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Variation in skin and environmental survival of hospital gentamicin-resistant enterobacteria

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SUMMARY

During a period when 245 patients were infected by or harboured gentamicin-resistant enterobacteria, random sampling showed hand carriage in 33% of affected patients but in only 5% of attendant staff. Only klebsiellae were isolated from the latter. Recovery was commoner from the hands of bed-ridden patients or faecal carriers and significantly more frequent for klebsiellae (37%) and enterobacter (33%) than citrobacter (5·6%) and *E. coli* (5·0%). Similarly, survival on forearms of volunteers was much longer for klebsiellae and enterobacter than for citrobacter or *E. coli* (means respectively were 70, 45, 10 and 13 min), and on dry surfaces (means respectively were 28, 26, 3 and 7 h). Klebsiellae were isolated from 17 of the 56 dry environmental surfaces sampled. The presence of plasmid resistance determinants had no effect on survival times, either on the skin or following drying onto formica surfaces. On dry surfaces 9·5% of *E. coli* but only 1·3% of klebsiellae lost resistance to gentamicin. These findings accord well with recent experience in which gentamicin-resistant klebsiellae have been involved to a much greater extent than other resistant enterobacteria in hospital infection.

INTRODUCTION

The Royal Liverpool Hospital opened in October 1978 and since January 1979 has experienced an increase in infection with gentamicin-resistant Gram-negative bacteria. By the beginning of December 1980, 245 patients had been involved. Most infections were caused by klebsiella species (30 different serotypes) but a smaller number were due to enterobacter, citrobacter and *E. coli*.

Previous reports (Salzman, Clark & Klemm, 1967; Knittle, Eitzmann & Baer, 1975; Casewell *et al.* 1977; Curie *et al.* 1978; Haverkorn & Michel, 1979; Riser, Noone, & Howard, 1980; Casewell, 1980) have stressed the importance of hand carriage of klebsiellase by hospital staff in transmission of infection. Casewell & Phillips (1977) showed that klebsiella, which were not gentamicin-resistant, could survive for up to 150 min on the hands of volunteers. Little importance has been attached to environmental sources other than foodstuffs (Shooter *et al.* 1971; Casewell & Phillips, 1978).

Here, the results of investigations on the carriage of gentamicin-resistant enterobacteria on the hands of staff and patients are compared with environmental surveys. In addition, we describe appreciable differences in the abilities of various

Gram-negative bacteria, both resistant and sensitive, to survive on the skin of volunteers, and drying on formica surfaces.

MATERIALS AND METHODS

Ward and patient survey

The hands of patients harbouring gentamicin-resistant enterobacteria and their attendant staff were sampled using a modification of the method described by Casewell & Phillips (1977). Briefly, 10 ml of sterile normal saline was poured into an extra-large sterile disposable glove (Disposa-Glove Ethicon Ltd), the subject's hand was placed in the glove, rubbed for 1 min and removed. Using the same glove, the procedure was repeated with the other hand. The fluid was then poured into sterile screw-capped bottles and taken to the laboratory. 1 ml was spread onto two plates (9 cm in diameter) containing MacConkey agar alone or MacConkey agar with gentamicin added to a final concentration of 4 mg/l. Thus, numbers of 'transient' enterobacteria removed by washing were estimated. Faecal carriage was investigated using rectal swabs inoculated onto the same media. Prior experiments had shown that well taken rectal swabs were as sensitive as faecal cultures for detecting these organisms.

The environment (floors, formica surfaces, dressing trolleys) was sampled by rubbing absorbent swabs, which had been moistened with sterile nutrient broth, over measured areas. Swabs were cultured on the same media.

Gentamicin-resistant enterobacteria (R) were identified using Donovan's medium (Donovan, 1966) and API 20E system (API Products Ltd.). Antimicrobial susceptibilities were determined by a controlled disk diffusion method (Stokes & Waterworth, 1972) on sensitivity agar (Oxoid Ltd.), containing 7% lysed horse blood. The gentamicin-resistant enterobacteria (M.I.C. 200 –> 500 mg/l) were not inhibited at all by disks containing trimethoprim (1·25 μg), sulphamethoxazole (50 μg), tetracycline (10 μg), tobramycin (10 μg), neomycin (10 μg), streptomycin (10 μg), spectinomycin (50 μg), ampicillin (25 μg) or chloramphenicol (25 μg). Resistance to each of these antibiotics was transferable to *E. coli* K12.

