Epidem. Inf. (1987), 99, 659–667
Printed in Great Britain

Typing of *Acinetobacter calcoaceticus* strains isolated from hospital patients by cell envelope protein profiles

By L. DIJKSHOORN, W. VAN VIANEN, J. E. DEGENER and M. F. MICHEL

Department of Clinical Microbiology, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands

(Accepted 10 July 1987)

SUMMARY

The usefulness of sodium dodecyl sulphate–polyacrylamide gel electrophoresis patterns of cell envelope proteins for classifying strains of *Acinetobacter calcoaceticus* was studied using 129 isolates from 16 in-patients in a teaching hospital. In 11 patients, all of the isolates from each patient exhibited the same pattern irrespective of the body site or time of isolation. The patterns of the isolates from four other patients were indistinguishable, with the exception of one isolate per patient. In the isolates from one patient five patterns were observed. In several cases isolates from different patients exhibited the same pattern. The relative frequency of some of these patterns was low. Epidemiological data were compatible with the assumption that the concurrent presence of bacteria of these patterns in the patients was the result of cross-infection. For one pattern, which was seen in seven patients, cross-infection could not be substantiated. On the basis of analysis of electrophoretic patterns in combination with epidemiological data on a number of strains it is concluded that cell-envelope protein profiles appear to be a useful aid in studying the dissemination of *Acinetobacter* in the hospital environment.

INTRODUCTION

In recent years several epidemic outbreaks of *Acinetobacter calcoaceticus* isolations have occurred in hospital departments (Holton, 1982; Stone & Das, 1985) and reliable typing systems are needed in order to be able to study the dissemination of *Acinetobacter* in such situations. Different methods of typing acinetobacters have been described, such as serotyping (Das & Ayliffe, 1984), bacteriocin typing (Andrews, 1986) and the combined use of biochemical tests and phage typing (Joly-Guillou *et al.* 1984).

The technique of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS–PAGE) of membrane proteins has been found to be a useful aid in distinguishing between bacterial strains (Loeb & Smith, 1980; Blaser *et al.* 1983). This method can also be used to establish identity or non-identity of strains of *Acinetobacter* (Alexander *et al.* 1984; Dijkshoorn, Michel & Degener, 1987). Studies of certain Gram-negative bacteria have shown, however, that intra-strain variation of SDS–PAGE patterns can occur. For example, individual strains of *Neisseria gonorrhoeae* can vary in pattern when isolated from different body sites in a patient or after transfer to another host (Zak *et al.* 1984).

In this study the use of SDS–PAGE cell-envelope protein patterns as an epidemiological marker was examined. To obtain an impression of the stability or instability of the expression of the proteins, the patterns of multiple isolates from a number of patients were compared. In analysing the patterns both the time of isolation and the location of the patients were taken into account.

MATERIALS AND METHODS

Microorganisms

A total of 129 isolates from 16 in-patients in a 1000-bed teaching hospital were studied. Clinical isolates were cultivated from samples which were sent to the bacteriological laboratory for clinical reasons at different points of time throughout the hospitalization period. If clinical samples were positive for *Acinetobacter*, epidemiological samples were taken from different body sites of the patient in question within a period of 1–2 weeks, using a standard procedure. Moist swabs were taken from the scalp, the forehead, the external auditory canal, the nose, the throat, the axilla, the palm, the groin, the rectum and the toe web. The swabs were shaken in a liquid medium consisting of an S-2 saline solution (Monod & Wollman, 1947) enriched with 0·2% sodium acetate (w/v). The media were incubated for 24 h at 30 °C in a shaking water bath. Subcultures of these media on blood agar and on MacConkey agar (CM7b, Oxoid) were studied for *Acinetobacter*. The bacteria were identified as *Acinetobacter* by conventional methods (Cowan, 1974) and divided into phenotypic varieties on the basis of the aerobic acidification of glucose and haemolysis on blood agar enriched with 5% sheep blood.

SDS–PAGE of cell-envelope proteins

The preparation of the cell envelope and subsequent SDS–PAGE were carried out essentially as described by Lugtenberg *et al.* (1975), with some minor modifications (Dijkshoorn, Michel & Degener, 1987). Briefly, cultivated cells were disrupted by ultrasonic treatments. Cell envelopes were isolated by differential centrifugation. SDS–PAGE was performed with a Protean Dual 16 cm slab cell apparatus (Bio-Rad, Richmond, California, USA). Discontinuous systems with a stacking and running gel of 3% and 11% acrylamide respectively were used. The gels were run at a constant current of 30 mA for the stacking gel and 35 mA for the running gel in approximately 3·5 h. The proteins were stained with Fast Green FCF (Sigma Chemical Co., St Louis, MO, USA).

