J. Hyg., Camb. (1986), 97, 255–263
Printed in Great Britain

Plasmids in group JK coryneform bacteria isolated in a single hospital

By S. M. KERRY-WILLIAMS* and W. C. NOBLE

Department of Bacteriology, Institute of Dermatology, Homerton Grove, London E9 6BX

(*Received 19 May 1986; accepted 6 June 1986*)

SUMMARY

Investigation of 39 JK-type coryneform isolates from patients at a single hospital revealed that 23 possessed plasmids, which formed six groups on restriction endonuclease analysis. Four of the groups were associated with production of similar bacteriocin-like substances, and shared a minimum of 6·4 kilobase pairs of DNA. These plasmids, found in isolates from different patients, provide strong direct evidence that person-to-person transmission of JK bacteria had occurred within the hospital.

INTRODUCTION

The JK group of coryneform bacteria has been characterized as an important cause of infection in immunosuppressed patients (Riley *et al.* 1979; Stamm *et al.* 1979). These organisms, which are typically multiply antibiotic-resistant, are often present on the skin of susceptible patients, but are rarely isolated from normal individuals (Stamm *et al.* 1979; Gill *et al.* 1981; Tompkins, Juffali & Stamm, 1982). Stamm *et al.* (1979) reported that colonization was hospital-acquired in some individuals, and suggested that this was due to cross-infection, as patients in laminar airflow rooms had lower rates of JK coryneform bacteraemia. An alternative possibility was also suggested: that antibiotic therapy selects multi-resistant JK strains which are present in low numbers as part of the normal skin flora.

The former hypothesis is supported by a recent investigation by Quinn *et al.* (1984) of an outbreak of JK infections. These authors showed that not only were a large proportion of patients in a haematology ward colonized, but that environmental surfaces, air, and hands of hospital personnel were contaminated with these organisms. Empirical infection control measures were effective in halting the outbreak, despite continuing colonization of the patients.

Epidemiological studies of JK coryneforms are difficult because there is no reliable means of distinguishing between strains (Riley *et al.* 1979). Several studies have failed to reveal plasmids (Stamm *et al.* 1979; Young *et al.* 1981; Quinn *et al.* 1984), but we have recently reported a plasmid associated with production of a

* Present address: Department of Microbiology, The Medical School, University of Bristol, Bristol BS8 1TD.

bacteriocin-like substance (BLS) in a JK-type isolate from the Royal Marsden Hospital, Surrey (Kerry-Williams & Noble, 1984).

In this study the plasmid profiles and degree of relatedness of plasmids in JK isolates from this hospital were investigated.

MATERIALS AND METHODS

Bacterial strains

Thirty-nine multiresistant coryneforms isolated from patients were received from the Royal Marsden Hospital (RMH). Sites from which strains were isolated were: blood cultures (14 isolates), vagina (9), axillae (5), toes (3), intravenous catheters (2) and groin (1). Site of isolation and source patient were unknown for five isolates obtained before 15 October 1981. The isolates were taken from at least 24 different patients, all of whom were suffering from soft cancers, and the majority of whom had received bone marrow transplants. Data available on 22 of the patients indicated that all were receiving immunosuppressive therapy, and 19 were also being treated with broad-spectrum antibiotics. It is noteworthy that despite the high proportion of isolates from blood culture, only three patients had illnesses which could be attributed to infection with a JK-type coryneform. These were one case of encephalitis of unknown cause and two cases of pyrexia, each coincident with the isolation of JKs from blood cultures.

The remainder of the patients either had no symptom of infection or suffered from infections typical of immunocompromised individuals, including Gram-negative and streptococcal septicaemia and disseminated candidosis.

Information on periods of hospitalization of patients from whom strains were isolated and the underlying illnesses and treatment of patients were provided by Drs R. Lewis and B. Jameson of the Royal Marsden Hospital.

C433, a BLS-sensitive (BLS^s) isolate, and C483, a BLS-producing (BLS^+) isolate (both from RMH) have been described previously as RM062 and K411 respectively (Kerry-Williams & Noble, 1984). C483 has also been deposited with the National Collection of Type Cultures, as NCTC 11915.

Many of the strains used in this study were also included in a study of coryneform taxonomy by Jackman & Pelczynska, 1986.

Two hundred and thirty coryneform strains were obtained from sources other than RMH in the course of larger study of plasmids in coryneform bacteria, and 46 strains possessed extrachromosomal DNA (Kerry-Williams, 1985).

