SURVIVAL OF DIFFERENT AUXOTYPES OF *NEISSERIA GONORRHOEAE* IN SIX COMMERCIAL TRANSPORT SYSTEMS

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BACKGROUND

We have previously demonstrated variability in survival of different auxotypes of *Neisseria gonorrhoeae* in a commercial transport medium.[1] Here we determine to what extent six commercial transport swab systems preserve different auxotypes of *N. gonorrhoeae*.

METHODS

Strains Six clinical isolates of *N. gonorrhoea* and two control strains, ATCC 43069 and NCTC 8375, were studied. The clinical strains included one non-requiring (NR), and one strain each with requirement for: proline (P^-); arginine (A^-); hypoxanthine (H^-); proline + arginine (P^-A^-), and arginine +hypoxanthine (A^-H^-); these were strains considered to have survival curves typical of their auxotype from the previous study.[1]

Swab products Six swabs from three manufacturers were evaluated: Copan Diagnostics 'M40 Transystem' with (CC) and without (C) charcoal; Medical Wire & Equipment 'Transwab' with (MC) and without (M) charcoal, and Starplex Scientific 'Starswab II' with (SC) and without (S) charcoal.

Swab inoculation Each strain was grown for 18h on chocolate agar (Oxoid, Basingstoke, UK) in 5% CO_2 at 37°C. A suspension was made inphosphate-buffered saline (PBS), and adjusted to 0.5 McFarland standard using a colorimeter; tenfold dilutions to 10^{-4} in PBS were prepared and, for each dilution, 50 µL was inoculated onto each of three chocolate agar plates using a spiral plater (Don Whitley, Shipley, UK) to quantify the initial inoculum. Each strain was tested against all swabs in a single experiment. For each strain, nine swabs of each product were inoculated, using a Gilson pipette, with 100 µL of the 10^{-1} dilution of the initial inoculum, to provide three swabs for sampling at each of times 0, 24 and 48h. The swabs for 24 and 48h sampling were kept at 22.5°C in a chilling incubator.

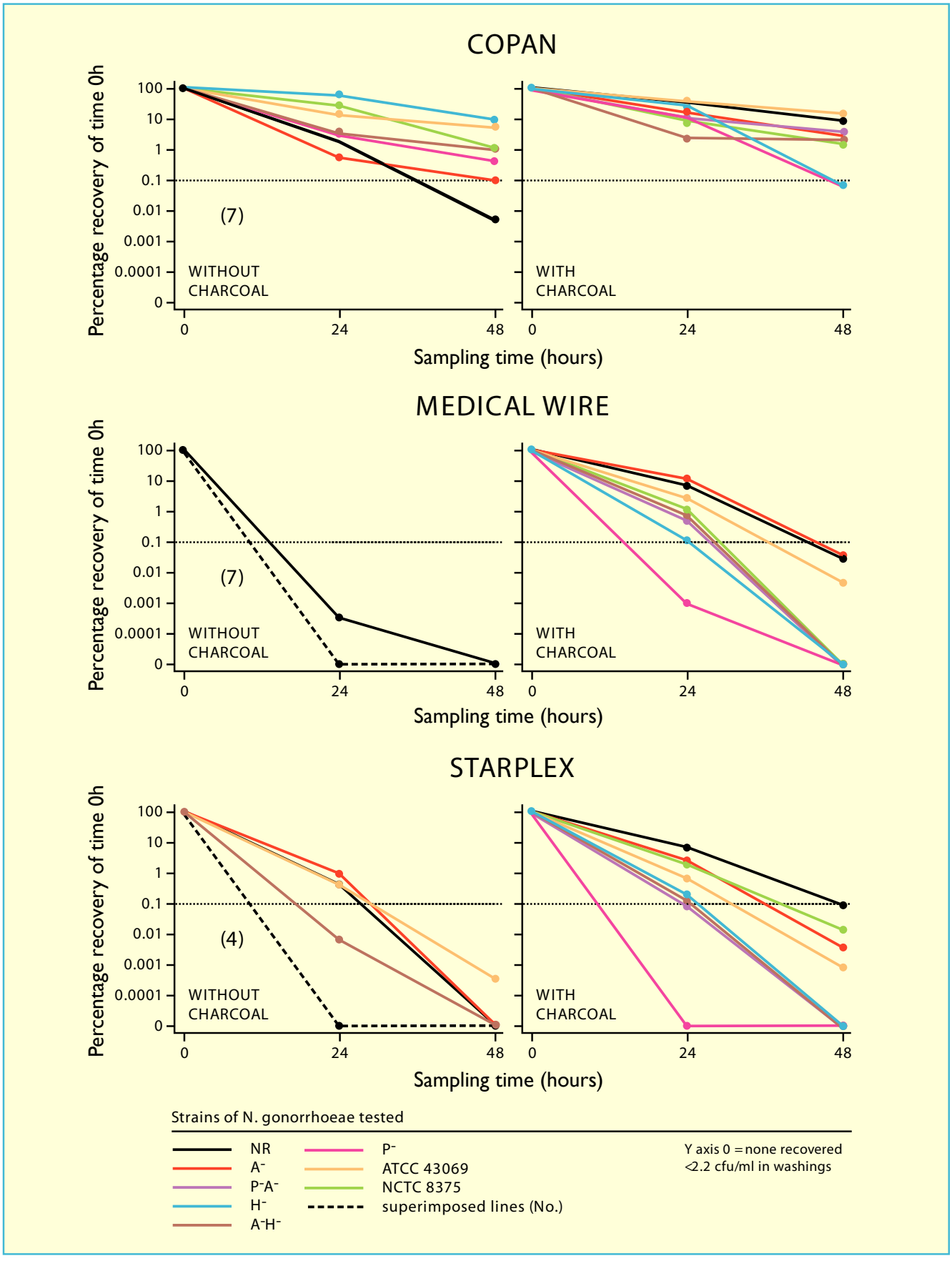
Vortex elution Time 0h samples were taken <15 min after inoculation. At each time point bacteria were recovered by vortexing the swab tips in 1mL of PBS for 15 seconds and expressing fluid from the swab. For each washing, serial tenfold dilutions to 10^{-3} were prepared and plated (50µL in triplicate for each dilution) on to chocolate agar using a spiral plater.