The relative molecular mass (M_r) of the proteins was determined by comparing their electrophoretic mobility with that of the standard proteins phosphorylase B (97400 (97·4K)), bovine albumin (66K), ovalbumin (45K), glyceraldehyde-3-phosphate dehydrogenase subunit (36K), carbonic anhydrase (29K), trypsinogen (24K), trypsin inhibitor (20·1K) and lactalbumin (14·2K) (Sigma).

Classification of protein patterns

The patterns were classified by visual inspection on the basis of similarity. The primary criterion for distinguishing between the patterns was the most deeply stained protein band with an M_r in the 40–52K range. Other bands, for most patterns in the M_r range of 18–55K, were also evaluated. The patterns were coded

https://doi.org/10.1017/S0950268800066516 Published online by Cambridge University Press

Table 1. *Patients whose isolates were uniform in protein pattern*

Patient	Protein pattern*	Number of samples	Origin of samples†		Sampling period (range in days)
			Clinical (*n*)	Epidemiological	
I	A	8	sp	ea, no, th, ax, hp, gr, an	6
II	A	8	vg, ur	sc, fr, no, ax, hp, an	22
III	A	11	sp (2), td	fr, no, th, ax, hp, gr, an, to	13
IV	A	2	pu	to	10
V	B2	11	ea, lq(7)‡	sx, ax, gr	15
VI	G	5	ur (3)	th, gr	27
VII	H	10	um	fr, ea, no, th, ax, hp, gr, an, to	9
VIII	D3	7	ct, sp (2)	ax, hp, gr, to	35
IX	J	2	df	th	8
X	K	3	ur	th, an	8
XI	E5	4	wo (3), sp	—	46

* SDS–PAGE protein patterns of cell envelopes are shown in Fig. 2.

† Clinical samples: (*n*), number of multiple samples; sp, sputum; vg, vagina; ur, urine; td, thorax drain; pu, pus; ea, external auditory canal; lq, liquor cerebrospinalis; um, umbilicus; ct, tip of catheter; df, draining fluid; wo, wound; epidemiological samples; sc scalp; fr, forehead; no, nose; th, throat; ax, axilla; hp, palm of the hand; gr, groin; an, anus; to, toe web.

‡ One to three colonies from each sample were separately subcultured and investigated for protein pattern.

with capital letters. Patterns which were similar but not identical were designated by capital letters followed by a number.

RESULTS

Electrophoretic patterns of the isolates

The first study was to determine whether multiple isolates from individual patients exhibited variations in their protein patterns. All the isolates from the different patients were then compared and classified. Uniform patterns per patient were found for 11 of the 16 patients (Table 1). The patterns of four isolates from each of patients III, VII and VIII are shown in Fig. 1. The profiles of patients XII–XV were indistinguishable with the exception of one protein pattern (Table 2). Five different patterns were found in the isolates of patient XVI.

The patterns observed in the present study (Fig. 2) were compared with the patterns A, B1–4, C, D1–3, E1–4 and F described in a previous report (Dijkshoorn, Michel & Degener, 1987). In a number of cases, patterns in the present study were found to be concordant with patterns described previously. For example, it can be seen in lanes 5 and 6 of Fig. 2 that the pattern of an isolate from patient III corresponds to pattern A as defined in the earlier study. Similarly, the profiles of lanes 3, 4, 11, 12 and 13 in Fig. 2 could be classified as patterns B2, B1, D3, D1 and E5. Patterns not previously observed were coded with the letters G–P. Patterns M, G, B2, B1, A and L differed from one another

https://doi.org/10.1017/S0950268800066516 Published online by Cambridge University Press

