful colonization of infants with the 502A strain, resulted in the spontaneous elimination of the 80/81 Staphylococcus from all but four of the nursing personnel. Despite continued intimate contact between the infants and these permanent staphylococcal carriers, infants colonized with the 502A strain did not acquire the pathogenic strain. This supports the original observations of Shinefield et al who noted that infants naturally colonized with 502A strain did not subsequently acquire 80/81 staphylococci from nursing personnel known to be carriers of the organism.

The low virulence of the 502A organism in newborn infants has been established. The frequency of pustular skin lesions in infants colonized with the 502A strain has repeatedly been reported as less than 5%. The earlier studies also demonstrated that lesions were most prevalent with the 80/81 organisms and least prevalent with the 502A organisms. With other unspecified coagulase-positive staphylococci or no such staphylococci, the prevalence of lesions was intermediate.

In the present study 36 (4.7%) of 772 infants deliberately colonized with the 502A strain developed vesiculo-pustular skin lesions. The frequency of 502A skin lesions was related to the number of organisms in
the inoculating broth. Vesiculo-pustular lesions were significantly increased from 3.5% in infants receiving the dilute broth (2.5 × 10^3 502A organisms/ml) to 14% in infants receiving the concentrated broth (10^5 502A organisms/ml). Occasional infants had a mild conjunctivitis from which the 502A strain was isolated. In none of the infants colonized with the 502A strain were severe pustular lesions or systemic staphylococcal infections noted. In contrast, in infants colonized with 80/81 staphylococci skin lesions were more severe and more frequent than those associated with the 502A strain. Additional lesions, caused by phage type 80/81 staphylococci were encountered; these included breast abscesses and staphylococcal pneumonia.

In infants colonized with 80/81 staphylococci the frequency of skin lesions increased in males. This finding is similar to the observation of Gezon et al. who noted that male infants acquire staphylococci more readily than females, and that in those who acquire the organism a higher proportion of males develop disease.

The possibility has existed that individuals may harbor more than one strain of *S. aureus*. The apparent good results observed following deliberate colonization with the 502A strain might then be misleading, and related to the relative number of infecting organisms. To test this hypothesis, nasal and umbilical cultures obtained at discharge from 44 infants were plated on media with and without penicillin. Isolation of a second penicillin-resistant strain in the presence of the 502A strain occurred in only 5% of the cultures tested. These penicillin resistant staphylococci were not lysed by the phages 80 or 81.

Thus, the mechanism of bacterial interference was further defined. The difference between the 502A strain of lower virulence and the Marion, Ind 80/81 strain(s) of relatively greater virulence was apparent. The 502A strain, though of low virulence, is probably not avirulent, for minor lesions were observed. The disease-potential of the strain, though small, precludes administration of the low-risk organism to infants during periods when they are not exposed to the greater risk of an epidemic. Although 502A inoculation was safe and effective in controlling a hazardous staphylococcal outbreak in a nursery, it is not a procedure to be followed routinely during nonepidemic periods. It may still be possible ultimately to provide each newborn infant with an avirulent bacterial flora tailored to prevent invasion by more dangerous organisms.

**Summary**

A nursery outbreak of a pathogenic strain of *Staphylococcus aureus* (80/81) was recognized in a nonuniversity affiliated community hospital. The outbreak was successfully controlled by purposely colonizing all infants with a previously described coagulase-positive *Staphylococcus* of relatively low pathogenicity (502A). Cultures from the nasal and umbilical sites streaked on media with and without penicillin demonstrated that infants colonized with the 502A strain rarely harbor other coagulase-positive staphylococci at the same site. Among the inoculated infants, the low incidence of pustular lesions (less than 5%) and the minor nature of the lesions confirmed the low virulence of the 502A strain. The finding that lesions occurred more frequently when more organisms were inoculated is unexplained.

Among the personnel, many of the nasal carriers of pathogenic staphylococci were transient carriers and lost the pathogenic strain when it disappeared from the infants.

A step-by-step description of the laboratory procedures and method of colonization is presented as a guide for future utilization of this procedure.

This program could not have been successful without the full cooperation of the nursing staff who carried out the procedures despite a devastating tornado which struck Marion, Ind on April 11, 1965. Phage typing was provided by Jessie Boots, MS of the Indiana State Board of Health. Technical assistance was rendered by Mrs. Lois Adams.

