Sensitivity of Providence to antiseptics and disinfectants

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SYNOPSIS Seventy isolates of Providence were obtained from urinary tract infections in patients from five hospitals. They were all identified as Providence B (*Providencia stuartii*). A typing system was devised for these organisms based on their production of and sensitivity to bacteriophage and bacteriocins. Using this system, 27 distinct strains were recognized, and it was clear that many patients had been infected with the same strain. The minimum inhibitory concentrations of six antiseptics and disinfectants for these strains were determined. Many of the strains were up to 300-fold more resistant to the cationic antiseptics chlorhexidine, cetrimide, and Resiguard than the control strain *Escherichia coli* NCTC 10418. The sensitivities of the Providence strains to Hycolin, glutaraldehyde, and phenyl mercuric nitrate, however, were similar to those of the control organism. There were significant correlations between the sensitivities of the strains to the three cationic antiseptics. The loss of chlorhexidine resistance from a Providence strain after exposure of the cells to *N*-methyl-*N'* nitro *N* nitrosoguanidine resulted in a concomitant loss of resistance to cetrimide and Resiguard, but there was no significant increase in the sensitivity of the mutants to the other three antibacterials.

The main clinical importance of the Providence group of bacteria stems from their capacity to produce urinary tract infections, especially in patients having some underlying urological problem. Dutton and Ralston (1957), for example, found that 21% of infections in a urological ward, occurring after insertion of an indwelling catheter into the bladder or other instrumentation of the urinary tract, were caused by Providence. Milner (1963) reported that Providence was responsible for 38% of urinary tract infections in paraplegic patients whose bladder management involved either intermittent or indwelling catheterization. Similar findings have been reported by Fields *et al* (1967), Li and Miller (1970), Solberg and Matsen (1971), and Dobrey (1971).

Hospital isolates of Providence are well known to be resistant to a wide range of antibiotics (Middleton, 1958; Tomaschoff, 1969; Dobrey, 1971; Graevenitz and Nourbakhsh, 1972), and Li and Miller (1970) have suggested that this property of multiple antibiotic resistance could well favour the increasing significance of this organism as an agent of infection

in the hospital environment. The particular situation that the organism seems to favour, the urinary tract that is undergoing instrumentation, is one where it is likely to be exposed to antiseptics (Gillespie *et al*, 1967; Desautels, 1969; Stickler *et al*, 1971). It would therefore be of considerable ecological advantage to this organism to have a degree of resistance to such antibacterial agents. In this connection O'Flynn and Stickler (1972) reported that some strains of Providence isolated from the urethral meatuses of paraplegic patients undergoing intermittent catheterization of the bladder by a procedure which involved the application of chlorhexidine to the external genitalia, could grow in the presence of 200 µg/ml of the antiseptic, a level well above that of 10-50 µg/ml of the agent normally considered to inhibit Gram-negative bacteria (Davies *et al*, 1954).

In view of the clinical significance of these organisms at sites where conventionally antiseptics play an important role in the prevention of infection, it is surprising that there is so little information in the literature on the sensitivity of Providence to antiseptics and disinfectants. The object of this study was to make a collection of isolates of Providence from a

number of hospitals and to test their sensitivity to a range of these agents.

Material and methods

STRAINS USED

The clinical isolates of Providence used in this study were all isolated from urinary tract infections, the 70 isolates coming from 70 patients in five hospitals. Seven of the isolates were obtained from paraplegic patients undergoing intermittent catheterization of the bladder at the National Medical Rehabilitation Centre, Our Lady of Lourdes Hospital, Dublin; 14 were obtained from Dr C. Keane, of the Adelaide Hospital, Dublin, and were from urinary infections in patients having indwelling bladder catheters. A total of 48 of the isolates came from three Cardiff hospitals, 25 of these coming from a paraplegic unit and most of the balance from gynaecological wards. The NCTC strains 2481, 6344, 10286, and 10318 were used as reference organisms. In some experiments *Escherichia coli* NCTC 10418 and *Proteus mirabilis* NCIB 60 were used as control organisms. The clinical isolates were identified as Providence using the methods of Cowan and Steel (1965).