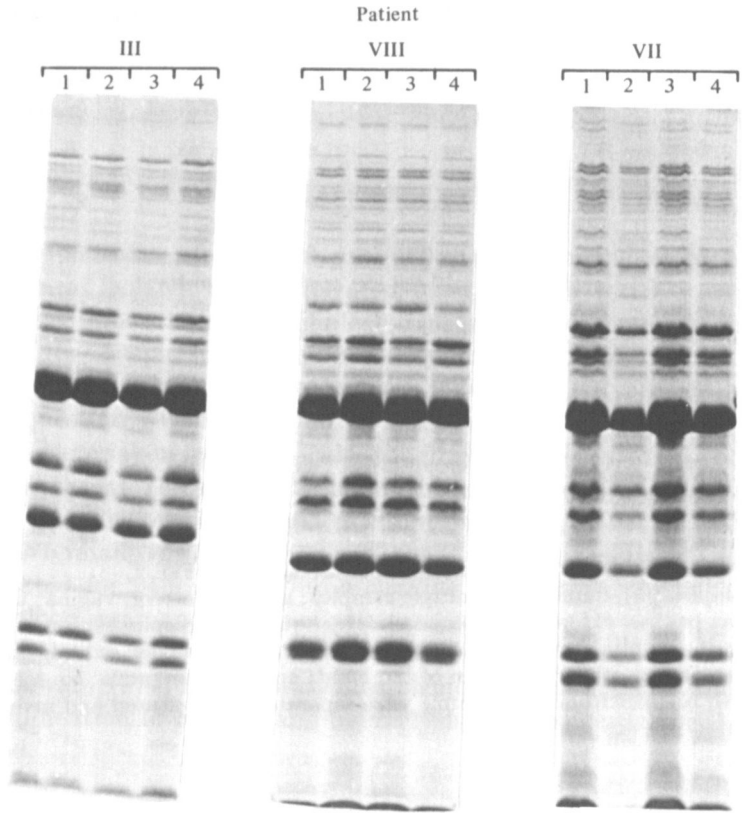

Fig. 1. SDS–PAGE cell-envelope protein patterns of multiple isolates of *A. calcoaceticus* from three patients. Patient III: 1, sputum; 2, thorax drain; 3, sputum of a later date; 4, forehead. Patient VIII: 1, axilla; 2, toe web; 3, venous catheter; 4, sputum. Patient VII: 1, umbilicus; 2, forehead; 3, external auditory canal; 4, anus.

in minor protein bands of $M_r > 45$K. In the case of patterns L, J, N, H, D and E5, differences were also observed in minor protein bands of M_r 18–34K. Patterns P, K and O were distinguished by major protein bands with an M_r range of 43–52K and also by various minor protein bands.

Phenotypic varieties

Like the previously isolated acinetobacters with the patterns designated as E1–4, the isolates of pattern E5 acidified glucose and were haemolytic. The acinetobacters of patterns O and P were asaccharolytic and non-haemolytic. The acinetobacters of the other patterns were saccharolytic and non-haemolytic.

Variation and non-variation of protein patterns of isolates from individual patients

The isolates of the patients with uniform patterns, patients I–XI (Table 1), were cultivated from a variety of samples. In most of the patients, after primary isolation from clinical samples the bacteria were also cultivated from various parts of the skin, from the nose and throat and from anal swabs. Positive samples were obtained over a period varying from 6–46 days.

https://doi.org/10.1017/S0950268800066516 Published online by Cambridge University Press

Table 2. *Patients whose isolates were assigned to different patterns*

Patient	Protein pattern*	Number of samples	Origin of samples†		Sampled on day‡
			Clinical (*n*)	Epidemiological	
XII	A	2	wo	—	1,15
	D1	1	—	an	43
XIII	G	6	sp	sc, no, th, gr, to	1, 7
	A	1	—	an	7
XIV	L	7	wf (4), ur, ab	an	1, 8, 10, 17
	O	1	—	sc	8
XV	H	15	um	fr (2), ea (2), no, th, ax, hp (3), gr, an, to (2)	1, 4, 9
	P	1	—	ax	9
XVI	A	1	ur	—	ba
	M	1	wf	—	ba
	B1	10	sp (3)	sc, fr, ea, no, th, an, to	1, 3, 10, 21
	L	11	bl (6), al, dr	ax, hp, gr	ba, 1, 11, 16, 17, 18
	N	1	bl	—	17

* SDS–PAGE protein patterns of cell envelopes are shown in Fig. 1.

† See corresponding notes to Table 1 ; ab, abdomen; bl, blood; al, arterial line; dr, drain; wf, wound fluid.

‡ Samples were obtained on several days. The numbers indicate the day on which samples were obtained; ba, recovered from clinical samples in another hospital before admission.