The klebsiellae were subjected to capsular serotyping by counter-immunoelectrophoresis (Palfreyman, 1978). The serotyping of klebsiellae with capsular antigens K2, K39, K43 and K68 was subsequently performed by us using counter-immunoelectrophoresis before and after each drying experiment.

Organisms

The organisms used were all isolated, except where stated, from patients in the Royal Liverpool Hospital and had not previously been subcultured more than three times. They were grown in nutrient broth for 18 h at 37 °C, washed twice and resuspended in sterile distilled water.

Skin survival

Suspensions were diluted to give an inoculum of approximately 10^3 organisms in 50 μl. The forearm of human adult volunteers was chosen as an accessible site where several organisms could be tested simultaneously under similar conditions. Subjects

were asked not to use disinfectant soaps for washing for several days prior to sampling and not to wash their arms at all during the preceding 18 h. 50 μl was placed on each of ten marked spots and allowed to dry. The sites were sampled sequentially at 10 min intervals for the first hour and at 15 min intervals thereafter by means of absorbent swabs, moistened with sterile nutrient broth. The swabs were then spread on blood agar plates. The survival time was defined as the last time at which the inoculated enterobacteria could be recovered.

Drying on surfaces

On the day prior to the experiment, formica surfaces were cleaned with a phenolic detergent and, before starting, with isopropanol (70% v/v) followed by two rinses with sterile distilled water. 50 μl (containing approximately 10^5 to 10^6 organisms) of suspensions were placed on pre-marked spots and allowed to dry (30–45 min). Using moistened swabs, the sites were sampled sequentially every hour for the first 10 h and thereafter every 2 h. The swabs were spread onto blood agar plates. Surviving colonies of the inoculated organisms were subcultured onto MacConkey agar containing either gentamicin (4 mg l^{-1}) or tetracycline (10 mg l^{-1}) or onto sensitivity agar containing lysed blood (7% v/v) and trimethoprim (10 mg l^{-1}). Colonies not growing on subculture were tested for their susceptibility to a variety of antimicrobials by the disk diffusion method (Stokes & Waterworth, 1972).

The room temperature during drying varied between 19 and 24 °C and the relative humidity between 40 and 50%, conditions similar to those found on the hospital wards.

RESULTS

Handwashing – Staff

The hands of 138 members of staff working on affected wards were sampled prior to the introduction of routine hand cleansing with chorhexidine (Hibiscrub, ICI Ltd). The members of staff included doctors (21), ward nurses (82), colostomy care nurses (3), physiotherapists (10) and ward orderlies and cleaners (22). The sampling was random and was not necessarily carried out immediately after the subject had attended an affected patient. Only eight (5·8%) members of staff were found to be carrying gentamicin-resistant klebsiellae, on their hands (Table 1). The numbers recovered were small. The hands of nurses were significantly less contaminated than those of ward orderlies ($t = 2{\cdot}99$, $P < 0{\cdot}05$, by Student's t-test). On only one occasion was a klebsiella isolated from a member of staff's hands that had the same serotype as an organism affecting a patient in the same ward. Klebsiellae isolated from staff hands did not affect patients on those wards in the ensuing 18 months. Gentamicin-resistant organisms other than klebsiellae were not isolated from the hands of members of staff.

Handwashing – Patients

In contrast, gentamicin-resistant klebsiellae were more readily isolated from the hands of patients infected by or carrying such organisms (χ^2, 36·2, $P < 0{\cdot}001$). The organisms were isolated from the hands of 74 (32·9%) of 225 patients tested. The

Table 1. *Carriage of gentamicin-resistant klebsiellae on the hands of 8 members of staff*

Subject	Serotype	Indole	Serotype present in ward: At the time of sampling	Serotype present in ward: After sampling	No. organisms recovered from hands
Nurse A	NT	+	−	−	20
Nurse B	K39	−	+	−	150
Nurse C	K35	+	−	−	30
Nurse D	NT	−	−	−	30
Nurse E	K8/35	+	−	−	90
Orderly A	K55	+	−	−	400
Orderly B	K5	+	−	−	500
Orderly C	K20	+	−	−	1000