**Generic and Trade Names of Drugs**

References


Appendix

Instructions for Nursing Personnel.—A preparation of 1 ml of a frozen broth culture stored in an individual tube is available for each infant admitted to the nursery. The freezer in which these tubes are stored should be used for no other purpose. The bacteriology laboratory will be responsible for maintaining an adequate supply of tubes of frozen broth in the freezer.

Colonization of Infants.—Colonization is carried out on every infant as soon as possible after birth, but no longer than two hours after birth. On admission to the nursery a tube of broth is removed from the freezer. Thawing of the frozen broth will occur in 20 to 30 minutes. A pack containing three long, sterile, wooden, cotton-tipped swabs is used. The cover of the broth tube is removed and the three swabs are placed in the tube. One of the swabs is removed from the broth and excess broth is removed from the swab by touching the inside of the tube. The moistened swab is gently inserted just inside one nostril with most of the cotton tip inside the nostril. The swab is turned through one complete revolution. The used swab is replaced in the paper envelope. In the same manner a second swab is removed from the broth, inserted in the second nostril, and returned to the envelope. The third swab is removed from the broth and touched to the inside of the tube. This moistened swab is applied to the umbilical site with a single sweep around the entire circumference at the junction of skin and membrane cord. This swab is placed in the envelope. The envelope containing the three used swabs is discarded in a paper-lined basket which is autoclaved before discarding.

The used broth tube is marked with the infant's name and placed in a basket marked "Discard broth tubes" to be returned to the bacteriology laboratory for autoclaving and cleaning. The date and time of colonization is to be recorded on the infant's hospital record and on the special form provided for this purpose.

Skin Care.—Routine bathing will be performed only with clear water. Hexachlorophene (pHisoHex) and soap will not be used. No alcohol or other medications should be applied to the cord. Alcohol may be used as usual for skin cleansing prior to injections. Hexachlorophene or any other routine skin cleansing measures may be used as precircumcision scrub or antiseptic. Silver nitrate may be applied to oozing or granulomatous cord bases. Alcohol may be applied to oozing or foul smelling cords after the first 3 to 4 days of life. If soap or hexachlorophene bathing is ordered by the physician for a specific medical indication after the first week of life, the infant may be bathed.

Use of Antimicrobial Drugs.—It should be recognized that the 502A Staphylococcus is very sensitive to antibiotics and that almost all infants given antibiotics will lose the protective 502A strain. Having lost their protective these antibiotic-treated infants may then acquire pathogenic organisms, be subject to disease.
themselves, and even without disease may constitute a hazard to the other infants in the nursery. Despite this, if an infant requires antibiotics, he should receive them.

Flagging Safe and Unsafe Babies.—It may help to flag the bassinets of safe (green) and unsafe (red) babies with colored stickers. Safe babies are inoculated babies. The green sticker (safe) should be removed from the bassinet of any infant who has received antimicrobial drugs and be replaced with a red sticker (unsafe). In this way, as the unsafe babies decrease in number they may be concentrated geographically, perhaps isolated, and efforts made to speed their discharge from the hospital.

Obtaining Cultures.—On the day of discharge from the hospital each infant is cultured. Cultures are obtained by the nurses from one nostril and from the base of the cord. In addition, on one day each week (Tuesday), similar cultures are taken from all infants in the nursery who are 7 days of age or older. This culturing procedure applies to all infants, both infants who have been inoculated and infants who were in the nursery before the inoculation procedure began.

To take the culture, a sterile swab is gently inserted inside one nostril and turned through one complete revolution. The swab is then streaked six times across one half of a Petri dish containing salt-mannitol (phenol red) media, rotating the swab as the plate is streaked so that all surfaces of the swab come in contact with the media. One half of the plate is marked with the baby’s name, the date, and N for nose. A second swab is swept three times about the base of the cord, taking care to sample any exudate which may be present. The second half of the culture plate is then streaked with the cord swab. The second half plate is marked with U for umbilicus. The plate is then sent to the bacteriology laboratory with the special information sheet on babies being discharged from the hospital and with a routine bacteriology requisition sheet on the older infants.