In order to investigate whether the intra-patient variation observed in patients XII–XVI (Table 2) could be connected with the presence of different strains, data on the origin, the protein pattern and other phenotypic characteristics of the isolates was compared. In the case of patient XII acinetobacters of pattern A were isolated, twice from the fluid of a leg wound, at an interval of 15 days. The deviant isolate with pattern D1 was isolated 28 days after the last wound culture from an anal swab. The bacteria with the deviant patterns O and P in patients XIV and XV (Table 2) were isolated from the skin. These isolates deviated from the others not only in protein pattern but also in their inability to acidify glucose.

Identical patterns found in isolates from different patients

The occurrence of common patterns (A, G, H and L) in acinetobacters isolated from different patients (Tables 1 and 2) could be the result of cross-contamination or contamination from common sources. The hospital records were therefore examined to discover whether patients with isolates with the same pattern had been nursed in the same ward in the same period. Four such cases were found. Acinetobacters of pattern A were isolated successively in patients I, II and XII (Fig. 3). The patients had been nursed in several surgical intensive-care units (ICUs). For a short time patients I and II were together in the surgical ICU I. A few days later patient XII spent a day together with patient II in the surgical ICU I. Though cross-infection between the patients may have taken place, this cannot

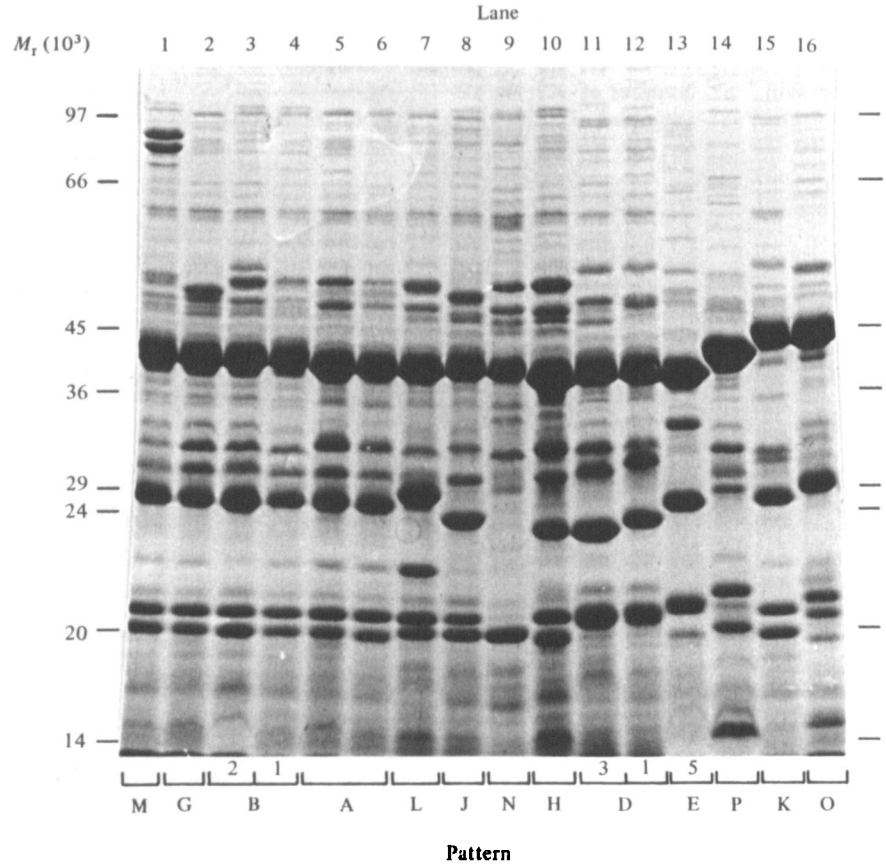

Fig. 2. SDS–PAGE patterns according to which the isolates of *A. calcoaceticus* were classified. The samples were arranged on the gel on the basis of their similarity. The lane 6 isolate was classified in a previous study as pattern type A. The others are described in this study. M_r, relative molecular mass of calibration proteins.

be proved, as the relative frequency of pattern A was rather high (7 cases out of 16 patients, Tables 1 and 2). Acinetobacters of pattern G were cultivated from various samples from patients VI and XIII, who spent 2 weeks together in the surgical ICU II. In patients XIV and XVI, who were some time together in the same ICU, acinetobacters of pattern L were found. The patterns G and L were exclusively observed in these patients and had not been detected in the hospital before. On the basis of epidemiological data and the low frequency of these patterns it is suggested that cross-infection between the patients took place.