Table 2. *Recovery of gentamicin-resistant enterobacteria from the hand of affected patients*

Organism	Number examined	Recovery from hands: numbers (%) positive	P*
Klebsiellae	195	73 (37·4)	—
Enterobacter spp.	6	2 (33·3)	> 0·5
Citrobacter spp.	20	1 (5·0)	< 0·01
E. coli	18	1 (5·6)	< 0·01
Totals	239	77 (32·2)	

* P is the probability that the carriage rate for the organisms was different from the rate for klebsiellae (by χ^2 test).

numbers of organisms recovered varied between 10 and 10000 (mean 964); more than from staff members' hands though not significantly so ($P > 0·1$).

Patients harbouring gentamicin-resistant klebsiella or enterobacter species were more likely to carry these organisms than those patients harbouring gentamicin-resistant citrobacter species or *E. coli* (Table 2). The total of 239 shown in Table 2 is greater than the number of patients examined because of a few patients were harbouring more than one gentamicin-resistant strain. When organisms were isolated from hands of patients infected by both klebsiellae and citrobacter or klebsiellae and *E. coli*, klebsiellae alone were recovered.

If the patient was bed-ridden or showed faecal carriage of the organisms, there was an increased chance of hand washings being positive (Table 3).

Environment

Gentamicin-resistant klebsiellae were isolated from 17 (30·4 %) of 56 horizontal dry surfaces sampled in proximity to patients (locker tops, floors, shelves) or sites in the sluice room and dressing trolleys. In contrast resistant citrobacter, enterobacter or *E. coli* were never isolated.

Table 3. *Relationships between hand carriage of gentamicin-resistant enterobacteria and faecal carriage or being bed-ridden*

	Hand washing +ve	Handwashing −ve	P*
Faecal carriage	60/144 (41·7%)	15/81 (18·5%)	< 0·001
Bed-ridden	70/76 (92·1%)	27/159 (17%)	< 0·001

* P is the probability that the proportion of patients carrying gentamicin-resistant organisms on their hands was different from the proportion not showing hand carriage (by χ^2 test).

Table 4. *Survival of gentamicin-resistant enterobacteria on the skin of the human forearm*

Organism	Survival time*	P†
Klebsiella aerogenes (K39)	70·7 ± 28·6 (7)	—
Klebsiella aerogenes (K39) 'cured' variant	50·0 ± 19·6 (7)	> 0·1
Klebsiella aerogenes var. oxytoca (K55)	37·5 ± 7·5 (8)	< 0·05
Enterobacter cloacae	45·0 ± 7·5 (8)	> 0·1
E. coli	12·9 ± 7·0 (7)	< 0·001
Citrobacter freundii	9·4 ± 1·7 (8)	< 0·001

* The results are expressed as the survival time in minutes ± the standard deviation for the number of experiments in parentheses.

† P is the probability that the survival of the organism was different from the survival of *Klebsiella aerogenes* (K39) by Student's t-test.

Skin survival

From Table 4 it can be seen that there was no significant difference between the survival times of a gentamicin-resistant klebsiella (K39) and its 'cured' sensitive variant, when inoculated onto the forearms of volunteers. The initial inoculum was approximately 10^3 organisms which is slightly higher than the numbers isolated from patients' hands. An *Enterobacter cloacae* survived for a similar period. A gentamicin-resistant strain of klebsiella (K55) that had been isolated from a sluice survived significantly less well than the strain (K39) isolated from an infected patient. Both a *Citrobacter freundii* and an *E. coli* survived for significantly shorter periods than the klebsiella (K39). There was no loss of resistance observed in surviving colonies. Preliminary experiments revealed that, even with an inoculum of 10^6 organisms, the gentamicin-resistant klebsiella survived for only 90 min.

Survival on dry surfaces

With inocula of either 10^5 or 10^6 organisms, gentamicin-resistant strains of *Klebsiella aerogenes* (15 different serotypes) and *Enterobacter cloacae* survived for significantly longer periods than similarly resistant strains of *Citrobacter freundii* and *E. coli* (four different biotypes) (Table 5). There was no significant difference in the survival times of the 15 different serotypes of gentamicin-resistant klebsiellae (data not shown).

