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Bacteriuria in a Scottish island community. A comparison of chemical and cultural tests for bacteriuria applied in remote surroundings

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SUMMARY

Four hundred and thirty-eight overtly healthy adults on Tiree were screened for bacteriuria by dip-inoculum culture and a tetrazolium reductase test.

Dip-inoculum culture affords a simple and effective means of providing a service in quantitative urine bacteriology for communities remote from a laboratory.

The pattern of bacteriuria on Tiree is much the same as in other communities surveyed. Criteria are discussed for assessing the sensitivity of the tetrazolium test in terms of quantitative urine culture.

INTRODUCTION

In April 1969 a health screening team visited Tiree as part of a study of several distinctively different Scottish communities. Islanders attending the screening clinics were asked to bring with them a freshly voided sample of urine to be tested for protein and other abnormal constituents. This provided an opportunity to screen the population for bacteriuria and it was decided to compare the results of parallel tests by dip-inoculum culture, using the Mackey–Sandys spoon (Mackey & Sandys, 1965, 1966) with triphenyltetrazolium reduction (Simmons & Williams, 1962), using B.D.H. bacteriuria reagent.

METHODS

Dip-inoculum cultures were taken on plastic spoons (Medical Wire & Equipment Co., Potley, Corsham, Wilts.) charged with Oxoid brand CLED medium (Mackey and Sandys, 1966). This is an indicator-lactose medium with electrolyte content

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sufficiently low to prevent the spreading of *Proteus*. Cysteine is added to promote the growth of exacting coliforms. Sterile spoons were loaded with medium, placed in wide-mouthed one-ounce vials containing a moist pad of plastic foam and pasteurized in batches for 4 hr. at 70° C. in a thermostatic oven. At the screening clinics, cultures were prepared by momentarily dipping a culture-spoon in each urine sample. After dipping, the spoon was replaced at once in its vial to drain in the upright position onto the foam pad. The accumulated cultures were sent in three batches by air and rail to Bristol and thence by road to the laboratory at Bath. Each batch spent 4–5 days in transit at ambient temperatures prevailing in late April. After arrival at the laboratory the cultures were incubated overnight at 36° C. before being examined. Urine bacterial counts were estimated from the dip-spoon colony counts, using the established relationship that the urine count in organisms per ml. is 500 times the total surface viable count on the dip-spoon (Mackey & Sandys, 1965). Organisms were identified by their distinctive colonial appearances on CLED medium, supplemented when necessary by stained smears or further examination of subcultures. Drug sensitivity tests were done by a conventional disk-diffusion method.

Triphenyltetrazolium chloride (TTC) reductase tests were done on 2 ml. amounts of urine in tubes containing buffered TTC (British Drug Houses), incubated for 4 hr. in a thermostatic water bath at 37° C. A red precipitate of formazan indicates urine containing 100,000 organisms per ml. Tests were done by the island general practitioner in his surgery. Urine samples from the clinics were kept at 4° C. until tested.

RESULTS

Colony counts on dip-inoculum cultures are known to be unaffected by delays in transit of up to 48 hr. before incubation at 36° C. (Mackey & Sandys, 1965). In view of the extra delay between Tiree and Bath tests were made on the effect of storage for 5 days at 15° C. before incubating. Serial ten-fold dilutions were made in broth from cultures of selected pathogens. Replicate dip-inoculum cultures were made from each dilution over a range likely to include one producing between 10 and 50 colonies on each culture-spoon. A number of spoons were incubated at 36° C. immediately, while the remainder were held at 15° C. in a dark wine-cellar

Table 1. *Effect on D.I.C. spoon colony counts of storage at 15° C. before incubation overnight at 36° C.*

Organism	Mean dip-inoculum colony counts per spoon		
	Initial	After 48 hr. storage	After 5 days storage
E. coli	32	32	26
P. mirabilis	53	56	51
Klebsiella sp.	59	NT	54
Micrococcus sp.	14	NT	17

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for 2–5 days before being incubated. Table 1 shows that 5 days storage had no significant effect on the colony count.

Each dip-inoculum culture was placed in one of the following five assessment categories according to the kinds of organism present and the estimated urine bacterial count.

1. Obscured by contamination (heavy confluent growth of non-pathogens).
2. No significant bacteriuria (less than 10,000 organisms/ml.).
3. Active infection indicated (more than 100,000 potential pathogens/ml.).
4. Contamination rather than infection (mixed pathogens/non-pathogens totalling more than 10,000/ml.).
5. Equivocal viable count (between 10,000 and 100,000 potential pathogens/ml.).

Table 2 shows the distribution of TTC test results in relation to assessment by dip-inoculum culture. Potential primary pathogens are regarded as comprising *Escherichia coli*, lactose non-fermenting (LNF) coliforms, *Klebsiella* sp., *Proteus* sp., *Pseudomonas aeruginosa*, *Staphyloccocus aureus*, and *Micrococcus* sp.