Acinetobacters of pattern H were cultivated from the navels of twin brothers, patients VII and XV, immediately after birth (Fig. 4). Later the bacteria were cultivated from epidemiological samples from various skin and mucous membrane surfaces. The twins were probably colonized from a common source immediately after birth.

DISCUSSION

Variation in the expression of cell-envelope proteins could be of clinical importance, because it may enable bacteria to escape from immunological surveillance (Sparling, Cannon & So, 1986). If acinetobacters were to display such

https://doi.org/10.1017/S0950268800066516 Published online by Cambridge University Press

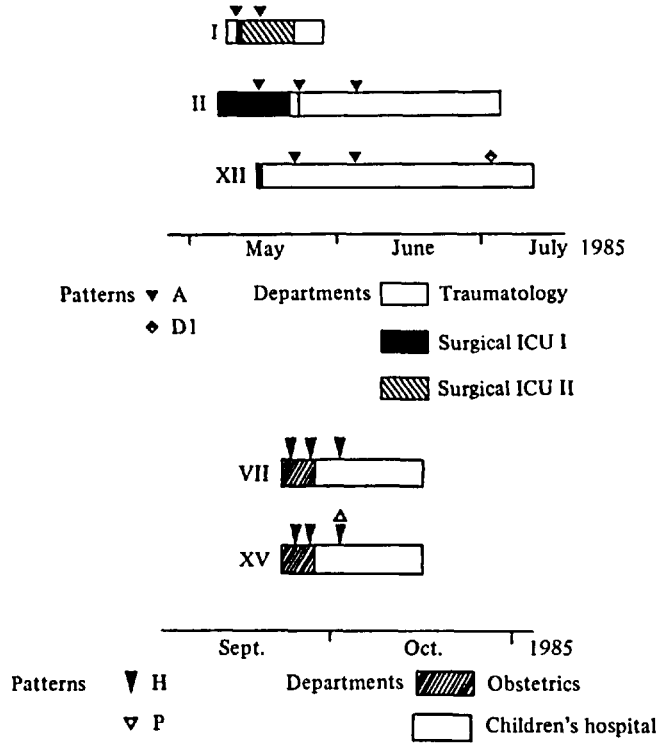

Fig. 3–4. Times and places at which *A. calcoaceticus* was isolated from patients. Only one of various concurrent isolates of a given pattern from a patient is shown. Patients are indicated by Roman numerals. Further details are given in Tables 1 and 2.

a variation, the cell-envelope protein patterns of the bacteria would not be usable as epidemiological markers. To investigate the variation or non-variation of the protein patterns multiple isolates of acinetobacters from 16 patients were studied. For the majority of patients the isolates were indistinguishable. Some instances of intra-patient variation seemed to be connected with the concurrent presence of different strains (patients XII, XIV and XV). These findings suggest that for strains of *Acinetobacter calcoaceticus* the variability of expression of cell-envelope proteins over a limited period is low.

Acinetobacters of the same pattern were isolated from patients who had been nursed in the same unit in the same period. The presence of strains of rare patterns in several patients was suggestive for cross-infection. One pattern (pattern A) was observed in 7 of 16 patients and also in a previous investigation in the same hospital. Although isolation of acinetobacters of this pattern may have been the result of cross-infection, it may also have reflected the general dissemination of the microorganisms in the hospital. Acinetobacters are widespread in nature (Henriksen, 1973). A number of epidemic increases of isolations of *A. calcoaceticus* in hospitals have been described (Holton, 1982; Stone & Das, 1985). For preventive purposes it is important to determine the routes by which the patients acquire the bacteria in these situations and the sites of the body which are colonized. Recently, Allen & Green (1987) reported the isolation of acinetobacters from patients and from the environment during an epidemic. Although for most

https://doi.org/10.1017/S0950268800066516 Published online by Cambridge University Press

isolates only the antibiogram was used as an epidemiological marker, it seemed that the bacteria were spread by air.

The patients in our study were sampled for acinetobacter for epidemiological reasons following primary isolation of the microorganism from one or more clinical samples. The simultaneous recovery of identical bacteria from various skin and mucous membrane samples from various patients suggests that large areas of the external surfaces may be colonized by acinetobacters for days to weeks. Longitudinal studies of patients are desirable to obtain detailed knowledge of the primary site and the chronological course of colonization by acinetobacters.

