Culture diagnosis of meningococcal carriers

Yield from different sites and influence of storage in transport medium

PER OLCÉN, JAN KJELLANDER, DAN DANIELSSON, AND BO L. LINDQUIST

From the Department of Clinical Microbiology, Infectious Diseases and Paediatrics, Central County Hospital, Örebro, Sweden

SUMMARY Different specimens and techniques have been used in the diagnosis of carriers of *Neisseria meningitidis*, reflecting the uncertainty about the optimal diagnostic procedure. In the present investigation the culture yield of meningococci from throat specimens was compared to that from nasopharyngeal specimens in 178 persons: 44 carriers were diagnosed. All of them were detected by culture of throat specimens while 34% of them would have remained undiagnosed if only nasopharyngeal specimens had been examined. Storage of throat specimens in a transport medium for 24 hours before culture gave a negative culture for meningococci in 41% of the carriers. This loss was surprisingly high, the reasons for which are discussed.

The diagnosis of meningococcal carriers is of interest in, for example, epidemiological investigations of meningococcal disease. The general routine in the search for meningococcal carriers has been culture of nasopharyngeal specimens in order to avoid overgrowth and other interactions of the normal flora in the tonsillo-oropharyngeal area. Since the introduction of selective culture medium for pathogenic *Neisseria* (Thayer and Martin, 1964; Martin *et al.*, 1965) no investigations have compared the yields by culturing nasopharyngeal and throat specimens with the use of such media. Due to this lack of information, it is far from clear which location will give the highest yield. Also, the transport of specimens to the laboratory, in currently used transportation media, before they are cultured, will influence the results. Thus, Holten *et al.* (1978) mentioned a loss of at least 10% after transport as compared to direct inoculation ('at the bedside').

The present study was undertaken to compare the culture yields of meningococci from nasopharyngeal and throat specimens inoculated 'at the bedside'. The culture yield of meningococci after storage of clinical specimens in modified Stuart transport medium (Stuart, 1946; Gästrin *et al.*, 1968) was also investigated as well as the survival of meningococcal

organisms in the same transport medium in a laboratory study.

Material and methods

DESIGN OF CLINICAL STUDY

The study was carried out during the period July 1975 to May 1978 in Örebro County, Sweden, and comprised 178 persons. These included 64 family members of 23 consecutive patients with meningococcal disease, 64 family members of 24 consecutive patients with meningitis due to *Haemophilus influenzae* or *Streptococcus pneumoniae*, and 50 healthy military service men.

Three specimens were taken from each person by one of us according to the following procedure: (1) throat swab taken from both tonsils with a dry cotton-tipped wooden applicator. The specimen was immediately inoculated onto culture plates (see below); (2) throat swab taken from both tonsils with a dry charcoaled cotton-tipped wooden applicator and put into Transport Medium SBL (TMSBL) (Gästrin *et al.*, 1968). This specimen was inoculated onto plates after 24 hours' storage at room temperature; (3) nasopharyngeal swab taken via the transnasal route with a dry cotton-tipped wire applicator. The applicator was held for a few seconds towards the posterior wall of the nasopharynx, rotated in both directions, and then withdrawn and immediately inoculated onto plates.

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BACTERIOLOGICAL AND SEROLOGICAL METHODS

The specimens were cultured on a medium prepared from BBL GC agar base enriched with Isovitalex and Bacto Haemoglobin with and without VCN (vanomycin, colistin, nystatin) inhibitor as described before (Danielsson and Johannisson, 1973). The plates were incubated in high humidity 5% CO₂ at 36°C within 30 minutes after inoculation and read after two days. Diagnosis and serogrouping of meningococci was performed as described by Olcén *et al.* (1978) with sugar degradation tests, ONPG, immunofluorescence, and co-agglutination.

DESIGN OF LABORATORY STUDY CONCERNING SURVIVAL OF MENINGOCOCCI IN TRANSPORT MEDIUM

Altogether three strains of meningococci group A, three of group B, and three of group C were examined. One international reference strain (group A NCTC No. 10025, batch 7; group B NCTC No. 10026, batch 3; group C NCTC No. 8554, batch 3), one strain freshly isolated from cerebrospinal fluid, and one freshly isolated from the throat were examined within each group.

The strains had been preserved at -63°C (Danielsson and Sandström, 1979) and were subcultured three to four times on the solid culture medium described by Kellogg *et al.* (1963). After 18 hours' growth the culture was harvested in phosphate buffered saline with 5% heat-inactivated (56°C, 30 min) horse serum to a turbidity corresponding to about 9×10^8 CFU/ml; 0.1 ml of this suspension was pipetted to each of 14 dry charcoaled cotton-tipped applicators (identical with those used in the clinical study). Twelve of the applicators were transferred to TMSBL while two were vigorously shaken for 30 seconds in 3 ml of dextrose-broth using a rotatory shaker. A series of 10-fold dilutions in dextrose-broth were prepared from the suspension, and 0.1 ml of each dilution was inocu-

lated onto enriched GC agar base with and without VCN and incubated for 48 hours. The number of colonies was counted in those plates which showed growth of 30 to 300 colonies, and the viable count per applicator was calculated. The applicators stored in TMSBL were withdrawn in pairs and treated in the same way after storage for 1 minute, 4, 8, 24, 48, and 72 hours. In some experiments other times for storage were used, as indicated below.