The possibility that the presence of a large resistance plasmid (*c.* 75×10^6) might also be associated with differences in survival was tested by comparing a

Table 5. *Survival of gentamicin-resistant enterobacteria on dry formica surfaces*

Organism	Inoculum (orgs)	Survival (h ± S.D.)	*P*1*	*P*2*
Klebsiella spp.	10^5	13·7 ± 9·2 (44)†	—	—
	10^6	28·1 ± 7·0 (65)	—	—
Enterobacter cloacae	10^5	5·5 ± 2·6 (4)	> 0·05	—
	10^6	26·0 ± 4·0 (4)	—	> 0·5
Citrobacter freundii	10^5	1·8 ± 0·4 (4)	< 0·02	—
	10^6	3·0 ± 1·0 (4)	—	< 0·001
E. coli	10^5	3·6 ± 2·5 (17)	< 0·001	—
	10^6	6·9 ± 2·5 (17)	—	< 0·001

* *P*1 and *P*2 are the probabilities respectively that inocula of 10^5 and 10^6 organisms survived less well than a similar inoculum of klebsiellae (by Student's *t*-test).

† In parentheses: number of experiments.

Table 6. *Comparison of the survival on dry formica surfaces of klebsiellae and* E. coli *with and without antibiotic resistance plasmids*

Organisms	Survival time (h ± S.D.)	*P*1*	*P*2†
Gentamicin-resistant klebsiella (K39)	30·0 ± 7·6 (10)	—	NA*
'Cured' variant	36·5 ± 8·3 (6)	> 0·05	NA
Sensitive klebsiella (K15)	33·5 ± 8·7 (4)	> 0·5	NA
E. coli K12	2·1 ± 0·8 (9)	< 0·001	—
Transconjugant K12 (from K39)	2·0 ± 0·8 (3)	< 0·001	> 0·5

* *P*1 is the probability that the survival of the organisms differed from that of the gentamicin-resistant klebsiella (K39).

† *P*2 is the probability that the survival of the transconjugant differs from that of K12.

NA, not applicable.

In parentheses: number of experiments.

gentamicin-resistant klebsiella (K39) with its cured variant and with a sensitive klebsiella that had been isolated from an infected urine. In addition, the resistance to drying of *E. coli* K12 was compared with that of K12 that had received the resistance plasmid from the resistant klebsiella (K39). There was no significant difference in the survival times of the three kelbsiellae. The survival of *E. coli* K12 was not altered by the presence of a resistance plasmid (Table 6).

The possibility that the thick hydrophilic capsule of klebsiellae might affect survival was tested by comparing a gentamicin-resistant strain (K5) and a similarly resistant non-capsulate strain, both of which had been isolated from the same sample from a patient (both isolates had the same biotype and the same klebecine type). In four separate experiments the capsulate strain (K5) survived for 27·8 ± 3·2 h and the non-capsulate strain for 25·8 ± 4·6 h. This difference was not statistically significant by Student's *t* test. In addition, the *Enterobacter cloacae* included in Table 5 survived as long as capsulate strains of klebsiellae but unlike the klebsiellae did not show capsules on nigrosin testing. As a control for the drying experiments, colonies were serotyped before and after drying (for K39, K2, K68, K43) and their resistance pattern determined. It appeared that some of the

surviving colonies had lost some or all of their resistance. Therefore, surviving colonies were replicated on sets of plates containing gentamicin (4 mg l^{-1}), tetracycline (10 mg l^{-1}) and trimethoprim (10 mg l^{-1}). Out of 211 surviving colonies of resistant *E. coli*, 20 (9·5%) had lost resistance to gentamicin, trimethoprim and tetracycline and a further two had lost resistance to trimethoprim alone. By contrast, only 16 (1·3%) colonies of resistant klebsiellae out of the 1196 survivors tested had lost resistance to gentamicin, trimethoprim and tetracycline. Loss of resistance occurred maximally between 20 and 28 h (for kebsiellae) when the numbers of surviving organisms were small (approx. 0·001% of the original inoculum). Loss of resistance occurred significantly more frequently in *E. coli* than in klebsiellae (χ^2, 47·7, $P < 0·001$).