TYPING OF THE PROVIDENCE ISOLATES

The Providence isolates were typed using a method similar to that devised by Farmer and Herman (1969) for the typing of *Pseudomonas aeruginosa*. It involved the characterization of strains by (a) their sensitivity to a set of bacteriophage and bacteriocin preparations, and (b) the host range of the bacteriocin or bacteriophage produced by each strain. In order to produce preparations of phage and bacteriocins for typing, cultures of each of the isolates were grown to early log phase in broth

(Oxoid No. 2 nutrient broth) at 37° with aeration in a shaking waterbath. They were then induced with mitomycin-*c* (1-10 µg/ml) (Otsuji *et al.*, 1959). The cultures generally lysed after 1½ to 2 hours. Bacterial debris was removed by centrifugation at 3000 *g* for 15 minutes and the supernatant fluid, containing the phage or bacteriocin, was stored at 4° before use. In order to demonstrate the phage or bacteriocin activity on the Providence isolates, inocula (0.5 ml) from 18-hour broth cultures of each isolate were added to aliquots (4 ml) of molten nutrient agar (Oxoid Ltd) at 45° and then poured as a top layer, onto nutrient agar plates. Standard drops of the phage and bacteriocin preparations were then spotted onto these top layers using a multi-applicator similar to that designed by Tarr (1958). The plates were incubated overnight at 37° and examined for plaques or zones of bacteriocin activity.

MINIMUM INHIBITORY CONCENTRATION DETERMINATIONS

The strains to be tested were grown for 24 hours at 37° in broth. Volumes (5 µl) of a 10⁻⁴ dilution, each containing approximately 10⁸ viable cells, were then dropped, using the multi-applicator, onto the surface of nutrient agar plates containing a range of doubling dilutions of antibacterial agents (table I). A standard method was adopted for the preparation of the plates. The antibacterial agents were added to molten nutrient agar that had been allowed to cool to 50°. The plates were dried at 37° for 20 minutes and then used directly. After inoculation the plates were incubated at 37° and examined for growth after 24 hours. The lowest concentration of the agent that prevented colony formation was taken as the minimum inhibitory concentration (MIC) for that strain. The tests were performed in duplicate, *E. coli*

Agent	Source	Concentrations incorporated into nutrient agar for MIC testing
Chlorhexidine	A 5% w/v solution of Hibitane (chlorhexidine digluconate) ICI Ltd	Various volumes of this concentrate were added to agar (5-1600 µg/ml)
Cetrimide	Cetavlon, ICI Ltd	A stock solution (100 mg/ml) in distilled water was used to prepare plates containing 25-1600 µg/ml
Glutaraldehyde	Sigma Ltd	A stock solution (10% w/v) in 0.3% w/v sodium bicarbonate was used to prepare plates containing 100-1600 µg/ml
Resiguard	The commercial concentrate (Nicholas Laboratories Ltd) containing picloxydine digluconate 1.0%, octylphenoxy polyethoxyethanol 11.0%, benzalkonium chloride 12.0%	Various volumes of the commercial concentrate were added directly to molten agar to produce dilutions of 1/100 to 1/3200 of Resiguard
Hycolin	The commercial concentrate (William Peason Ltd, Clough Hill, Hull) containing a combination of synthetic phenols	A 10% v/v solution of concentrate in distilled water was used to prepare agar containing 0.01 to 0.16% v/v of commercial concentrate
Phenyl Mercuric Nitrate	BDH Ltd	A stock solution, 100 µg/ml in distilled water, was used to prepare agar containing 0.125 to 2 µg/ml

Table I Antibacterial agents used in minimum inhibitory concentration tests

10418 and *P. mirabilis* 61 (Stickler, 1974) being included as reference control organisms with each batch of strains tested.

BACTERICIDAL EFFECT OF ANTISEPTICS ON PROVIDENCE

The bactericidal effect of six antiseptics was tested by performing log reduction experiments on selected strains of *Providencia*, *E. coli* 10418, and *P. mirabilis* 60. The organisms were grown for 18 hours in broth culture, and 0.5 ml volumes were mixed with solutions of the various antibacterials in distilled water (4.5 ml). The cells were exposed to the agents for 10 minutes at ambient temperatures, and then 0.5 ml samples were withdrawn and added to 4.5 ml of the appropriate neutralizer (Kelsey and Maurer, 1972; Russell, 1974). After 10 minutes in the neutralizer further dilutions were made in nutrient broth in order to perform viable counts on the survivors. Viable counts were also performed on the cultures used as a source of inocula for these tests. From the number of cells added to the antiseptic and the number surviving the 10 minute exposure the log₁₀ of the reduction in viable count was calculated. The antibacterial agents and neutralizers used in this experiment are shown in table II.