Curing. Plasmid curing was attempted by inoculating one colony of the desired strain into 10 ml CB broth containing 0·4 μg/ml ethidium bromide and incubating overnight at 42 °C. Cultures were then diluted and plated on CA medium to give single colonies, which were tested for loss of BLS production by replica plating on to CA medium inoculated with a lawn of C433. BLS^- isolates failed to give a zone of inhibition of the BLS^s lawn.

General methods. The media is used, and the methods used for determination of BLS production and sensitivity, preparation of plasmid DNA, and agarose gel electrophoresis, were as described previously (Kerry-Williams & Noble, 1984). Antibiotic susceptibility testing was by Oxoid disc diffusion on CA medium.

Restriction endonuclease digestion. Restriction endonuclease *Bam* HI was used

in single digests according to the manufacturer's instructions. *Eco* RI and *Hind* III (Sigma Chemical Company Ltd, Poole, Dorset) were used in single and double digests according to the manufacturer's instructions, except that Core buffer (Bethesda Research Laboratories, Cambridge, UK) was used instead of the specific buffers. Bacteriophage lambda DNA was separately digested with either *Hind* III or *Eco* RI alone, or with *Eco* RI plus *Hind* III to give molecular weight markers. Fragment sizes were determined by the method of Sealey & Southern (1982).

RESULTS

Characteristics of strains

The 39 RMH isolates were all multiresistant. Antibiotic resistance was as follows: penicillin G, 38 isolates; neomycin, 39; gentamicin, 39; erythromycin, 39; tetracycline, 12; clindamycin, 39; chloramphenicol, 34; fusidic acid, 27; and novobiocin, 0.

Jackman & Pelczynska (1986) compared the whole cell protein patterns of 27 RMH isolates with those of authentic JK strains from the Centers for Disease Control, Atlanta, USA. The patterns were extremely similar, both to each other and to those of CDC JKs, indicating that RMH isolates were indeed JK types. A further four RMH isolates have been confirmed as JKs on the same basis (S. Pelczynska, personal communication). The 12 untested isolates from the same source possessed very similar resistance patterns, morphology and culture characteristics; so these are presumed also to be JKs.

Production of a bacteriocin-like substance

All RMH isolates were tested for production of a BLS active against C433, a sensitive strain. Nineteen isolates produced a BLS; 3 of the 20 non-producers were BLS-resistant (BLS^r), and the remainder BLS^s, although 2 of the latter showed very limited sensitivity.

Plasmid comparisons

All RMH isolates were screened for plasmids: 23 were found to contain extrachromosomal DNA, with no more than one plasmid in each. All BLS producers possessed a plasmid, and only four non-producers possessed a plasmid; these were C429, C433, C440 and C442.

Comparison of the plasmids by restriction endonuclease cleavage after purification by CsCl–ethidium bromide gradient centrifugation revealed that they fell into six groups (Table 1) (pKW23, in C206, was from a different hospital), a group being defined as plasmids with indistinguishable restriction digest patterns. One plasmid, of about 4·6 kb, was lost from its host strain, C440, and could not be compared with the other plasmids.

The groups characterized by pKW4, pKW10, pKW15 and pKW37 appeared to be correlated with BLS production, in that all isolates with these plasmid-types were BLS producers (BLS^+) and all producers possessed these types of plasmid. These plasmids had restriction fragments in common (Fig. 1, Table 2).

pKW4 was the least similar to the other plasmids, possessing only the minimum

Table 1. *Plasmid groups*

Prototype plasmid	Size (kb)	Strains	BLS production
pKW4	13·4	C483	+
		K410*	+
		C204	+
		C434	+
		C438	+
		C439	+
		C487	+
		C489†	+
		C490†	+
		C491†	+
		C508†	+
pKW6	9·0	C433*	−
		C422	−
		C429‡	−
		C512	−
pKW10	16·3	C441	+
		C475	+
		C477§	+
		C485	+
		C488	+
		C492	+
pKW15	19·9	C476	+
		C479	+
pKW16	4·5	C442	−
pKW23	19·9	C206	+
pKW37	18·1	K488§	+

Unless otherwise indicated, strains were isolated from different individuals. Sizes were determined by summation of the constitutive fragments, and are weighted averages including data from *Bam* HI digestions (Kerry-Williams, 1985).

* Isolated in mixed culture from the same patient.

