Physical and Biochemical Characterization of the *qacA* Gene Encoding Antiseptic and Disinfectant Resistance in *Staphylococcus aureus*

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We have previously cloned a 3.5 kb fragment from the *Staphylococcus aureus* multiresistance plasmid pSK1 which carries the *qacA* determinant responsible for linked resistance to acriflavine (Ac^r), ethidium bromide (Eb^r), quaternary ammonium compounds (Qa^r), propamidine isethionate (Pi^r), and diamidinodiphenylamine dihydrochloride (Dd^r). This report presents a biochemical and physical analysis of *qacA* and shows the widespread carriage of this gene on *S. aureus* resistance plasmids. Tn5 insertion mutagenesis defined the extent of *qacA* to within 2.40 kb of pSK1 DNA. Examination of the expression of insertion and deletion mutants of the cloned *qacA* sequences in both maxicells and minicells led to the association of a 50 kDa protein, designated QacA, with the Ac^rEb^rQa^rPi^rDd^r phenotype. Based on fluorimetric and isotopic assays used to determine the extent of accumulation of ethidium bromide by *S. aureus* strains harbouring pSK1, we propose that the basis of Ac^rEb^rQa^rPi^rDd^r in *S. aureus* is a *qacA*-mediated efflux system.

INTRODUCTION

Previous genetic analyses have established that, in addition to resistance to the aminoglycosides gentamicin, tobramycin and kanamycin and to trimethoprim, the commonly detected *Staphylococcus aureus* plasmid pSK1 encodes resistance to acriflavine (Ac^r), ethidium bromide (Eb^r), quaternary ammonium compounds (Qa^r) such as cetrime (cetyltrimethylammonium bromide) and benzalkonium chloride, and diamidines such as propamidine isethionate (Pi^r) and diamidinodiphenylamine dihydrochloride (Dd^r) (Lyon *et al.*, 1984; Gillespie *et al.*, 1986; Lyon & Skurray, 1987). It has been suggested that the emergence of staphylococci resistant to these and related compounds may have been due to the frequent inclusion of such agents, particularly acriflavine and cetrime, in antiseptics and disinfectants (Foster, 1983; Tennent *et al.*, 1985). The genetic determinant carried by pSK1 responsible for Ac^rEb^rQa^rPi^rDd^r has been designated *qacA* (Lyon & Skurray, 1987). Restriction endonuclease analysis and DNA–DNA hybridization have established that *qacA* is also present on β -lactamase/heavy metal resistance plasmids detected in *S. aureus* strains collected during the early 1980s (Gillespie *et al.*, 1986). Furthermore, we believe that *qacA* is equivalent to the determinants responsible for the type I nucleic acid binding resistance (Emslie *et al.*, 1985, 1986) and for the antiseptic-resistant phenotypes of *S. aureus* isolates which have appeared in London (UK) hospitals in the last few years (Townsend *et al.*, 1987; Cookson & Phillips, 1988).

There exist up to four determinants besides *qacA* which mediate resistance to antiseptics and disinfectants in *S. aureus*. Of these, *qacB* shares restriction site identity and DNA sequence

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homology on the basis of hybridization experiments with *qacA* and is carried by β -lactamase/heavy metal resistance plasmids (Gillespie & Skurray, 1986, and unpublished data; Lyon & Skurray, 1987). However, we have distinguished between these two determinants on the basis of the levels of resistance they confer towards Ac, Eb, Qa, Pi and Dd. A third gene, designated *qacC*, encodes Qa^r and low-level Eb^r. *qacC* has been detected on a variety of 2–3 kb plasmids identified in *S. aureus* strains from Australia, Italy and North America (Emslie *et al.*, 1986; Lyon & Skurray, 1987; M. Gillespie & R. Skurray, unpublished data). Phenotypically similar to *qacC*, we have now defined the *qacD* and *qacE* antiseptic-resistance determinants as those genes carried by conjugative aminoglycoside-resistance plasmids isolated in North America (pSK41, pUW3626; Lyon *et al.*, 1987a) and West Germany (pJE1; Evans & Dyke, 1988), respectively. Although it is clear that *qacC*, *qacD* and *qacE* are related on the basis of DNA hybridization studies, and are physically distinct from either of *qacA* or *qacB* (Lyon & Skurray, 1987; M. Gillespie, J. Tennent, M. Midgley, G. Jones, A. Purewal & R. Skurray, unpublished data), the exact relationship between the three former genes is yet to be fully examined.