ANTIBIOTIC SENSITIVITY

The antibiotic sensitivity patterns of the *Providencia* strain were determined by seeding plates of DST agar (Oxoid Ltd) with young log phase broth cultures and applying U3 Multidiscs (Oxoid Ltd), which are impregnated with the following antibiotics: gentamicin (10 µg), colistin (10 µg), nitrofurantoin (200 µg), sulphafurazole (500 µg), kanamycin (30 µg), ampicillin (25 µg), sulphamethoxazole/trimethoprim (25 µg), and tetracycline (50 µg). *E. coli* 10418

was used as a sensitive control with each batch of strains tested.

ISOLATION OF CHLORHEXIDINE-SENSITIVE MUTANTS

Chlorhexidine-sensitive mutants of *Providencia* strain 66 were isolated using the mutagen *N*-methyl-*N'*-nitro *N* nitrosoguanidine (NTG) (Sigma Chemical Co Ltd), according to the procedure of Adelberg *et al* (1965). A log phase culture of *Providencia* 66 was harvested by centrifugation, and the cells were washed in 0.05 M phosphate buffer (pH 6.0) and then resuspended in the buffer at a density of about 2×10^8 cells/ml. NTG was added to a final concentration of 50 µg/ml, and the culture was shaken at 37° for 30 minutes. The cells were then washed and resuspended in buffer and finally plated for single colonies on nutrient agar. After incubation at 37° for 18 hours the colonies were replica plated using velvet pads onto nutrient agar containing chlorhexidine (50 µg/ml). Colonies which failed to replicate on this medium were picked off and re-examined for their sensitivity to chlorhexidine. Approximately 4000 colonies were screened in this way and a total of eight sensitive clones was isolated. These sensitive mutants were confirmed as derivatives of the parent strain 66 by the typing procedure.

Results

TYPING OF PROVIDENCE ISOLATES

A total of 70 isolates of *Providencia* were obtained from five hospitals and all were identified as *Providencia* B (Cowan and Steel, 1965). *Providencia stuartii* (Edwards and Ewing, 1972), as they produced acid from inositol and failed to produce gas from glucose. All 70 of the isolates together with four NCTC strains were induced with mitomycin-c. Sixty-nine of the 74 cultures tested lysed approximately 2 hours after induction and produced high titres of phage or bacteriocin. These preparations were then used to type the isolates. Table III is an abbreviated summary of the results of this typing, showing the results with respect to 58 of the clinical isolates. Examination of the table will show that all the strains listed produce an interaction of some sort and that 23 distinct patterns of sensitivity and activity were recorded, indicating the presence of at least 23 distinct strains. When all results from the 70 isolates were examined, 26 distinct patterns of sensitivity and activity were recorded among the clinical isolates. Four isolates, which are not included in table III, produced no interaction, and while it is possible that these could represent four different strains, they could also be repeated isolates of the same strain. Only one of these untypable isolates

Agent	Neutralizer
Chlorhexidine 5% Hibitane (ICI Ltd)	Nutrient broth (Oxoid Ltd) containing 3% lecithin and 10% Tween 80
Benzalkonium chloride (Winthrop Laboratories Ltd)	"
Resiguard (Nicholas Ltd)	"
Hycolin (William Peason Ltd)	"
Betadine (Napp Laboratories Ltd)	Nutrient broth containing 0.5% sodium thiosulphate
Commercial concentrate 10% povidone-iodine containing 1% available iodine	"
Hypochlorite, a commercial concentrate Milton (Richardson-Merrell Ltd) containing 1% w/v sodium hypochlorite and 16% w/v NaCl	"