† Isolated from the same patient.

‡ Isolated from the same patient as those marked '*'.

§ Isolated in mixed culture from the same patient.

similarities to pKW10, pKW15 and pKW37, which were more extensively related. Indeed the only difference between pKW10 and pKW37 was, in the *Eco* RI digests, the loss of pKW10 fragment C and the gain of two new fragments in pKW37; and in the *Hind* III digest, an increase in size of fragment A in pKW37 (Fig. 2). This is best explained as a single deletion/insertion of a segment containing an *Eco* RI site.

In contrast, neither pKW6 nor pKW16 from BLS^- strains possessed restriction fragments in common with the other plasmids (Kerry-Williams, 1985).

Restriction endonuclease maps of the BLS-associated plasmids were constructed using standard procedures (Kerry-Williams, 1985). The data were consistent with the common fragments forming large contiguous segments, which are shown on the map of pKW10 (Fig. 2). The map shows that it is theoretically possible for each of the other BLS-associated plasmids to have been derived from pKW10 by a single recombinational event.

Curing

Curing experiments have previously demonstrated a direct correlation between pKW4 and BLS production (Kerry-Williams & Noble, 1984): loss of pKW4

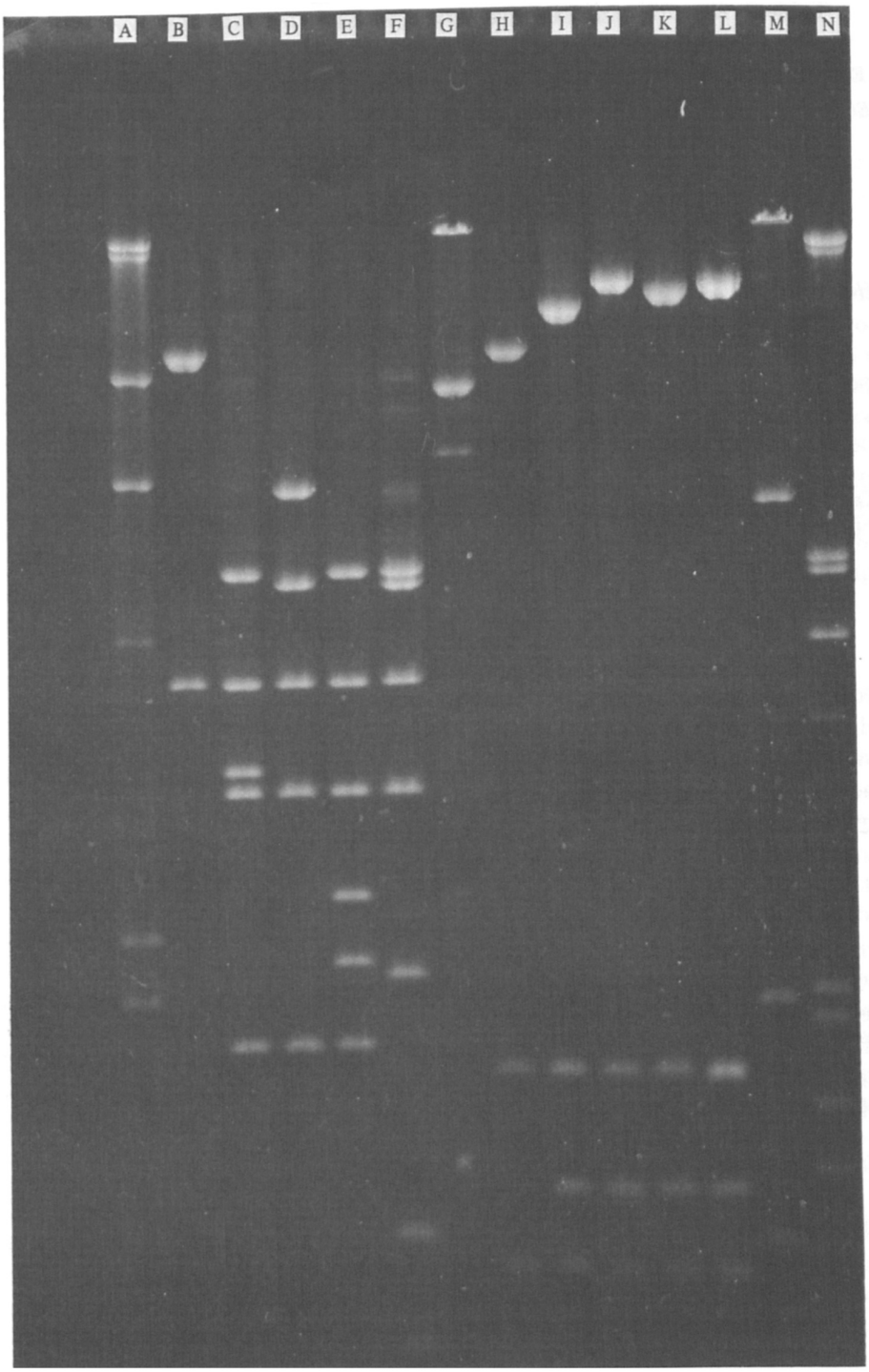


Fig. 1. Restriction endonuclease digestion of RMH plasmids. B–G, *Eco* RI digestions; H–M, *Hind* III digestions. A, lambda + *Hind* III (fragments of sizes 23·1, 9·42, 6·68, 4·36, 2·32 and 2·03 kb); B & H, pKW4; C & I, pKW10; D & J, pKW15; E & K, pKW37; F & L, pKW23; G & M, pKW6, N, lambda + *Eco* RI + *Hind* III (fragments of sizes 21·2, 5·15, 4·97, 4·28, 3·53, 2·03, 1·90, 1·58, 1·33, 0·98 and 0·83 kb).

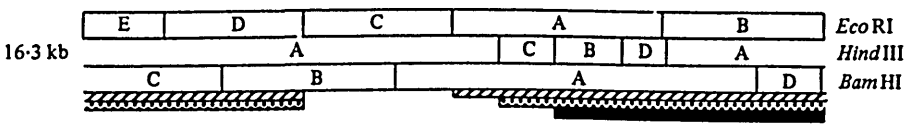
https://doi.org/10.1017/S0022172400065347 Published online by Cambridge University Press

Table 2. *Restriction digest fragments of plasmids from JK-type coryneforms*

