roundings, including treatment centres for the chronically ill. Samples analysed at the third laboratory were from the University Hospital with 1100 beds. The sampling area represented half of Finland; the samples were collected in March-May, 1981.

The bacteria were identified using routine microbial methods, including the API 20E procedure.³ The sensitivity of the bacteria was determined using sensitivity test discs (A/S Rosco, Denmark). The sensitivity test disc for trimethoprim contained 5.2 µg trimethoprim.²

Table 1 shows that the resistance of the bacteria to trimethoprim varied as follows: *E coli* 5-7%; *Klebsiella* sp 18-40%; *Proteus* sp 59-100%. Table 2 shows that the resistance to sulphatrimethoprim was lower than to trimethoprim alone. The resistance of the three pathogens to other antimicrobial agents was as follows (*E coli*; *Klebsiella* sp; *Proteus* sp): to nitrofurantoin 1-7%; 28-42%; 61-100%; to ampicillin 12-27%; 67-94%; 31-100%; and to cephalosporins 3-21%; 23-33%; 16-100%.

The results show that the resistance of the bacteria to all the antimicrobial agents

including trimethoprim varied at the three laboratories. In most cases the resistance rate was highest in Oulu. This reflects the problems of a university hospital without its own specialist microbiologists, but which treats patients with chronic UTI. Our results indicate that the increasing number of bacteria resistant to trimethoprim in Turku⁴ is a local problem and may be due to the intensive use of trimethoprim alone in the prophylaxis of UTI. In other parts of Finland trimethoprim is a safe alternative in the prophylaxis of UTI in open care, but it should not be used in institutions for the chronically ill.

*SIMO RÄISÄNEN

†NIILO KERÄNEN

‡HANNU SYRJÄLÄ

*Clinical Laboratory,

Savonlinna Central Hospital,

57120 Savonlinna 12,

†Rovaniemi Central Hospital,

‡Department of Medical Microbiology,
University of Oulu,
Finland

Table 1 Sensitivity of urine pathogens to trimethoprim in different parts of Finland

No of urine pathogens		Sensitivity of pathogens (%)			Source of pathogens
		S	I	R	
<i>E coli</i>	624	93	2	5	Savonlinna
	604	95	0	5	Rovaniemi
	200	91	2	7	Oulu
<i>Klebsiella</i> sp	69	73	9	18	Savonlinna
	65	60	0	40	Oulu
<i>Proteus</i> sp	49	29	8	63	Savonlinna
	70	41	0	59	Rovaniemi
	42	0	0	100	Oulu

S = Sensitive.

I = Insensitive.

R = Resistant.

Table 2 Sensitivity of urine pathogens to sulphatrimethoprim in different parts of Finland

No of urine pathogens		Sensitivity of pathogens (%)			Source of pathogens
		S	I	R	
<i>E coli</i>	624	93	3	4	Savonlinna
	604	96	1	3	Rovaniemi
	200	94	5	1	Oulu
<i>Klebsiella</i> sp	69	71	9	20	Savonlinna
	65	76	2	22	Oulu
<i>Proteus</i> sp	49	45	4	51	Savonlinna
	70	51	2	47	Rovaniemi
	42	4	0	96	Oulu

S = Sensitive.

I = Insensitive.

R = Resistant.

References

- ¹ Kasanen A, Anttila M, Elfving R, et al. Trimethoprim—pharmacology, antimicrobial activity and clinical use in urinary tract infections. *Ann Clin Res* 1978;10 suppl 22:1-39.
- ² Hamilton-Miller JMT, Gooding A, Brumfit W. Resistance to trimethoprim in 1978-79 compared with 1973-1975. *Clin Pathol* 1981;34:439-42.
- ³ Apparcils et procédures d'identification. Imprimerie nouvelle, 01300 Belley, France.
- ⁴ Huovinen P, Toivanen P. Trimethoprim resistance in Finland after five years' use of plain trimethoprim. *Br Med J* 1980;280:72-4.

Growth of *Neisseria Gonorrhoeae* at 25°C

In a previous report¹ it was recorded that contrary to general teaching, isolates of *Neisseria gonorrhoeae* grew well at temperature below 30°C. The organisms yielded heavy growth in a semi-solid medium incubated at 28°C but no growth was observed at 24°C. The observations made at this lower temperature (24°C) however, were rather cursory and the tubes were observed for only 3-4 days as a general control for contamination and for the inadvertent inclusion of *Neisseria* species which grow at temperatures at or below this level. Also it was not imagined that gonococci would grow at temperatures close to such a level but now it has been shown that many isolates do indeed grow slowly but profusely at a temperature of 25°C.

Thirty-five gonococcal strains were tested, several freshly isolated and the remainder from stored desiccates. Inocula for the tests were obtained from cultures grown in semi-solid agar incubated at 37°C.

The organisms were cultured on three media: semi-solid agar;¹ chocolate agar slopes; chocolate agar plates. Incubation was carried out in various constant temperature baths and cabinets. Slopes and semi-solid media were incubated in water baths (23°C, 25°C and 28°C) and the plates in cabinets (25°C and 28°C). All monitoring thermometers were calibrated against standard instruments. Plates were incubated in sealed containers but with no added carbon dioxide.