Table 3 summarizes the bacteriological findings from 22 specimens with more than 100,000 potential pathogens per ml. It should be noted that both strains of lactose non-fermenting coliform required cysteine.

Two to three months after the screening survey a second dip-inoculum urine culture was obtained from each of the 22 subjects whose first sample showed more than 100,000 potential pathogens per ml., taking particular care that freshly taken urine was examined. In 15 instances the results of the second culture were substantially the same as the first, but in seven the second examination did not fully confirm the first. Table 4 sets out the actual extent of the discrepancies noted. Subjects 1, 3 and 7 showed only small differences within the range of variation to be expected. Two subjects (2 and 6) showed a distinct fall in the number of organisms present, but the infecting organism remained unchanged. One subject showed no evidence of significant bacteriuria at the second test, while the seventh produced a lactose non-fermenting coliform quite distinct from the initial proteus

Table 2. *Tetrazolium reductase results in relation to dip-inoculum culture assessment*

Dip-inoculum culture assessment	Total	Tetrazolium reductase test positive	negative
1. Obscured by contamination	23	18	5
2. No significant bacteriuria	374	8	366
3. Active infection indicated	22	18	4
4. Contamination rather than infection	14	7	7
5. Equivocal viable count	5	1	4

Table 3. *Organisms identified in urines containing more than 100 thousand potential pathogens per ml.*

Organism	Total cases
E. coli	17
Lactose non-fermenting coliform	2
Klebsiella sp.	2
Proteus sp.	1

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infection, and present in rather smaller numbers. Repeat cultures were also taken from 31 subjects randomly chosen from the group with negative initial urines. In all cases the second culture proved unequivocally negative.

Since the TTC test is designed to detect urines containing more than 100,000 organisms per ml., Table 5 has been constructed for all samples on which the dip-inoculum culture enabled the count to be assessed, discarding the results from 23 samples so heavily contaminated that bacteriological appraisal was impossible.

DISCUSSION

Experience on Tiree bears out the practical value of dip-inoculum culture as the means of providing a service in quantitative urine bacteriology for communities remote in distance or travelling-time from a bacteriological laboratory. Dixon & Clarke (1968) and Dixon (1970) have drawn attention to the value of the technique in Canada. Dip-inoculum cultures prepared in the laboratory from broth with known counts of pathogens have shown little change in viable count despite standing for periods of up to 5 days at ambient temperature before being incubated at 36° C.

The comparatively small population on Tiree limits the conclusions that can be drawn concerning the prevalence of bacteriuria among the islanders, but the

Table 4. *Discrepancies noted in seven replicate dip-inoculum cultures*

Case	Sex	Age	First DIC assessment	Second DIC assessment
1	F	74	*E. coli* 10^5/ml.	*E. coli* 40,000/ml.
2	F	57	*E. coli* 10^5/ml.	*E. coli* 6000/ml.
3	F	59	LNF coliform 10^5/ml.	LNF coliform 30,000/ml.
4	F	72	*Proteus* sp. 10^5/ml.	LNF coliform 20,000/ml.
5	M	72	*E. coli* 10^5/ml.	No significant bacteriuria
6	M	18	*E. coli* 10^5/ml.	*E. coli* 6000/ml.
7	M	81	*E. coli* 10^5/ml.	*E. coli* 70,000/ml.

Table 5. *Sensitivity of tetrazolium test in relation to urine colony count and DIC assessment*

		TTC		
DIC assessment	Bacterial count	Total	Pos.	Neg.
Active infection indicated	More than 100,000 pathogens/ml.	22	18	4
Contamination rather than infection	More than 100,000 organisms/ml.	10	4	6
	Total	32	22	10
No significant bacteriuria	Less than 10,000 organisms/ml.	374	8	366
Contamination rather than infection	Less than 100,000 organisms/ml.	4	2	2
Equivocal viable count	Between 10,000 and 100,000 pathogen/ml.	5	1	4
	Total	383	11	372

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general pattern resembles other reported surveys using dip-inoculum spoons (Grob, Manners & Dulake, 1970) and dip-slides (Heinonen *et al.* 1969). Asscher (1970) estimates that about 4% of women between 16 and 65 show significant bacteriuria. On Tiree there is the same marked preponderance of women over men and a general tendency for the condition to be commoner as age increases. Although the island population shows some excess of hypertension by comparison with the mainland communities (Hawthorne *et al.* 1969), the small numbers seen with bacteriuria permit no conclusion to be drawn on any possible association between hypertension and bacteriuria such as has been reported in larger studies (Asscher, 1970).