Enzyme	Plasmid group	Fragment sizes (kb)												Total size	Number of digestions
Eco RI	pKW4*	9·6				3·8								13·3	5
	pKW10*			5·0		3·8	3·1	3·0			1·8			16·6	7
	pKW15*		6·6		4·9	3·8		3.0			1·8			19·9	3
	pKW23*			5·0	4·9	3·8		3·0		2·1		1·1	0·9	20·6	3
	pKW37*			5·0		3·8		3·0	2·4	2·1	1·8			18·0	4
	pKW6		8·5											8·5	2
	pKW16				NC										1
Hind III	pKW4*			9·5				1·7		1·0	0·8			13·0	3
	pKW10*		12·0					1·7	1·2	1·0				15·9	6
	pKW15*	15·7						1·7	1·2	1·0				19·6	1
	pKW23*	14·5						1·7†	1·2	1·0				20·1	3
	pKW37*	14·3						1·7	1·2	1·0				18·2	3
	pKW6				6·0		1·9			1.1				9·1	4
	pKW16					3·2						0·7	0·6	4·5	2

Sizes are averages of the number of digestions shown, and may differ from those shown in Table 1 and Fig. 1. They were calculated to 2 d.p. and rounded to 1 d.p., so the total may not equal the sum of the fragments above.

NC, not cleaved; *, BLS plasmids; †, doublet band.



Key:

Segments composed of fragments common to all BLS-associated plasmids.

Segment composed of fragments common to group 10 and 15 plasmids.

Segment composed of fragments common to group 10 and 37 plasmids.

Fig. 2. Restriction endonuclease map of pKW10 (based on Kerry–Williams, 1985). Letters indicate restriction fragments, in size order (largest to smallest) for given restriction endonucleases.

resulted in loss of the BLS$^+$ phenotype. Similar experiments were carried out for the other plasmid groups reported here (Table 3). All isolates which were BLS$^-$ had also lost their plasmid, and BLS$^+$ co-isolates which were tested retained their plasmid.

Cross immunity

A number of BLS$^+$ strains including C483, C441 and C476, containing pKW4, pKW10 and pKW15 respectively, were tested for deferred antagonistic activity against each other. None of the isolates was sensitive to the BLS of any other isolate, suggesting that producers were immune to the action of the BLSs, which may therefore be related or identical.

