Diagnosis and Incidence of *Neisseria gonorrhoeae* in Cape Coloured Females in the Western Cape

LABORATORY ASPECTS

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SUMMARY

Specimens were taken, using carbon-impregnated swabs, from the cervix, urethra and rectum of 945 Cape Coloured gynaecological patients, and from the cervix only of 1 276 pregnant Cape Coloured women. These specimens were submitted to the laboratory in a modified Stuart transport medium and cultured on Thayer-Martin medium. *Neisseria gonorrhoeae* was cultured in 5,3% of the specimens from the gynaecological patients and in 5,3% of specimens from the pregnant women. All cultures showed type I or II colony pattern. No strains showed resistance to any of the antibiotics tested.

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Although Leistikow first cultured *Neisseria gonorrhoeae* in 1882, this organism was for many years isolated only with difficulty on artificial culture media. Growth was rarely obtained unless the infected material was cultured immediately after the specimen was taken from the patient, and in the case of specimens taken from female patients, particularly, the additional problem of overgrowth by other organisms was frequently encountered.

According to Kraus and Yen[1] these methods detected only about 16% of suspected cases. After the use of selective antibiotics incorporated in the medium, and in particular the use of the medium devised by Thayer and Martin[2] in 1964, these difficulties were largely overcome. and Wende[3] was able to obtain 92% positive cultures from patients with clinical gonorrhoea. The problem of obtaining a growth of the gonococcus when delay had occurred between the taking of the specimen and the inoculation of the culture medium was overcome by the use of charcoal-impregnated swabs as recommended by Stokes,[4] and Stuart transport medium,[5] or by the use of Martin and Lester's Transgrow medium in which the gonococcus was not only transported but also grew during transportation.

The adoption of these techniques has revolutionised the laboratory diagnosis of gonorrhoea. During the past 18 months, we have used some of these methods in the Tygerberg Hospital and the results are described here. Patients from whom positive cultures were obtained have been treated, and follow-up cultures have been done.

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METHODS

Charcoal-impregnated swabs, prepared as described by Stokes,[4] were used. Three swabs, one each from the cervix, urethra and rectum of all gynaecological cases, were taken. In the case of obstetric patients, swabs were taken only from the cervix. The swabs, rolled on wooden sticks, were contained in glass test-tubes and immediately after the specimens were taken, plunged deeply into Stuart transport medium (Oxoid) which was contained to a depth of 4 - 5 cm in identical glass test-tubes.

After varying intervals, but usually within 6 hours after the specimen was taken, the swabs were plated out on a modified Thayer-Martin medium, the composition of which is as follows:

1. Base medium

Tryptone (Difco)	10 g
Proteose peptone (Oxoid)	20 g
Soluble starch (BDH)	2 g
K_2HPO_4	8 g
KH_2PO_4	2 g
NaCl	10 g
Agar No. 3 (Oxoid)	24 g
Distilled water	1 litre

2. Haemoglobin (Difco) 2% in distilled water
3. Supplements A and B (Difco)
4. VCN inhibitor.[6]

The medium was prepared by adding 250 ml of (2), 2,5 ml each of supplements A and B (3), and 5,0 ml of (4) to 250 ml of melted base (1).

Incubation was carried out in an atmosphere containing approximately 3% CO_2 (candle flame), for 48 hours at 35°C. After incubation the plates were examined and if suspicious colonies were present, these were tested with oxidase reagent (tetramethyl paraphenylenediamine). Colonies of *N. gonorrhoeae* were replated onto Thayer-Martin plates.

Pure cultures were tested for fermentation properties using sugars in cystine trypicase agar (BBL). Antibiotic sensitivity tests using the methods described by Garrod and Waterhouse[7] were carried out with the following antibiotics: penicillin, ampicillin, streptomycin, tetracycline,

erythromycin, lincomycin, gentamicin, cephalosporin, Nicene, carbenicillin, and trimethoprim-sulphamethoxazole. The antibiotic discs were placed on the surface of cultures grown on Wellcotest lysed blood agar. The antibiotic concentrations were those recommended by Garrod and Waterhouse.[7] Nicene was tested at a concentration of 25 μg/ml and trimethoprim-sulphamethoxazole at a concentration of 25 μg/ml.

All strains of *Neisseria* isolated, fermented glucose only, after incubation for 72 hours.

RESULTS

Swabs from 945 patients admitted to the gynaecological wards and from 1 276 obstetric patients were cultured. The results are shown in Table I.

TABLE I. *N. GONORRHOEAE* ISOLATIONS — DECEMBER 1972 TO JUNE 1973

Department	Total investigations	Total isolation	Percentage isolation
Gynaecology — urethral, cervical and rectal swabs	**945**	**50**	**5,3**
Obstetrics — cervical swabs	**1 276**	**68**	**5,3**
Total	**2 221**	**118**	**5,3**

It will be seen that from 5,3% of both the gynaecological and the obstetric patients, *N. gonorrhoeae* was cultured. All cultures showed colony types I or II, as described by Kellog *et al.*[8] and Jephcott and Reyn.[9]

Sensitivity tests, according to the paper disc method as described by Garrod and Waterhouse,[7] were carried out on all strains. None of the strains showed resistance to any of the 11 chemotherapeutic substances tested.

An attempt was made to follow up the patients from whom *N. gonorrhoeae* were isolated. These patients were treated as described by Hayward and after an interval of 5 - 16 weeks repeat swabs from 42 patients were examined. In each case swabs were taken from the cervix, urethra, rectum and throat. From 2 of the patients *N. gonorrhoeae* was isolated from the cervical swabs only.

DISCUSSION

It is of interest that our findings are in agreement with those of Kraus and Yen,[1] who examined cervical cultures from 1 309 antepartum patients in Cleveland, Ohio, USA, and discovered an incidence of *N. gonorrhoeae* of 5,73%. Of the patients examined 93% were non-White and none of them showed symptoms of gonorrhoea. The presence of *N. gonorrhoeae* in symptomless females was also reported by Harris *et al.*,[10] who took cultures from 213 female prisoners, and using a delayed fluorescent antibody method, detected *N. gonorrhoeae* in 20,6% who had no symptoms of *N. gonorrhoeae* infection.

The sensitivity tests carried out by us showed no strains of *N. gonorrhoeae* resistant to penicillin, streptomycin or any of the other 9 chemotherapeutic substances tested. This is at variance with the findings of Arya *et al.*[11] and Masawe *et al.*[12] in Uganda, the former finding 18% and the latter 20 - 30% of the local strains of *N. gonorrhoeae* resistant to penicillin *in vitro*. In many countries in Europe and also in the USA an increased resistance of *N. gonorrhoeae* to penicillin has been reported, while in Sweden Bergman and Tarnvick[13] have also reported an increase in resistance to streptomycin. In the USSR, Lurie and Kvassnaya[14] have reported increased resistance to tetracycline.

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