DISCUSSION

The isolation rates for klebsiellae from the hands of attendant staff were less than those reported by Curie *et al.* (1978) but their sampling was carried out immediately after attendance on infected patients. However, in our experience, there was only one instance when a klebsiella of the same serotype as caused infection on the same ward was isolated from staff hands. Otherwise, with one exception, all klebsiellae from staff hands were indole positive. Such strains were found more frequently in sluices than anywhere else and contamination of orderlies' hands was heavier. Subsequent testing of those members of staff found to be carrying gentamicin-resistant klebsiellae on their hands was negative on at least three occasions indicating that these organisms had not become part of the resident hand flora. This has been described previously (Casewell *et al.* 1977; Parry *et al.* 1980). The low mean counts of gentamicin-resistant klebsiellae recovered from hands confirms the findings of Casewell *et al.* (1977) and suggests that the dose required to establish carriage or even infection may be low.

Our investigations on skin survival confirm and extend those of Casewell & Phillips (1977) to show that gentamicin-resistant kelbsiellae can survive on skin. We found that the presence of a large resistance plasmid did not affect this survival Our observation that a *Klebsiella oxytoca* (K55) isolated from a sluice room survived less well than a *Klebsiella aerogenes* (K39) from an infected patient, is similar to the findings of others (Cooke *et al.*, personal communication) that environmental strains of klebsiellae survive less well on skin than do epidemic strains isolated from infected patients. From the shorter skin survival of *E. coli* and citrobacter species, it might be expected that those organisms would be less often isolated from patients harbouring them, and this was so (Table 2). However, other factors determine carriage of gentamicin-resistant enterobacteria on hands. Patients who were bed-ridden and those who were faecal carriers were more likely to carry the organisms on their hands. It is possible that such patients are more likely to disseminate resistant organisms.

It has been shown previously that Gram-positive organisms survive longer than Gram-negative on drying (Pettit & Lowbury, 1968), but little is known of the comparative ability of Gram-negative organisms to survive drying. The isolation of gentamicin-resistant klebsiellae from dry surfaces agrees with the report by Cook *et al.* (1979) who found klebsiellae on 12 of 129 dry surfaces. These findings

correlate with the ability of klebsiellae to survive drying (Table 5). Unlike klebsiellae, enterobacter species which also survived drying for relatively long periods, were not found on environmental surfaces. However, only relatively small numbers of patients harboured this organism (6 of 245 studied). The *in vitro* experiments on skin survival and resistance to drying seem to correlate well with epidemiological data. Clusters of infections by gentamicin-resistant klebsiellae occurred but infections by *E. coli* and citrobacter species were sporadic. The inocula used for drying experiments would be equivalent to 1 ml of infected urine falling on a floor. Preliminary experiments revealed that a similar inoculum of klebsiellae in serum broth (50% v/v) survived for more than 2 weeks and this would possibly be equivalent to 1 ml of purulent material drying on a surface. The mechanisms responsible for death of the organisms on dry surfaces are presumably analogous to those observed for nebulized bacteria (Cox & Baldwin, 1966; Strange & Cox, 1976). That all the enterobacteria tested survived much longer on dry surfaces than on the human arm (Tables 4 and 5) indicates the bactericidal potential of the latter for this type of organism.

The loss of plasmid mediated resistance appeared to have been due to loss of, or damage to, the plasmid rather than the increased stability of R minus strains. Resistant and R-minus 'cured' variants survived for similar periods and the plasmid was stable both *in vivo* and *in vitro*. Less than 0·001% of cells grown in continuous culture for 1 week lost resistance. Since it is known that phage DNA is more sensitive to damage by desiccation than chromosomal DNA (Webb, 1968), it is not surprising that plasmid-mediated antibiotic resistance was also lost on drying the host organism. Perhaps more gentamicin-resistant strains would have been isolated from horizontal dry surfaces if they had not been 'cured' by desiccation. In fact, drying these resistant strains of klebsiella on formica surfaces, proved the only effective method for 'curing' them. Prolonged incubation of the klebsiellae in the presence of sub-inhibitory concentrations of ethidium bromide, acridine orange or mitomycin C, had no effect but did 'cure' wild strains of *Citrobacter freundii* and *E. coli*.

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