Strains from other sources

Those plasmids isolated from strains from sources other than RMH which were of similar mol. wt. to those of the RMH isolates were compared with the RMH

Table 3. *Curing strains of BLS production*

Plasmid group	Strain	Colonies screened	Cured isolates
pKW4	C483	1060	3*
pKW10	C475	2501	2
pKW15	C479	248	10
pKW23	C206	1010	0
pKW37	K488	1010	1

* Kerry-Williams & Noble (1984).

plasmids using restriction endonuclease cleavage. Four strains, all identified as JKs (S. Pelczynska, personal communication), possessed plasmids similar or identical to RMH plasmids. C475, which was received in December 1983 from a patient in Bristol, possessed a pKW10-group plasmid, and was BLS^+. C206, received in February 1983 from a patient in London, was also BLS^+, and showed co-immunity with RMH BLS producers, but could not be cured of the BLS^+ phenotype. However, its plasmid, pKW23, was extensively similar to pKW10, and possessed the core segment common to all the BLS plasmids (Figs. 1 and 2). Two BLS^- strains, C422 and C512, received in July 1983 and May 1984 respectively, possessed plasmids indistinguishable from the RMH pKW6 group. No connection could be established between these patients or the hospitals.

Comparison of plasmid frequencies

Coryneform isolates possessing a high level of multiresistance are almost invariably members of the JK group, and 58 such strains were found among the 230 strains from sources other than RMH. Three of these isolates, C422, C475 and C512, possessed plasmids indistinguishable from those of the RMH strains, so that 3/58 (5%) can be taken as the expected proportion of strains with RMH-type plasmids in a group of independent strains. At RMH, 23 of 39 JK coryneforms possessed plasmids, and many of these were indentical or could be presumed to have a common origin. If, for the sake of statistical argument, indistinguishable isolates from the same patient are considered as non-independent, and other isolates as independent, then there were 35 independent RMH isolates, 19 of which possessed plasmids that had been grouped. The proportions 3/58 and 19/35 can be compared, to test the hypothesis that the percentage of RMH isolates with these plasmids is significantly different from that expected if the isolates were truly independent. When calculated, the value of chi-squared with Yates' correction is 26·50, with $P<0{\cdot}0005$, indicating that the RMH isolates are most unlikely to be independent. This is strongly indicative that individual strains have been transmitted between patients within RMH.

DISCUSSION

The majority of the 39 highly multiresistant coryneform isolates received from the Royal Marsden Hospital, Surrey, have been identified as JK types by comparison of protein patterns with those of known JKs from the CDC (Jackman & Pelczynska, 1986). Almost half the isolates produced a BLS active against C433.

Twenty-three of the 39 isolates possessed plasmids, which could be divided into six groups on the basis of restriction digest patterns. Plasmids within a group (with indistinguishable restriction patterns) were undoubtedly extremely similar, and were in all probability identical.

Curing experiments showed that the BLS$^+$ phenotype was wholly dependent in RMH isolates on the presence of a plasmid of the pKW4, pKW10, pKW15 or pKW37 groups, which presumably encode BLS production. C206, a BLS$^+$ isolate from St Paul's Hospital, London, possessed a related plasmid.

These plasmid groups possessed restriction fragments in common, which when mapped formed large single segments, such that each plasmid could be derived from another by a single recombinational event. A core segment of 6·4 kilobase-pairs (kb) was present in all the BLS plasmids. pKW4 had only this segment in common with the other BLS plasmids, but pKW10, pKW15, pKW23 and pKW37 had more extensive similarities.

The pKW6 group plasmids and pKW16 bore no resemblance to the BLS plasmids or to each other on the basis of restriction endonuclease cleavage.

It should be stressed, however, that although common restriction fragments are likely to have nearly identical DNA sequences, the common sequences could almost certainly extend into dissimilar fragments. Furthermore, dissimilar plasmids could contain regions of common DNA.

It is clear that in many cases isolates with indistinguishable plasmids were recovered from different individuals, indicating that either plasmid or strain transmission between patients must have occurred. Plasmid transfer is unlikely in comparison to strain transfer; the skin environment is unlikely to be conducive to transformation or transduction, and there has been no report to date of bacteriophages in the JK group of organisms. Furthermore, any conditions which lead to person-to-person transfer of naked DNA or bacteriophages are also likely to bring about transfer of bacterial cells. It is therefore highly probable that the BLS$^+$ strains have spread between individuals.