Table II Antibacterial agents and neutralizers used in log reduction experiments

Source and strain number of indicator lawns		Bacteriophage or bacteriocin preparations obtained from numbered strains of <i>Providencia</i>																								
		23	59	7	8	18	2	6	26	71	3	27	34	35	66	68	74	75	77	84	87	88	90	95	101	103
Dublin Hosp. 1	23 (5) ¹	-	-	-	-	+	-	+	-	-	+	-	-	+	-	+	-	-	-	-	-	-	+	-	-	-
	59 (1)	-	-	-	-	+	-	+	-	-	-	-	-	+	-	+	-	-	-	-	-	-	+	-	-	-
Dublin Hosp. 2	7 (12)	-	-	-	-	+	-	-	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-
	8 (1)	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	18 (1)	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cardiff Hosp. 1	2 (1)	+	+	+	+	+	-	-	-	-	+	-	-	+	-	+	-	-	-	-	+	-	+	-	+	+
	6 (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	26 (1)	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	-	+	-	-	-
	71 (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cardiff Hosp. 2	3 (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	27 (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
	34 (1)	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-
	35 (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	66 (1)	+	+	+	+	+	-	+	-	-	+	-	-	+	-	+	-	-	-	-	+	-	+	-	+	+
	68 (3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	74 (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	75 (1)	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-
	77 (1)	-	+	-	-	+	-	-	-	-	+	-	-	+	-	+	+	-	-	-	-	-	+	-	+	+
Cardiff Hosp. 3	84 (1)	+	+	+	+	+	-	-	-	+	+	-	-	+	-	+	+	-	+	-	-	-	+	-	+	+
	87 (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	88 (1)	-	-	-	-	+	+	-	-	-	+	-	-	+	-	+	-	-	-	-	-	-	+	-	-	-
	90 (14)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	95 (1)	+	+	+	+	+	-	+	-	-	-	+	-	+	-	+	-	+	-	-	+	-	+	-	+	+
	101 (1)	+	+	+	+	+	-	-	-	-	-	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+
	103 (1)	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+	-	-	-	-	-	-	-

Table III Bacteriophage and bacteriocin typing of 58 of the 70 clinical isolates of *Providencia*¹The figure in brackets indicates the number of isolates giving the identical pattern to that shown for the numbered strain.

+ Indicates activity of the phage or bacteriocin produced by the designated strain on the lawn of indicator; - indicates no activity.

was taken for further study as the possible repeated use of MIC data from the same strain could introduce a bias into the calculation of the Spearman and Kendal rank correlation coefficients. The results of this typing therefore indicate that the clinical isolates obtained from the five hospitals represented at least 27 distinct strains.

SENSITIVITY OF STRAINS TO ANTISEPTICS

MIC determinations were performed on cultures of the 27 clinical strains together with the four NCTC strains and the control organisms *E. coli* 10418 and *P. mirabilis* 61. Summaries of these results are shown in figures 1 and 2. Inspection of the MIC values obtained for the 31 strains of *Providencia* seemed to indicate that resistance to chlorhexidine was associated with resistance to the other cationic agents. Fig. 3, for example, shows the results of plotting the MIC of Resiguard for each strain against their MICs for (a) cetrimide, and (b) Hycolin. The possibility of significant correlations between sensitivities of the strains to the six antibacterials was examined using non-parametric correlation tests. The strains were ranked on the basis of their MIC values to the various disinfectants, and Kendal and Spearman correlation coefficients were calculated between all possible pairs of rankings using the computer Statistical Package for the Social

Sciences (Nie *et al.*, 1970). The results of such analysis are shown in table IV.

In a further series of experiments the bactericidal activity of disinfectants was tested against four strains of *Providencia* designated as chlorhexidine resistant on the basis of their MICs, each strain having a distinct typing pattern and coming from a different hospital. Two chlorhexidine sensitive *Providencia* strains and two control organisms, *E. coli* 10418 and *P. mirabilis* NCIB 60, were also examined. In these tests the log₁₀ of the reduction in viable count occurring on exposure of each strain to the disinfectant for 10 minutes was determined. The concentrations of the agents were chosen so as to produce about a 2-3 log reduction in counts of the sensitive strains. In the cases of the cationic antiseptics, however, higher concentrations than this had to be used in order to register any significant killing of strains 5, 8, 66, and 95. The eight strains were tested as a batch against each antiseptic in turn. Table V shows the mean of three logreduction values obtained for each strain against each antibacterial agent.