All isolates grew on plates, slopes and semi-solid media at 28°C, heavy growth being evident after 2-3 days. At 25°C

Letters to the Editor

approximately 80% of the isolates grew well in semi-solid media. Growth was evident after 2-4 days and reached a maximum in 5-8 days. On the plates and slopes at 25°C about 60% of the isolates produced visible colonies; these were evident in 3-4 days and reached a maximum in 5-8 days. With some of the isolates, the colonies grew to a size comparable to that attained at the higher temperature. With others smaller ones only were produced. Slopes and semi-solid media showed no growth at 23°C within 20 days of incubation. Plates were not tested at this temperature.

As yet no attempt has been made to culture gonococci at temperatures below 36°C direct from clinical material. It is apparent however, that once isolated a large proportion of strains with little or no "training" will grow well, albeit slowly, at a temperature as low as 25°C. Information in current texts on this characteristic of the species is therefore inaccurate and misleading.

DI ANNEAR
WB GRUBB

Department of Microbiology,
Royal Perth Hospital,
Perth, Western Australia
School of Medical Technology,
Western Australian Institute of Technology,
Bentley, Western Australia

Reference

- ¹ Annear DI. Growth of *Neisseria gonorrhoeae* in a simple medium at 28°C. *J Clin Pathol* 1981;34:688.

Growth of *Neisseria gonorrhoeae* in brain heart infusion

Although relatively complex liquid media are available for growing *Neisseria gonorrhoeae*^{1,2} a simply prepared, heat stable liquid medium would be welcomed by venereologists for a variety of general purposes. Attention was recently drawn to a simple semi-solid medium consisting of Brain Heart Infusion, agar and starch which supported growth of all 65 isolates of gonococci tested.³ Although this medium was very useful, its agar content was a disadvantage for some procedures such as those requiring centrifuged deposits of cells. Further work showed what gonococci grew well in unmodified Brain Heart Infusion (BHI) (Difco) but

that the physical conditions under which the organisms were grown greatly influenced the yield. Growth was remarkably increased by growing liquid cultures in shallow layers. While this was in part, undoubtedly due to the aeration provided it was also attributable to the heavy growth which occurred at the medium-vessel interface, an observation which does not appear to have been made previously.

Fifty isolates of gonococci, some freshly isolated and others from desiccates, were tested and found to grow well in BHI. Many of the isolates were tested in several batches of the same medium and all gave similar and satisfactory results. Inocula were usually taken from a semi-solid culture and the final concentration of organisms in the broth before incubation was approximately 10⁵ colony forming units (CFU)/ml.

In conventional tube cultures at 36°C, growth gradually occurred at the surface of the medium as it did in semi-solid media. On prolonged incubation and if left undisturbed, growth gradually progressed down the interface of medium and tube.

Shallow layer cultures were carried out in 200 ml flat-sided glass bottles. The depth of the medium was approximately 2-3 mm. In such cultures turbidity developed both in the free medium and at the interface of the medium and glass surface. During the early stages of incubation faint patches of turbidity developed at the interface before any clouding of the medium itself was obvious. As incubation continued the interface layer became quite dense and the medium above it turbid. Gentle shaking of the vessel at this stage released the loosely adherent interface layer and yielded a dense homogeneous suspension. As would be expected there was considerable variation between strains but even the lightest growth was reasonably turbid. Several cultures yielded counts of approximately 10⁸ CFU/ml within 24-48 h of incubation.

As would be expected from established work in this field, aeration of the cultures by shaking also resulted in copious growth. No additional CO₂ was supplied in these experiments as it was obvious that heavy growth was attainable without such a measure. Also relatively heavy inocula were used and no attempt was made to determine minimal inocula required to initiate growth.

It was somewhat paradoxical that in deep unagitated culture, the organisms had a strong predilection for growth at the

medium-air interface, whereas in shallow layers the preference was for growth at the medium-vessel interface at the bottom of the culture. Numerous factors including CO₂:O₂ ratios were probably important factors in determining growth responses. However, it appeared that the solid surface *per se* provided a stimulus for the proliferation of the organism.

Many formulations of gonococcal media include proteose peptone which is a basic constituent of BHI. Although the latter medium is recommended for growing "fastidious" organisms, it does not appear to have been used for growing gonococci except in blood culture media where its properties are unavoidably modified by addition of the blood inoculum itself. While it is probable that BHI could be improved by various measures such as addition of starch, haemin and yeast extract, the results here demonstrate that the unmodified medium is adequate for growing gonococci and should be useful for at least some purposes. Auxotrophic mutants would of course pose their own specific nutritional requirements.

If the full potential of BHI for work with gonococci is to be realised, manufacturers must ensure that the medium is consistently suitable for the purpose. It may well be rewarding to investigate other types of unmodified dehydrated media currently available for their ability to support the growth of gonococci. Whatever the medium used it would seem that the propensity of the gonococcus to flourish at the vessel-medium interface is a characteristic worth exploiting.

DI ANNEAR
BERYL WILD

Department of Microbiology,
Royal Perth Hospital,
Perth,
Western Australia

References

- ¹ MacFarlane DE, Elias-Jones TF. Improved media for the culture of *Neisseria gonorrhoeae*. *J Med Microbiol* 1980;13: 597-607.
- ² Soltesz LV, Mardh PA. Serum-free liquid medium for *Neisseria gonorrhoeae*. *Curr Microbiol* 1980;4:45-9.
- ³ Annear DI. Growth of *Neisseria gonorrhoeae* in a simple medium at 28°C. *J Clin Pathol* 1981;34:688.