The frequency of plasmids at RMH is significantly higher than expected, indicating that the spread of strains occurred *within* RMH. Patients colonized or infected with JKs with particular plasmids were rarely present in the hospital simultaneously (data not shown), so it must be assumed that either colonization of staff or patients went undetected, or the patients' environment was contaminated with these JKs, or both.

It is conceivable that all the RMH isolates could be derived from a single strain originally introduced into the hospital. Certainly the introduction of a JK strain with a pKW10 group plasmid could explain the presence of all the BLS$^+$ isolates, as pKW4, pKW15 and pKW37 could all have been derived from pKW10 by a single recombinational event; indeed the sole strain with pKW37 was iolated in mixed culture with a pKW10 group strain. The pKW10 group was also the only RMH BLS plasmid to be found outside the hospital, in C475, a strain from a Bristol hospital.

C475 (with a pKW10 group plasmid), C422 and C512 (both with pKW6 group plasmids) were all isolated between 2 and 14 months after the appearance of the corresponding strains in RMH, which may have been the source for all these plasmids.

This study has demonstrated that plasmids in JK-type coryneform bacteria can be used as epidemiological markers, and indeed they are the only markers reported in JK types to date. Strong, direct evidence that strains have spread between patients within a hospital has been presented for the first time. Plasmid evolution may also have occurred within the hospital. This evidence was provided by a number of plasmids, all of which encoded a similar bacteriocin-like substance, and shared at least 6·4 kb of DNA, found in a large proportion of isolates at the hospital.

Further studies should provide more detailed information on the relationships of the BLS plasmids, the pKW6 group plasmids, and pKW16, using DNA hybridization techniques; and a prospective analysis of JK colonization and infection at the Royal Marsden Hospital should now be possible.

We are grateful to all those who provided isolates, unpublished data and information, in particular R. Harding, Royal Marsden Hospital; M. Almeida, The London Hospital; G. Browning, The Maudsley Hospital; D. A. Lewis, Southmead Hospital and I. M. Gould, St Paul's Hospital.

Thanks are also due to Dr K. G. H. Dyke; for a gift of lambda DNA and for most helpful discussion.

We are indebted to the MRC and the Dunhill Trust for grants to support this work.

REFERENCES

Gill, V. J., Manning, C., Lamson, M., Woltering, P. & Pizzo, P. A. (1981). Antibiotic-resistant Group JK bacteria in hospitals. *Journal of Clinical Microbiology* **13**, 472–477.

Jackman, P. J. H. & Pelczynska, S. (1986). Characterization of Corynebacterium Group JK by whole-cell protein patterns. *Journal of General Microbiology*, **132**, 1911–1915.

Kerry-Williams, S. M. (1985). Plasmids in coryneform bacteria isolated from human sources. Ph.D. thesis, University of London.

Kerry-Williams, S. M. & Noble, W. C. (1984). Plasmid-associated bacteriocin production in a JK–type coryneform bacterium. *FEMS Microbiology Letters* **25**, 179–182.

Quinn, J. P., Arnow, P. M., Weil, D., & Rosenbluth, J. (1984). Outbreak of JK diphtheroid infections associated with environmental contamination. *Journal of Clinical Microbiology* **19**, 668–671.

Riley, P. S., Hollis, D. G., Utter, G. B., Weaver, R. E. & Baker, C. N. (1979). Characterization and identification of 95 diphtheroid (Group JK) cultures isolated from clinical specimens. *Journal of Clinical Microbiology* **9**, 418–424.

Sealey, P. G. & Southern, E. M. (1982). Gel electrophoresis of DNA. In (eds) D. Brickwood & B. D. Hames *Gel Electrophoresis of Nucleic Acids: a Practical Approach*, p. 39 Oxford: IRL Press.

Stamm, W. E., Tompkins, L. S., Wagner, K. F., Counts, G. W., Thomas, E. D. & Meyers, J. D. (1979). Infection due to *Corynebacterium* species in marrow transplant patients. *Annals of Internal Medicine* **91**, 167–173.

Tompkins, L. S., Juffali, F. & Stamm, W. E. (1982). Use of selective broth enrichment to determine the prevalence of multiply resistant JK corynebacteria on skin. *Journal of Clinical Microbiology* **15**, 350–351.

Young, V. M., Meyers, W. F., Moody, M. R. & Schimpff, S. C. (1981). The emergence of coryneform bacteria as a cause of nosocomial infections in compromised hosts. *American Journal of Medicine* **70**, 646–650.