The first investigation into the biochemistry of staphylococcal antiseptic and disinfectant resistance established that the mechanism of resistance attributable to *qacE* encoded by pJE1 involved an efflux system (Jones & Midgley, 1985). In this study we have examined the physical and biochemical characteristics of *qacA*-mediated Ac^rEb^rQa^rPi^rDd^r in *S. aureus*.

METHODS

Bacterial strains and plasmids. Plasmid-free strains of *S. aureus* (SA113, SK982, SK983) used as recipients in DNA transfer experiments have been described previously (Lyon *et al.*, 1984; Tennent *et al.*, 1985). A description of the *S. aureus* strains and plasmids examined in this study is presented in Table 1. *Escherichia coli* K12 derivatives used for the preparation of maxicells and minicells were CSR603 (Sancar *et al.*, 1979) and ORN103 (Orndorff *et al.*, 1985), respectively. Construction of the *S. aureus*–*E. coli* hybrid plasmid pSK449 has been detailed elsewhere (Tennent *et al.*, 1985).

General methods. Bacteria were routinely grown in L-broth or on L-agar containing appropriate antibiotics. All media and the method for the determination of antibiotic susceptibility have been previously described (Lyon *et al.*, 1983; Tennent *et al.*, 1985). Propamidine isethionate and diamidinodiphenylamine hydrochloride were generously donated by May & Baker Australia Pty Ltd.

DNA manipulation procedures. The isolation of plasmid DNA, digestion with restriction endonucleases and agarose gel electrophoresis were performed as previously described (Lyon *et al.*, 1983; Ray & Skurray, 1983). Transposon mutagenesis with Tn5 was done essentially according to Weaver *et al.* (1981) using phage λ -467 (Berg *et al.*, 1975) as a source of the transposon. *Hind*III digests of λ bacteriophage DNA were employed as standards; fragment sizes in kilobase pairs (kb) were as published (Daniels *et al.*, 1983). DNA was transferred to nitrocellulose for hybridization analysis by the bidirectional procedure of Smith & Summers (1980). A 0.85 kb *Hpa*II DNA fragment which spans the *qacA* determinant was prepared from pSK449 by electroelution, nick-translated with [α -³²P]dATP (1800 Ci mmol⁻¹, 66.6 TBq mmol⁻¹) and hybridized in 50% (v/v) formamide with

Table 1. *S. aureus* strains and plasmids

Strain	Background	Plasmid			Reference
		No.	Size (kb)	Genotype*	
SK982	SA113	—			Lyon <i>et al.</i> (1984)
SK983	SA113	—			Tennent <i>et al.</i> (1985)
SK2267	SK982	pSK1	28.4	<i>aacA-aphD qacA dfrA</i>	Skurray <i>et al.</i> (1988)
SK257	Clinical isolate	pSK9	25.7	<i>aacA-aphD qacA</i>	Skurray <i>et al.</i> (1988)
SK74	Clinical isolate	pSK14	24.4	<i>aacA-aphD qacA</i>	Skurray <i>et al.</i> (1988)
SK2408	SA113	pSK16	35.1	<i>aacA-aphD qacA dfrA blaZ</i>	Skurray <i>et al.</i> (1988)
SK2414	SK982	pSK42	29.75	<i>aacA-aphD qacA dfrA</i>	This paper
SK2392	SK983	pSK78	25.9	<i>aacA-aphD dfrA</i>	Tennent <i>et al.</i> (1985)

* Other than *qacA*, the genes shown encode resistance to: *aacA-aphD*, gentamicin, tobramycin and kanamycin; *blaZ*, penicillin; *dfrA*, trimethoprim.

nitrocellulose filters carrying target DNA, using previously described techniques (Maniatis *et al.*, 1982; Lyon *et al.*, 1987a). Washed and dried filters were exposed to Fuji RX film at -70°C for sufficient time to produce an acceptable autoradiograph.