The organisms isolated in association with significant bacteriuria were representative potential urinary tract pathogens. It should be noted that both the strains of lactose non-fermenting coliform encountered were cysteine requiring, but produced colonies easily seen on CLED medium. This is specially formulated to include cysteine hydrochloride in view of reported association between urinary infection and cysteine-requiring coliforms, which produce only minute colonies on MacConkey agar and other traditional urine culture media (Gillespie, 1952). Discrepancies of the kind listed in Table 4 are to be expected when quantitative urine cultures are repeated. Even if attention is confined to subjects producing high counts of potential urinary pathogens, the possibility remains that these may occasionally appear as a consequence of accidental contamination rather than as evidence of active infection. It has also to be accepted that authentic active infection is not invariably attended on all occasions by high bacterial counts. Levy & Kass (1970) have proposed dealing with this source of uncertainty by examining two additional specimens following an initial positive sample. This

Table 6. *TTC sensitivity in detecting high pathogen counts*

(Specificity 0·82, 95% confidence limits lie between 0·60 and 0·95. Sensitivity 0·96. $J = 0{\cdot}6$ (S.E. 0·10).)

	TTC test		
Dip-inoculum culture assessment	Positive	Negative	Total
Active infection indicated	18	4 (18%)	22
No significant bacteriuria Contamination rather than infection Equivocal viable count	15 (4%)	378	393

Table 7. *Detection of high bacterial counts by TTC test, without regard to status as pathogens*

(Sensitivity 0·69. 95% confidence limits 0·53 to 0·85. Specificity 0·97. $J = 0{\cdot}64$ (S.E. 0·08).)

	TTC test		
Bacterial count	Positive	Negative	Total
More than 100,000 per ml.	22	10 (31%)	32
Less than 100,000 per ml.	11 (3%)	372	383

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was not attempted on Tiree. Whilst implying that estimates of incidence are open to some error, this does not affect the validity of comparisons between the TTC test and dip-inoculum culture when applied to the same urine sample.

The results from dip-inoculum culture can be applied in two ways for the appraisal of the TTC test, assessing efficiency at detecting urines with high counts of potential pathogens or by the detection of any urine with a high count whether of pathogens or commensals. In Table 6 the data from Table 5 are arranged to provide a comparison in relation to the detection of urines with more than 100,000 potential pathogens per ml. Among 22 such urines the TTC test detected 18 (82%). This may be compared with values ranging from 79% to 91% reported by other observers examining various population groups. (Bulger & Kirby, 1963; Deutch & Jesperson, 1964; Kincaid-Smith *et al.*, 1964; Pinkerton, Gibson & Houston, 1964; Simmons & Williams, 1962; Williams & Simmons, 1963; Williams, Leigh, Rosser & Brumfitt, 1964; Wright, 1968). However, this estimate is based on a small number of positive samples and has a wide 95% confidence interval, lying between 60% and 95%. Youden's Index (J), which takes account jointly of sensitivity and specificity, is 0·60 (S.E. 0·10). At the upper level of sensitivity the test would be satisfactory for survey purposes, but there could be drawbacks in using for diagnostic purposes a test which may miss up to 40% of positive samples. Moreover, like all other chemical tests for bacteriuria the TTC test must work in terms of a single threshold level. To the possible loss of a number of cases with significant bacteriuria must be added the likely loss of information about urines with counts in the equivocal range between 10,000 and 100,000 potential pathogens per ml. In the Tiree series five urines gave counts of this order, of which the TTC test detected only one.

The TTC test is probably not uniformly responsive to all organisms. Norden & Kass (1968) observed that a test able to detect 100,000 *E. coli* per ml. may fail to indicate the presence of *Ps. aeruginosa* even when in 10-fold greater numbers. Williams *et al.* (1965) noted that their form of the test detected only 22% of significant bacteriuria due to Gram-positive cocci. The Tiree series included 10 urines with high counts due to non-pathogens (mainly diphtheroids and saprophytic Gram negative bacilli) of which only five gave positive TTC reactions. In view of this Table 7 was prepared to show the results from tests on all urines with high bacterial counts, whether due to pathogens or not. Appraisal in these terms leads to much the same conclusion as one based only on high pathogen counts. The sensitivity of the test is 69% (95% confidence limits 0·53 to 0·85) while Youden's Index is 0·64 (S.E. 0·08), values which do not differ significantly from those of Table 6. It appears that specificity of the TTC test is satisfactory, but that sensitivity is certainly too low for diagnostic purposes and possibly also for screening surveys.

No practical difficulty arose from using dip-inoculum culture as a screening procedure. All the facilities of a fully staffed bacteriological laboratory were successfully deployed on examining specimens collected from a community 500 miles and 5 days travel away. The method offers the substantial advantages that bacteriuria can be related precisely to the organisms responsible, and its

degree can be estimated in terms of the actual bacterial count rather than by reference to a fixed threshold. Information can be obtained about urines with counts in the equivocal range. Since the survey a diagnostic service has been provided for the island general practitioner on the basis of dip-inoculum urine culture. The time taken in the screening clinics by the dip-inoculum technique is a matter of only a few seconds per patient. No further testing need be done in the field and no additional equipment is required.

The whole of the laboratory examination is carried out in the centre to which the cultures are despatched. The overall results from the dip-inoculum survey in Tiree were in good agreement with other surveys for bacteriuria in overtly healthy communities.

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