OBSERVATIONS ON CHLORHEXIDINE-SENSITIVE MUTANTS OF PROVIDENCIA

A total of eight chlorhexidine-sensitive mutants were isolated from *Providencia* strain 66 after treat-

Fig 1 Sensitivity of clinical isolates of *Providencia* to the antibacterials chlorhexidine, cetrimide, and glutaraldehyde.

Fig 2 Sensitivity of clinical isolates of *Providencia* to the antibacterials Resiguard, Hycolin, and phenyl mercuric nitrate.

Fig 3 A comparison of the sensitivity of *Providencia* strains to (A) Resiguard and cetrimide and (B) Resiguard and Hycolin. The figures in the open circles represent the number of strains having that level of resistance (the scales are $\log_2$ of MIC values). R_k refers to the Kendall rank correlation coefficient.

		Kendal's rank correlation coefficient											
		Chlorhexidine	Cetrimide	Resiguard	Glutaraldehyde	Hycolin	Phenyl mercuric nitrate						
Spearman's rank correlation coefficient	Chlorhexidine	—	(—)	0.64 ¹	(0.64 ¹)	0.62 ¹	(0.52 ¹)	0.17	(0.18)	0.15	(0.18)	—0.11	(0.35)
	Cetrimide	0.71 ¹	(0.69 ¹)	—	(—)	0.70 ¹	(0.68 ¹)	0.04	(0.05)	0.05	(0.24)	0.04	(0.36)
	Resiguard	0.68 ¹	(0.57 ¹)	0.76 ¹	(0.74 ¹)	—	(—)	0.15	(0.01)	0.10	(0.18)	0.03	(0.26)
	Glutaraldehyde	0.20	(0.20)	0.05	(0.06)	0.18	(— 0.01)	—	(—)	0.77 ¹	(0.36)	0.32	(0.37)
	Hycolin	0.17	(0.21)	0.07	(0.28)	0.12	(0.20)	0.81 ¹	(0.39)	—	(—)	0.51 ¹	(0.53 ¹)
	Phenyl mercuric nitrate	—0.11	(0.40)	0.06	(0.42)	0.04	(0.29)	0.36	(0.40)	0.56 ¹	(0.58 ¹)	—	(—)

Table IV *Kendal and Spearman rank correlation coefficients between sensitivities of Providence to pairs of antiseptics*¹Indicates that these correlations are highly significant ($P < 0.001$).

The values in brackets were obtained when the MIC screening was repeated on a separate occasion.

Log₁₀ of reduction¹ in viable count when bacterial suspensions were exposed to disinfectants for 10 minutes

Antiseptic	Chlorhexidine sensitive strains (group S)				Chlorhexidine resistant strains (group R)			
	<i>E. coli</i> 10418	<i>P. mirabilis</i> NCIB 60	<i>Pv strain</i> 59	<i>Pv NCTC</i> 2481	<i>Pv strain</i> 5	<i>Pv strain</i> 8	<i>Pv strain</i> 66	<i>Pv strain</i> 95
Chlorhexidine (100 µg/ml)	5.73	5.30	5.08	4.84	1.08	0.49	0.52	0.53
Benzalkonium chloride (100 µg/ml)	5.67	5.10	6.30	5.67	1.24	0.75	0.52	1.01
Resiguard (1 in 2000 dilution of commercial concentrate)	5.57	5.37	4.30	5.41	0.41	0.30	0.28	0.10
Betadine (1 in 400 dil. of commercial concentrate)	2.23	3.19	3.32	3.29	4.28	3.64	5.47	3.67
Milton (1 in 400 dil. of commercial concentrate)	3.11	1.49	0.86	1.08	1.67	1.41	1.91	1.37
Hycolin (1 in 2000 dil. of commercial concentrate)	1.40	3.73	0.65	1.42	0.87	0.45	1.03	0.04

Table V *Bactericidal action of six disinfectants against Providence strains and two reference organisms*

Mean of values obtained from three separate experiments.