Results

ISOLATION OF MENINGOCOCCI FROM THROAT AND NASOPHARYNGEAL SPECIMENS

The results are summarised in the Table. It can be seen from the Table that 44 of the 178 persons carried meningococci and that all the carriers had positive throat cultures while only 57% (25 persons) had positive nasopharyngeal cultures. If only the clinically important serogroups A, B, C, and Y are considered the corresponding nasopharyngeal isolation rate was 66%. The Table also shows that the culture yield from the throat was higher than from the nasopharynx for all serogroups except the three group A strains which were isolated from both sites.

SURVIVAL OF MENINGOCOCCI IN TMSBL

Clinical study

From the Table it can be seen that there was a total loss of 41% (18 out of 44 meningococcal carriers) after storage of the throat specimens for 24 hours in TMSBL. The same percentage was lost if only the serogroups A, B, C, and Y are considered.

Laboratory study

Nine meningococcal strains were examined for survival in TMSBL. The recovery from applicators that had not been inserted in TMSBL varied from 2.7×10^7 to 8.7×10^7 (mean 5.8×10^7) CFU/applicator. The recovery from applicators that had been inserted in TMSBL and withdrawn after 1 minute was 1.6×10^7 to 5.8×10^7 (mean 3.6×10^7) CFU/applicator lower than the uninserted ones. This means that 62% of the bacteria remained in the transport medium after withdrawal of the applicator. The changes in viable counts after different storage times in TMSBL for the nine strains are illustrated in Figure 1. It can be seen from the Figure that a continuous loss of viable bacteria was registered over time. The average loss by 24 hours was 1.3 log. If the mechanical effect of insertion and withdrawal of the applicator (including storage for 1 minute) is added the total average loss will be 1.8 log. It can

Table Yield of meningococci (MC) by culture from different sites in 178 persons

MC serogroup	Total no.	No. of specimens with MC		
		Th	Np	ThStu
A	3	3	3	3
B	11	11	7	6
C	14	14	11	8
Y	4	4	0	2
Others	12	12	4	7
Total	44	44	25	26

Th = throat culture, direct inoculation;

Np = nasopharyngeal culture, direct inoculation;

ThStu = throat culture stored in transport medium for 24 hours before culture.

Fig. 1 Changes in number of colony forming units (CFU) for nine meningococcal strains during storage in transport medium. The change is expressed as the difference between the $^{10}\log$ of the number of CFU per applicator after insertion of the applicator and withdrawal after 1 minute and the $^{10}\log$ of the number of CFU per applicator after the indicated storage time. Solid line = mean values. Dotted lines = extreme values.

also be seen that the reduction was more marked at the beginning.

The results illustrated in Fig. 1 are based on colony counts on GC agar without inhibitor. The corresponding counts on GC agar with VCN inhibitor were no more than 0.2 log lower.

Figure 2 shows that the reduction of colony counts increased with decreasing inoculum. This was especially pronounced at the beginning. At an inoculum of 4×10^7 CFU/applicator the loss after 6 hours was 0.7 log whereas the corresponding loss at an inoculum of 5×10^4 CFU/applicator was 3.8 log.

Discussion

Since the introduction of selective culture media for pathogenic *Neisseria* (Thayer and Martin, 1964; Martin *et al.*, 1965), surprisingly few data have been published concerning the site of meningococci in carriers. For a long time, culture of nasopharyngeal specimens has been the routine. If the approach to the nasopharynx was transoral it was recommended that the oropharyngeal structures should not be touched in order to avoid the normal flora in these areas (Slaterus *et al.*, 1963). In several investigations during recent years various techniques have been used, reflecting the lack of information concerning

Fig. 2 Number of colony forming units (CFU) for five different concentrations of a meningococcal group B strain after storage in transport medium for different periods of time.

the site of meningococci in carriers (Fraser *et al.*, 1973; Jacobson *et al.*, 1977; Holten *et al.*, 1978; Sivonen *et al.*, 1978). In the present study all meningococcal carriers were diagnosed by culture of throat specimens while 34% of them were not diagnosed by culture of nasopharyngeal specimens taken transnasally.

Hoeffler (1974) reported a higher yield with nasopharyngeal specimens taken transorally as compared to those taken transnasally. He did not, however, compare these results with cultures from the throat. Hoeffler's results could be explained by contamination of the applicator swabs with secretions from the oropharyngeal structures, which are difficult to avoid in order to reach the nasopharyngeal area. In the present study transoral nasopharyngeal specimens were not taken since many of the subjects were small children in whom this technique is technically difficult to carry out.