Analysis of plasmid-encoded proteins. Maxicells were prepared from various CSR603 transformants and plasmid-encoded proteins were labelled with [^{35}S]methionine according to the technique described by Sancar *et al.* (1979). Minicells were isolated from different ORN103 transformants and plasmid-encoded proteins were labelled with [^{35}S]methionine as described by Thompson & Achtman (1978). In both instances, radiolabelled polypeptide products were boiled for 3 min in sample buffer, separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and detected by autoradiography or fluorography.

Measurement of ethidium efflux. The methods for the fluorimetric and isotopic assays employed in these determinations were based on previous studies (Jones & Midgley, 1985; Midgley, 1986). The appropriate strain was grown for 16 h at 37°C in L-broth (50 ml). This culture was used to inoculate 200 ml of L-broth; after 90–120 min at 37°C , the cells were harvested, washed twice at 4°C in 20 mM-HEPES/NaOH (pH 7.0), and finally resuspended in the same buffer and kept on ice. Loading of the cells was by incubation for 35 min at 37°C at a cell density of 0.39 mg dry wt ml^{-1} in the presence of 20 μM -carbonyl cyanide *m*-chlorophenylhydrazone and 5 μM -ethidium bromide. For radiochemical experiments, [$6\text{-}^{14}\text{C}$]ethidium bromide was used at a specific activity of 3.3 $\mu\text{Ci } \mu\text{mol}^{-1}$ (122 kBq μmol^{-1}). The cell suspension was then chilled and the cells were washed once more in the same buffer at 4°C . Formate- or glucose-driven efflux of ethidium was assayed as previously described (Midgley, 1986).

Measurement of respiration. Rates of respiration were measured using an oxygen electrode as described by Midgley (1986).

RESULTS

Physical localization of the qacA determinant on pSK1

We have previously demonstrated that a 3.5 kb segment of pSK1 DNA contains the *qacA* determinant and can express $\text{Ac}^r\text{Eb}^r\text{Qa}^r$ in *E. coli* when cloned into the plasmid vector pBR322 (Fig. 1; Tennent *et al.*, 1985; Gillespie *et al.*, 1986). This *S. aureus*–*E. coli* hybrid plasmid, pSK449, has now been shown to confer on its *E. coli* host resistance to Pi (200 $\mu\text{g ml}^{-1}$) and Dd (150 $\mu\text{g ml}^{-1}$) also. To localize the *qacA* region of pSK1 more precisely within the cloned staphylococcal DNA, pSK449 was mutagenized with the kanamycin/neomycin resistance transposon Tn5. From one experiment, 80 transformants containing pSK449::Tn5 derivatives were obtained; 41 of these plasmids possessed Tn5 insertions within the cloned pSK1 segment. Of 24 insertion mutants subjected to restriction endonuclease analysis, 19 had Tn5 situated in pSK449 at distinguishable sites, with one mapping in the vector and the remaining 18 being located in the passenger DNA. The positions of the 19 Tn5 insertions in pSK449 are depicted in Fig. 1.

Susceptibility testing revealed that 15 distinct insertions of Tn5 in pSK449 led to the concomitant loss of $\text{Ac}^r\text{Eb}^r\text{Qa}^r\text{Pi}^r\text{Dd}^r$. This result confirmed the notion that a single determinant, *qacA*, mediated resistance to all of these agents. The insertions mapped within a 1.45 kb region of pSK1 DNA (between coordinates 19.40 and 20.85) which encompassed two sites for each of *Hae*III, *Hpa*II and *Pvu*II (Fig. 1). Three plasmids were identified which exhibited the full *qacA* phenotype of pSK449 and contained Tn5 insertions within the cloned staphylococcal fragment; one of these, pSK478, limited the leftward extent of the *qacA* determinant to 18.70 on the pSK1 map, whereas the locations of the other two (e.g. pSK480) suggested that the cloned portion of IS256L (Lyon *et al.*, 1987b) played no role in mediating this phenotype. Plasmid pSK467 possessed a Tn5 insertion in pBR322 sequences which did not affect the expression of *qacA*. On the basis of these data, the minimum and maximum extents of the *qacA* determinant on pSK1 have been limited to 1.45 kb (coordinates 19.40–20.85) and 2.40 kb (coordinates 18.70–21.10), respectively.