Organism	Minimum inhibitory concentration (MIC)					
	Chlorhexidine (µg/ml)	Cetrimide (µg/ml)	Resiguard (% v/v of commercial concentrate)	Glutaraldehyde (µg/ml)	Hycolin (% v/v of commercial concentrate)	Phenyl mercuric nitrate (µg/ml)
Providence 66	1600	1600	> 1.0%	800	0.02	0.5
Mutant strain 1	10	25	≧ 0.03	400	0.02	0.5
2	10	25	≧ 0.03	400	0.02	0.5
3	25	25	≧ 0.03	400	0.02	0.25
4	10	25	≧ 0.03	800	0.01	0.25
5	10	25	≧ 0.03	400	0.02	0.25
6	10	10	≧ 0.03	400	0.02	0.25
7	10	25	≧ 0.03	400	0.02	0.50
8	10	25	≧ 0.03	400	0.02	0.25
<i>E. coli</i> 10418	5	25	≧ 0.03	800	0.08	0.5
<i>P. mirabilis</i> 61	800	400	0.5	400	0.04	1.0

Table VI *Sensitivity of Providence strain 66 and its mutants to six antiseptics*

ment with NTG. These mutants were subjected to typing and gave the pattern of reactions characteristic of strain 66, indicating that they were derivatives of the parent strain. They were then examined

for their MICs to the six antibacterial agents shown in table I; *E. coli* 10418, *P. mirabilis* strain 61, and Providence strain 66 were included in these tests. The results presented in table VI show that the loss of

resistance to chlorhexidine has been accompanied by concomitant increase in sensitivity to cetrime and Resiguard. The mutants were not, however, markedly more sensitive to phenyl mercuric nitrate, glutaraldehyde or Hycolin than the parent strain.

Discussion

All 70 isolates of *Providencia* used in this work proved to be *Providencia B (Providencia stuartii)*. In fact during the collection of specimens two isolates of *Providencia A (Providencia alcalifaciens)* were also obtained but these were not reported on in this study. Many of the earlier studies on the incidence of *Providencia* infections did not distinguish between these two species. Dobrey (1971), however, reported that 140 isolates collected from 48 different patients in a Canadian hospital all proved to be *Providencia B*. Solberg and Matsen (1971) observed that 34 of 45 *Providencia* strains isolated mainly from urinary tract infections, but also from postoperative wound infections in a Minnesota Medical Centre, were type B. Overturf *et al* (1974) examined 114 isolates, 94 coming from 73 patients in a burns unit of a California hospital and the balance from other hospital services, and found that 102 were *Providencia stuartii*. These authors also found that strains characterized as *Providencia alcalifaciens* were susceptible to most antibiotics, whereas *Providencia stuartii* demonstrated marked antibiotic resistance, a finding that had been previously recorded by Middleton (1958). The results presented in table VII show that the strains isolated in this study are multiply antibiotic resistant.

Dutton and Ralston (1957) reported that a single biochemical type of *Providencia* was isolated from 17 of their patients with urinary tract infections. Milner (1963) similarly found that 38 strains from urinary infections in paraplegic patients belonged to biotype 27 (Ewing *et al*, 1954) and considered that this finding favoured the theory that infections of the urinary tract with this organism are nosocomial rather than autogenous infections from the patient's own bowel flora. Solberg and Matsen (1971) used biochemical and serotyping for *Pro-*

vidence infections and found that usually two or three patients were infected with strains of the same biotype at the same time and suggest that this again is strong evidence for the nosocomial nature of many infections. The bacteriophage/bacteriocin typing of strains performed in this study would seem to support these views. Examination of table III shows that strains 23 and 7, isolated from the Dublin hospitals, exhibit the same pattern of sensitivity to six different phage/bacteriocin preparations and further that the phage/bacteriocins produced by these strains had identical patterns of activity against lawns of five of the other strains. This evidence indicates that in the Dublin hospitals 17 of the 20 patients were infected with the same strain of *Providencia*. In two of the Cardiff hospitals the isolation of any one strain from two or more patients occurred much less frequently. In the paraplegic unit of the third Cardiff hospital, however, 14 patients were found to be infected with the same bacteriophage/bacteriocin type of *Providencia*.

Lysogeny and bacteriocinogeny appear to be an extremely common phenomenon in this group of bacteria, and it is possible that a typing system for this organism could be established by refining and extending the system used in this study.