Presentation of results For each strain-product-time combination, the mean of the nine counts (three swabs x three plate counts) was taken and from this the percentage recovery of, and $\log_{10}$ reduction from, the time 0h count were calculated.

RESULTS

The initial inocula were between 1.82×10^7 and 1.15×10^8 cfu/mL, and time 0h counts between 2×10^4 and 4.55×10^5 cfu. Results are represented graphically (Fig) and summarized in the Table, applying the NCCLS acceptability 'cutoff' for ATCC 43069 at 24h ($3 \log_{10}$-10% reduction from time 0h) to all strains and both sampling times.

RESULTS


COPAN
Percentage recovery of time 0h
100
10
1
0.1
0.01
0.001
0.0001
0
(7)
WITHOUT CHARCOAL
WITH CHARCOAL
0
24
48
Sampling time (hours)
MEDICAL WIRE
Percentage recovery of time 0h
(7)
WITHOUT CHARCOAL
WITH CHARCOAL
Sampling time (hours)
STARPLEX
Percentage recovery of time 0h
(4)
WITHOUT CHARCOAL
WITH CHARCOAL
Sampling time (hours)
Strains of N. gonorrhoeae tested
NR
A⁻
P⁻A⁻
H⁻
A⁻H⁻
P⁻
ATCC 43069
NCTC 8375
superimposed lines (No.)
Y axis 0 = none recovered
<2.2 cfu/ml in washings

CONCLUSIONS

Essentially, the methods in this study were as per the NCCLS M-40 protocol[2] except that:

1) inocula were delivered to swabs using a pipette;
2) 48h samples were taken;
3) counts were performed in triplicate,
4) counts were performed using a spiral plater

With the exception of product M, all swab systems performed satisfactorily with respect to the NCCLS M40 standard (for ATCC 43069 at 24h). However, although the NCCLS standard provides a categorical evaluation, determining the percentage reduction from time 0 provides more information about a product's performance.

In vitro and clinical evaluations of transport medium for *Neisseria gonorrhoeae* reveal variation in survival of uncharacterised isolates within and between such studies (3-5). As in our previous study, auxotypes P^- and H^- were amongst the least robust. Not only were products C and CC the only ones to preserve all strains at 24h, they also showed good and comparable results at 48h. Although our results confirm the benefits of charcoal, at 24h the Copan M40 without charcoal performed better than other manufacturers' charcoal-containing products. Copan product C would be suitable for clinicians or laboratories preferring not to use charcoal-containing media.

ATCC 43069 proved to be a relatively robust strain. NCTC 8375 (used for quality control purposes in the UK) behaved similarly. A less hardy control strain, such as a P^- or H^-, would prove a more exacting standard.

As well as the risk of missing diagnoses, transport media incapable of preserving all gonococci could have epidemiological implications: less robust strains – and any characteristics associated with them - may be under-represented in a sampled population.

Manufacturer and product	Number of strains with acceptable[#] recovery at:	
	24 hours	48 hours
COPAN		
Amies	8* / 8	6* / 8
Amies + charcoal	8* / 8	6* / 8
MEDICAL WIRE		
Amies	0 / 8	0 / 8
Amies + charcoal	7* / 8	0 / 8
STARPLEX		
Amies	3* / 8	0 / 8
Amies + charcoal	6* / 8	0 / 8

[#] applying the NCCLS criteria for ATCC 43069 (≤ 3log10–10% reduction from time 0h count at 24h) at 24 and 48h

* includes ATCC 43069 = 'pass' at 24h

ACKNOWLEDGEMENTS

We thank Copan Diagnostics Inc for supporting this study.

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Produced by the Dept of Medical Illustration, HPA.
e-mail: cphlmedill@hpa.org.uk Ref: H797