Demonstration of qacA sequences on S. aureus plasmids

Plasmid pSK1 represents a family of structurally related plasmids detected in Australian isolates of multiresistant *S. aureus*. Of these plasmids, all but one encodes $\text{Ac}^r\text{Eb}^r\text{Qa}^r\text{Pi}^r\text{Dd}^r$ and most additionally mediate resistance to the aminoglycosides, trimethoprim and/or penicillin (Lyon & Skurray, 1987; Skurray *et al.*, 1988). Restriction endonuclease maps established for

Fig. 1. Location of the *qacA* region of pSK1. Sites of Tn5 insertion into pSK449, with pSK numbers (e.g. pSK478) given only for those plasmids used in the polypeptide analysis (Fig. 3a), are shown by circles joined to the pSK449 linear map which has been aligned with a simplified physical map of pSK1; filled (●) and open (○) circles indicate Ac^rEb^rQa^rPi^rDd^r and Ac^sEb^sQa^sPi^sDd^s phenotypes, respectively. Thin lines represent pSK1 DNA sequences, whereas the IS256 inverted repeats of Tn4001 and the IS257 direct repeats of Tn4003 are shown as thick lines; pBR322 sequences of pSK449 are shown as thick (open) lines. Coordinates are in kb and refer to pSK1 map coordinates; we have recalculated the size of the cloned fragment in pSK449 to be 3.5 kb and not 3.4 kb as estimated previously by restriction endonuclease mapping (Tennent *et al.*, 1985). Restriction endonuclease sites are given by B (*Bam*HI), Bg (*Bgl*II), E (*Eco*RI), H (*Hind*III), Ha (*Hae*III), Hp (*Hpa*II), and PII (*Pvu*II). Only those *Hind*III sites of pSK1 within Tn4001 are indicated and only those *Hae*III and *Hpa*II sites of pSK1 lying within the cloned fragment (expanded) are shown. A detailed map of pSK1 has appeared elsewhere (Lyon *et al.*, 1987a). The dotted box in the lower part of the figure represents the maximum extent of the coding sequences of the *qacA* determinant, whereas the solid box indicates the minimum extent of this region (see text).

these plasmids identified a DNA segment on each that shared restriction identity with the *qacA* region of pSK1 (Skurray *et al.*, 1988; our unpublished data). When digested with *Hpa*II, five of the pSK1 family plasmids (pSK1, pSK9, pSK14, pSK16 and pSK42), together with pSK449, produced a 0.85 kb fragment (Fig. 2a, lanes B, C, D, E, F and H). In contrast, pSK78, an *in vitro* deleted derivative of pSK1 which does not encode Ac^rEb^rQa^rPi^rDd^r (Tennent *et al.*, 1985), failed to produce a *Hpa*II fragment of this size (Fig. 2a, lane G).

The transposon mutagenesis experiment described above also indicated that the 0.85 kb *Hpa*II fragment of pSK1 was located entirely within the minimum extent of *qacA* (Fig. 1). Therefore, to confirm the presence of *qacA* sequences in each of the pSK1 family plasmids, the 0.85 kb *Hpa*II fragment from pSK449 was radiolabelled and hybridized with the *Hpa*II-digested DNA in the gel shown in Fig. 2(a). As expected, the probe DNA hybridized with the 0.85 kb *Hpa*II fragment from the plasmids pSK1, pSK9, pSK14, pSK16, pSK42 and pSK449 (Fig. 2b, lanes B, C, D, E, F and H) but not with any fragment of pSK78 (Fig. 2b, lane G). Subsequent analyses of six other pSK1 family plasmids (pSK4, pSK7, pSK8, pSK15, pSK17 and pSK18) showed that each contained a similar-sized *Hpa*II fragment which was homologous to the probe DNA used here (data not shown).

These hybridization results are in agreement with restriction mapping data (Skurray *et al.*, 1988) and confirm the prevalence of the *qacA* determinant among members of the pSK1 family of *S. aureus* resistance plasmids.