The results of the MIC screening on the 31 strains of *Providencia* and the control organisms, *E. coli* 10418 and *P. mirabilis* 61, presented in figs 1 and 2, show that this organism can be even more resistant to cationic antiseptics than the strains of *P. mirabilis* reported as chlorhexidine resistant (Stickler, 1974). The majority of the strains in this study had MICs for chlorhexidine over 300-fold higher than that of the control *E. coli* strain. They are similarly considerably more resistant to cetrime and Resiguard. In the cases of Hycolin, glutaraldehyde, and phenyl mercuric nitrate, however, the MICs of the *Providencia* strains are similar to those of the control strain.

It is quite likely that the levels recorded for the MIC of glutaraldehyde are all raised due to the inactivation of this agent by the peptones present in nutrient agar (Russell and Munton, 1974).

Examination of the MIC data for the *Providencia* strains strongly suggested a correlation between the degree of resistance to the various cationic agents. Fig. 3 shows the relationship obtained when the MIC values for Resiguard for each strain are plotted against those for cetrime and Hycolin. The Kendal and Spearman rank correlation coefficients (table IV) show that there were highly significant correlations (the probability that such values for the correlation coefficients could be due to chance being $p < 0.001$) between the resistance of the strains to the three cationic antiseptics. There were no such highly

No. of strains tested	No. resistant to each antibiotic							
	CN	CT	F	G	K	Pn	SXT	Te
27	22	27	23	24	8	23	23	27

Table VII Antibiotic sensitivity of *Providencia* isolates

CN = gentamicin, CT = colistin, F = nitrofurantoin, G = sulphafurazole, K = kanamycin, Pn = ampicillin, SXT = sulphamethoxazole/trimethoprim, Te = tetracycline.

significant correlations between the cationic agents and any of the other three antibacterials.

The tests for bactericidal activity (table V) also show this pattern. The data presented in this table were analysed for differences between mean log reduction in counts by one-way analysis of variance and the sum of squares simultaneous test procedure (Gabriel, 1964). It was found that within the group S or group R there were no significant differences between the means of the log reduction values. However, there was a highly significant difference ($P < 0.001$) between the log reductions of the two groups for chlorhexidine, cetrимide, and Resiguard. For Betadine, Hycolin, and hypochlorite there was no significant difference in the mean values for the two groups.

The results of the MIC determinations on the chlorhexidine-sensitive mutants of Providence strain 66 (table VI) indicate that loss of chlorhexidine resistance is accompanied by loss of resistance to cetrимide and Resiguard but results in no substantial difference in sensitivity to the other three agents.

In summary, it appears that the results from the MIC determinations, the tests for bactericidal activity, and the examination of the properties of chlorhexidine-sensitive mutants, all indicate the existence of cross-resistance to cationic antiseptics in strains of Providence. The resistances of these strains to the cationic antiseptics were of course registered in laboratory tests which do not adequately reflect the conditions under which the antiseptics are required to work in practice. However, as many of these strains were isolated from situations in which antiseptics are extensively employed, it is not unreasonable to suppose that the levels of resistance recorded in the MIC and bactericidal tests are significant and reflect an enhanced ability of these organisms to survive in the hospital environment.

Resistance to quaternary ammonium compounds has been reported in *E. coli* (Chaplin, 1951), *Serratia marcescens* (Chaplin, 1952), and *Ps. aeruginosa* (MacGregor and Elliker, 1958; Adair *et al.*, 1971), and resistance to chlorhexidine in *Ps. aeruginosa* (Bentley *et al.*, 1968) and *P. mirabilis* (Stickler, 1974). In a number of these cases the resistance has been attributed to changes in the chemical structure of the Gram-negative envelope (Davies and Roberts, 1969; Anderes *et al.*, 1971), which prevent the adsorption of the cationic agents onto the underlying membrane, where they are believed to exert their antibacterial effect (Hugo, 1967; Davies and Field, 1969). It is possible that the cell envelope of Providence has a structure that protects the cytoplasmic membrane from disruption by cationic antiseptics and results in a level of intrinsic resistance to these agents.

In conclusion, it seems that Providence B (*Providencia stuartii*) is an organism extremely well equipped to survive in the hospital environment, being not only multiply antibiotic resistant but also having a degree of resistance to a number of extensively used antiseptics.

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