Inoculation of specimens 'at the bedside' gave the highest yield, which is in agreement with the report by Holten *et al.* (1978). We noted a loss of as much as 41% by storage of throat specimens in TMSBL for 24 hours at room temperature, while Holten *et al.* (1978) reported a loss of 10% after transport in a corresponding transport medium. The reason for the higher loss in our investigation is not clear. However, Holten *et al.* (1978) examined a semi-closed population which had a higher carrier rate than our groups and perhaps a heavier colonisation of meningococci.

Our laboratory experiments showed an average loss of 1.8×10^2 log during storage for 24 hours,

which is in agreement with the results reported by Gästrin *et al.* (1968). We found, however, losses of more than 2×10^6 log in experiments with lower viable counts of meningococcal organisms. We did not quantitate the number of meningococcal organisms collected by an applicator from a carrier, but our experiments indicate that in many cases the number of meningococcal organisms per applicator was lower than $10^7 - 10^8$, an inoculum commonly used in *in vitro* tests.

Our findings stress the need for testing transport media by laboratory experiments using a wide range of bacterial concentrations as well as field studies such as those in this report or those described by others (Danielsson and Johannisson, 1973; Danielsson *et al.*, 1978).

References

- Danielsson, D., and Johannisson, G. (1973). Culture diagnosis of gonorrhoea. A comparison of the yield with selective and non-selective gonococcal culture media inoculated in the clinic and after transport of specimens. *Acta Dermato-Venereologica*, **53**, 75-80.
- Danielsson, D., and Sandström, E. (1979). Serology of *Neisseria gonorrhoeae*: demonstration of strain-specific antigens by immunoelectrophoresis, immunofluorescence and co-agglutination techniques. *Acta Pathologica et Microbiologica Scandinavica*, **87B**, 55-64.
- Danielsson, D., Sandström, E., Kjellander, J., Moi, H., and Wallmark, G. (1978). Culture diagnosis of gonorrhoea: a comparison between two standard laboratory methods and a commercial gonococcal culture kit (Kvadricult). *Acta Dermato-Venereologica*, **58**, 69-73.
- Fraser, P. K., Bailey, G. K., Abbott, J. D., Gill, J. B., and Walker, D. J. C. (1973). The meningococcal carrier-rate. *Lancet*, **1**, 1235-1237.
- Gästrin, B., Kallings, L. O., and Marcetic, A. (1968). The survival time for different bacteria in various transport media. *Acta Pathologica et Microbiologica Scandinavica*, **74**, 371-380.
- Hoeffler, D. F. (1974). Recovery of *Neisseria meningitidis* from the nasopharynx. *American Journal of Diseases of Children*, **128**, 54-56.
- Holten, E., Bratlid, D., and Bøvre, K. (1978). Carriage of *Neisseria meningitidis* in a semi-isolated arctic community. *Scandinavian Journal of Infectious Diseases*, **10**, 36-40.
- Jacobson, J. A., Chester, T. J., and Fraser, D. W. (1977). An epidemic of disease due to serogroup B *Neisseria meningitidis* in Alabama: report of an investigation and community-wide prophylaxis with a sulfonamide. *Journal of Infectious Diseases*, **136**, 104-108.
- Kellogg, D. S., Jr., Peacock, W. L., Jr., Deacon, W. E., Brown, L., and Pirkle, C. I. (1963). *Neisseria gonorrhoeae* I. Virulence genetically linked to clonal variation. *Journal of Bacteriology*, **85**, 1274-1279.
- Martin, J. E., Jr., Peacock, W. L., Jr., and Thayer, J. D. (1965). Further studies with a selective medium for cultivating *Neisseria gonorrhoeae*. *British Journal of Venereal Diseases*, **41**, 199-201.
- Olcén, P., Danielsson, D., and Kjellander, J. (1978). Laboratory identification of pathogenic *Neisseria* with special regard to atypical strains: an evaluation of sugar degradation, immunofluorescence and co-agglutination tests. *Acta Pathologica et Microbiologica Scandinavica*, **86B**, 327-334.
- Sivonen, A., Renkonen, O. V., Weckström, P., Koskenvuo, K., Raunio, V., and Mäkelä, P. H. (1978). The effect of chemoprophylactic use of rifampin and minocycline on rates of carriage of *Neisseria meningitidis* in army recruits in Finland. *Journal of Infectious Diseases*, **137**, 238-244.
- Slaterus, K. W., Ruys, A. C., and Sieberg, I. G. (1963). Types of meningococci isolated from carriers and patients in a non-epidemic period in the Netherlands. *Antonie van Leeuwenhoek*, **29**, 265-271.
- Stuart, R. D. (1946). The diagnosis and control of gonorrhoea by bacteriological cultures. With a preliminary report on a new method for transporting clinical material. *Glasgow Medical Journal*, **27**, 131-142.
- Thayer, J. D., and Martin, J. E. (1964). A selective medium for the cultivation of *N. gonorrhoeae* and *N. meningitidis*. *Public Health Reports*, **79**, 49-57.

Requests for reprints to: Dr Per Olcén, Department of Clinical Microbiology, Central County Hospital, S-701 85 Örebro, Sweden.