Fig. 2. (a) Agarose gel (1.3%, w/v) electrophoresis of purified DNA cleaved with the restriction endonuclease *Hpa*II: lanes: A, λ phage DNA cleaved with *Hind*III as standards, with fragment sizes in kb shown at left; B, pSK1; C, pSK9; D, pSK14; E, pSK16; F, pSK42; G, pSK78; H, pSK449. It should be noted that pSK1, pSK16, pSK42, and pSK78 were prepared from laboratory-derived transferrants, whereas pSK9 and pSK14 were isolated from their clinical backgrounds which each carry two plasmids (see Table 1). In both cases, the additional plasmid was identified as pSK3 (which possesses a single *Hpa*II site); the linear form of pSK3 appears in lanes C and D as an intense band of DNA. (b) Autoradiograph of the gel shown in (a) after hybridization with the 0.85 kb *Hpa*II fragment of pSK449. No homologous sequences were detected in lane G (see text).

Polypeptide analysis of the *qacA* region of pSK1

In order to identify possible translation products of the *qacA* determinant of pSK1, polypeptides expressed by the vector plasmid pBR322 (Fig. 3a, lane A) and by pSK449 (Fig. 3a, lane B) were examined in [³⁵S]methionine-labelled maxicells prepared from strain CSR603. Polypeptides synthesized by pSK449 included proteins of apparent molecular mass 17 kDa, 24 kDa, 26 kDa and 30 kDa, and a diffusely-banded protein of approximately 50 kDa. Of these, the 30 kDa protein corresponded to the β -lactamase (BLA) protein encoded by pBR322. Considering that the size of the passenger DNA in pSK449 was 3.5 kb it was possible that the remaining polypeptides could all be encoded by the cloned pSK1 sequences.

The effect of selected Tn5 insertions on the synthesis of polypeptides by pSK449 was also examined in maxicells. The insertion mutants pSK467, pSK478 and pSK480 (Fig. 1), which retained the Ac^rEb^rQa^rPi^rDd^r phenotype, expressed three of the proteins specified by pSK449, namely 17 kDa, 26 kDa and 50 kDa (Fig. 3a, lanes C, D and J). In contrast, five Ac^sEb^sQa^sPi^sDd^s mutants (Fig. 3a, lanes E-I), which either defined the minimum extent of *qacA* (pSK463 and pSK476) or were located within these boundaries (pSK464, pSK465 and pSK477; Fig. 1), synthesized 17 kDa and 26 kDa polypeptides but lacked the heavily expressed 50 kDa protein. It should be noted that, in addition to those proteins described above, all of the transposon insertion mutants expressed several proteins, including the 29 kDa neomycin phosphotransferase (NPT), which were encoded by Tn5 (Rothstein *et al.*, 1980). Furthermore, longer exposure of the fluorogram shown in Fig. 3(a) revealed that the 24 kDa polypeptide was synthesized by strains carrying each of the pSK449 : Tn5 derivatives but in varying amounts. In

Fig. 3. Analysis of polypeptides synthesized by pSK449 and its derivatives. (a) Fluorograph of 15% (w/v) SDS-PAGE of proteins synthesized in maxicells containing the indicated plasmid. Lanes: A, pBR322; B, pSK449; C, pSK467; D, pSK478; E, pSK463; F, pSK464; G, pSK465; H, pSK477; I, pSK476; J, pSK480. (b) Autoradiograph of 15% (w/v) SDS-PAGE of proteins synthesized in minicells containing: lane A, pSK449; B, pSK516. The molecular masses (in kDa) of standard proteins are indicated. Positions of the vector-encoded proteins β -lactamase (BLA) and its precursor form (pre-BLA), and the Tn5-encoded neomycin phosphotransferase (NPT) are shown, together with the position of the 50 kDa QacA protein (see text for details).

any event, none of the 17 kDa, 24 kDa or 26 kDa polypeptides could be correlated with alterations in the *qacA* phenotype caused by the insertion of Tn5 into pSK449.

Although strains containing plasmids pSK463, pSK464, pSK465, pSK476 and pSK477 did not encode the intensely labelled 50 kDa protein, each showed weak expression of a similar-sized polypeptide as did the control, pBR322. Conceivably, this protein could represent a vector- or host-encoded protein obscured in those lanes which displayed the heavily expressed 50 kDa protein. This conclusion was supported by a densitometric comparison of the proteins encoded by pSK478 and pSK463 (Fig. 3a, lanes D and E), which suggested that the intense, albeit diffuse, 50 kDa protein encoded by pSK478 was indeed a unique pSK1-encoded polypeptide (data not shown).