On the basis of the foregoing, SDS–PAGE profiles of cell-envelope proteins appear to be a useful aid to studying the epidemiology of *A. calcoaceticus*. As patterns of unrelated strains have been found to be heterogeneous (Dijkshoorn, Michel & Degener, 1987), the presence of common patterns in several patients could be indicative for cross-infection or for increased occurrence in the hospital. For the detection of epidemiologically relevant strains in single isolates, however, more needs to be known about the dissemination of particular SDS–PAGE types of acinetobacters both inside and outside the hospital environment.

This study was financed by the 'Praeventiefonds' of the Netherlands, grant no. 28-1070. We thank Miss S. Schmidt for typing the script.

REFERENCES

ALEXANDER, M., ISMAIL, F., JACKMAN, P. J. H. & NOBLE, W. C. (1984). Fingerprinting *Acinetobacter* strains from clinical sources by numerical analysis of electrophoretic protein patterns. *Journal of Medical Microbiology* **18**, 55–64.

ALLEN, K. D. & GREEN, H. T. (1987). Hospital outbreak of multi-resistant *Acinetobacter anitratus*: an airborne mode of spread? *Journal of Hospital Infection* **9**, 110–119.

ANDREWS, H. J. (1986). *Acinetobacter* bacteriocin typing. *Journal of Hospital Infection* **7**, 169–175.

BLASER, M. J., HOPKINS, J. A., BERKA, R. M., VASIL, M. L. & WANG, W-L. (1983). Identification and characterization of *Campylobacter jejuni* outer membrane proteins. *Infection and Immunity* **42**, 276–284.

COWAN, S. T. (1974). *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 2nd edn. Cambridge: Cambridge University Press.

DAS, B. C. & AYLIFFE, G. A. J. (1984). Serotyping of *Acinetobacter calcoaceticus*. *Journal of Clinical Pathology* **37**, 1388–1391.

DIJKSHOORN, L., MICHEL, M. F. & DEGENER, J. E. (1987). Cell envelope protein profiles of *Acinetobacter calcoaceticus* strains isolated in hospitals. *Journal of Medical Microbiology* **23**, 313–319.

HENRIKSEN, S. D. (1973). *Moraxella*, *Acinetobacter*, and the *Mimeae*. *Bacteriological Reviews* **37**, 522–561.

HOLTON, J. (1982). A report of a further hospital outbreak caused by a multi-resistant *Acinetobacter anitratus*. *Journal of Hospital Infection* **3**, 305–309.

JOLY-GUILLOU, M. L., BERGOGNE-BÉRÉZIN, E., LABAN, P. & GAYRAL, J. P. (1984). Étude bioenzymatique et lysotypique de deux cens souches d'*Acinetobacter calcoaceticus*. *Pathologie Biologie* **32**, 177–181.

LOEB, M. R. & SMITH, D. H. (1980). Outer membrane protein composition in disease isolates of *Haemophilus influenzae*: pathogenic and epidemiological implications. *Infection and Immunity* **30**, 709–717.

LUGTENBERG, B., MEIJERS, J., PETERS, R., VAN DER HOEK, P. & VAN ALPHEN, L. (1975). Electrophoretic resolution of the 'major outer membrane protein' of *Escherichia coli* K12 into four bands. *FEBS Letters* **58**, 254–258.

MONOD, J. & WOLLMAN, E. (1947). l'Inhibition de la croissance et de l'adaptation enzymatique chez les bactéries infectées par le bactériophage. *Annales de l'Institut Pasteur* **73**, 937–956.

SPARLING, P. F., CANNON, J. G. & SO, M. (1986). Phase and antigenic variation of pili and outer membrane protein II of *Neisseria gonorrhoeae*. *Journal of Infectious Diseases* **153**, 196–201.

STONE, J. W. & DAS, B. C. (1985). Investigation of an outbreak of infection with *Acinetobacter calcoaceticus* in a special care baby unit. *Journal of Hospital Infection* **6**, 42–48.

ZAK, K., DIAZ, J.-L., JACKSON, D. & HECKELS, J. E. (1984). Antigenic variation during infection with *Neisseria gonorrhoeae*: detection of antibodies to surface proteins in sera of patients with gonorrhea. *Journal of Infectious Diseases* **149**, 166–174.