To positively associate the 50 kDa protein with the *qacA* sequences, a derivative of pSK449 was constructed by deleting approximately 4.1 kb DNA between the *Pvu*II site at coordinate 20.35 and the *Pvu*II site within the vector pBR322 (Fig. 1; data not shown). The resultant plasmid, pSK516, contained only 1.7 kb of pSK1 DNA (coordinates 18.65–20.35) and was deleted for the right-hand end of the *qacA* region defined by Tn5 mutagenesis, the cloned portion of IS256L and approximately 2.3 kb of pBR322. When further compared with pSK449, strains carrying pSK516 were found to be $\text{Ac}^s \text{Eb}^s \text{Qa}^s \text{Pi}^s \text{Dd}^s$ and unable to express the 50 kDa protein (Fig. 3b, lane B); no other polypeptide specified by pSK449 (Fig. 3b, lane A) was affected by the deletion that occurred in the construction of pSK516.

According to these data, we conclude that the 50 kDa protein encoded by pSK449 is the product of the pSK1 *qacA* determinant responsible for $\text{Ac}^r \text{Eb}^r \text{Qa}^r \text{Pi}^r \text{Dd}^r$ in *S. aureus*. Consequently, we refer to this protein as QacA.

Mechanism of antiseptic resistance in S. aureus

The *qacA*-mediated mechanism by which staphylococci evade the antiseptic actions of Ac, Eb and Qa was examined in two ways. A substrate-promoted efflux of ethidium from strain SK2267, which carries pSK1, was demonstrated in a fluorimetric assay (Fig. 4a) and also in an

Fig. 4. (a) Fluorimetric assay of ethidium efflux. Cells of strain SK982 or SK2267 (Table 1) were loaded with ethidium bromide as described in Methods. Traces A, B and C received no addition, glucose (10 mM) or sodium formate (10 mM), respectively, at the time indicated by the arrows. The traces are offset for clarity of presentation. The value at the beginning of each trace indicates the initial fluorescence intensity. (b) Isotopic assay of ethidium efflux. Cells of strain SK2267 were loaded with ethidium bromide as described in Methods. ●, Cell suspension to which sodium formate (10 mM) was added at the time indicated by the arrow; ○, no additions.

isotopic assay (Fig. 4b). The addition of formate to cells loaded with ethidium bromide supported a rate of respiration of $52 \mu\text{mol O}_2 \text{ min}^{-1} (\text{g dry wt})^{-1}$, whereas glucose supported the lower rate of $19 \mu\text{mol O}_2 \text{ min}^{-1} (\text{g dry wt})^{-1}$. The latter rate was established 3 min after the addition of glucose. A similar lag period was seen in the time course of efflux (Fig. 4b). Substrate-promoted efflux of ethidium from the background strain SK982 was not observed using either assay (Fig. 4a and data not shown). Furthermore, SK982 had equivalent rates of formate- and glucose-supported respiration to those seen with SK2267, thereby demonstrating that the differences in ethidium efflux between the pSK1 (*qacA*)-carrying derivative and its plasmid-free host were not attributable to differences in respiration.

DISCUSSION

Almost 20 years have elapsed since staphylococcal resistance to acriflavine and ethidium bromide was associated with the presence of β -lactamase plasmids (Ericson, 1969; Johnston & Dyke, 1969). More recently, $\text{Eb}^r \text{Qa}^r$ has been linked with a variety of *S. aureus* aminoglycoside-resistance plasmids in Australia, the UK, the USA, Ireland and Germany (Archer & Johnston, 1983; McDonnell *et al.*, 1983; Naidoo *et al.*, 1983; Lyon *et al.*, 1984, 1987a; Coleman *et al.*, 1985; Goering *et al.*, 1985; Townsend *et al.*, 1985, 1987; Cookson & Phillips, 1988; Skurray *et al.*, 1988).

The data presented in this paper clearly establish the involvement of a plasmid locus, *qacA*, in the linked expression of $\text{Ac}^r \text{Eb}^r \text{Qa}^r \text{Pi}^r \text{Dd}^r$ by *S. aureus* isolates from Australian hospitals. The *qacA* gene was found to be carried by most members of the pSK1 family of plasmids, which are widely distributed among clinical strains (Skurray *et al.*, 1988). The significance of this finding lies in the fact that *qacA* affords protection against many compounds that are part of commonly used clinical antiseptic/disinfectant formulations. As already mentioned, these agents include acriflavine (a mixture of euflavine and proflavine), cetrimide, benzalkonium chloride, propamidine isethionate and diamidinodiphenylamine dihydrochloride. Furthermore, in preliminary studies designed to explore the substrate specificity of the *qacA* determinant, it was found that *qacA* also conferred resistance to a number of other compounds including chlorhexidine diacetate, cetylpyridinium chloride, crystal violet, pentamidine isethionate, pyronin Y, quinaldine red, rhodamine 6G, and safranin O (our unpublished data). We believe

that variations in the patterns of resistance to these compounds will be useful in distinguishing and classifying other *qac* determinants, such as *qacB-E*, with respect to *qacA*.

The mechanism by which staphylococci evade the antiseptic actions of Ac, Eb and Qa has been postulated to be associated with decreased intracellular accumulation of these agents (Johnston & Dyke, 1969; McDonnell *et al.*, 1983). A mechanism such as this would most likely rely upon altered cellular permeability and as such may be affected by a specific protein(s) localized in the membrane matrix. In this paper, the *qacA*-determined phenotype was shown to be due to the operation of an efflux system. Sharing the same basis as those resistance mechanisms towards tetracycline, cadmium, arsenate and arsenite (Ball *et al.*, 1980; Tynecka *et al.*, 1981; Silver & Keach, 1982; Rosen & Borbolla, 1984), the *qacA* efflux system directed the rapid extrusion of ethidium from the staphylococcal cells. It is significant that tetracycline resistance in both the staphylococci and *E. coli* has been associated with a potentially membrane-bound protein (Levy & McMurry, 1974; Mojumdar & Khan, 1988); notably, the protein encoded by the tetracycline-resistance plasmid pT181 is also approximately 50 kDa in size (Mojumdar & Khan, 1988). We suggest that the 50 kDa QacA protein specified by the *qacA* determinant of pSK1 is a cytoplasmic-membrane-associated protein involved in an efflux system which actively reduces the intracellular accumulation of Ac, Eb, Qa, Pi and Dd. Our conclusion that QacA resides in the cytoplasmic membrane is based upon a comparison made between the characteristic diffuse appearance of QacA in SDS-PAGE and the similar migration of the *E. coli lac* carrier protein which catalyses transport of lactose through the plasma membrane of the cell (Kaback, 1983).

Since the *qacA*-mediated phenotype results from active transport, resistance to Ac, Eb, Qa, Pi and Dd must be due to the decreased exposure of these toxicants to intracellular targets only, as levels in the cell envelope would be unaffected. That the lethal target(s) for Ac and Eb is located in the cytoplasm is not unexpected considering their established major inhibitory effects on nucleic acid synthesis (Waring, 1966); Eb is also known to inactivate ribosomes (Ballesta *et al.*, 1976). However, the Qa compounds (benzalkonium chloride and cetyltrimethylammonium bromide) have been characterized as surface-active agents, i.e. their antiseptic properties result from disruption of cellular membranes (Petrocci, 1977). Thus, our data indicate that although Qa compounds are surface-active, major targets for their toxic activity also occur within the cell. Consistent with the intracellular action of Qa compounds, and the diamidines (Pi, Dd), is the observed binding of these types of agents to nucleic acids (Scott, 1962; Newton, 1970): the spectrum of their intracellular targets remains to be established.

Restriction mapping and DNA hybridization experiments using the *qacA*-specific probe detailed in this study have shown sequences homologous to this determinant to be present on a variety of plasmids from clinical isolates of coagulase-negative staphylococci (J. Tennent & R. Skurray, unpublished data). From these and other observations (Tennent *et al.*, 1984, 1986, 1988; Skurray *et al.*, 1988) it is becoming increasingly clear that members of the genus *Staphylococcus* within Australian hospitals share common genetic reservoirs. Such exchange, when considered along with the selective pressures imposed through the extensive application of agents such as acriflavine, cetrime and chlorhexidine as antiseptics and disinfectants, may well account for the emergence and continuing presence of plasmid vectors which primarily encode the *qacA* phenotype, but that are also capable of disseminating a variety of determinants such as those for resistance to gentamicin, tobramycin, kanamycin, trimethoprim and penicillin among hospital isolates of staphylococci.

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