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Hand carriage of aerobic Gram-negative rods may not be transient

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SUMMARY

In order to determine whether hand carriage of aerobic Gram-negative rods is continuous we used the glove-handwash technique to sample the hands of two groups (four each) of health care workers with normal hands (surgical intensive care unit, medical ward) and one group (four) with hand dermatitis (HD) and a group (five) of control subjects – secretaries with no exposure to patients. Each subject was sampled repeatedly over three to six weeks. The mean number of samples for each group was 25·2, 23·2, 19·8 and 25·8 respectively. The HD group had more samples positive for aerobic Gram-negative rods than did the other two groups of health care workers while the control group had more samples positive than any of the three health care groups.

Using various typing schemes and the following definition of continuous carriage (the isolation of an organism of the same serotype, pyocin type or biotype from more than two handwash samples) we found that 4 of 11 subjects from whom *Klebsiella pneumoniae* was isolated carried this organism continuously; 2 of 3 carried *Pseudomonas aeruginosa* continuously and 4 of 5 of the control subjects carried the same biotype of *Enterobacter agglomerans* continuously.

We conclude that continuous hand carriage of aerobic Gram-negative rods is common and, among health care workers, those with hand dermatitis carry Gram-negative rods more frequently and in greater numbers than health care workers without hand dermatitis.

INTRODUCTION

Most nosocomial infections are caused by Gram-negative bacilli (Schaffner, 1977; Goldman, 1981) and one of the major routes of transmission for these organisms is the contaminated hands of health care personnel (Salzman, Clark & Klemm, 1968; Bruun & Solberg, 1973; Maki, 1978; Casewell & Phillips, 1977; French *et al.* 1980). Most of the studies of hand carriage of aerobic Gram-negative rods by health care personnel have been carried out as part of the investigation

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of an outbreak of nosocomial infection. Maki (1978) has questioned the relevance of epidemic studies to endemic situations and emphasized the need for further investigation to elucidate the epidemiology of endemic hand carriage of nosocomial pathogens.

In a previous study we delineated the incidence of endemic hand carriage of aerobic Gram-negative rods by several groups of health care workers and control subjects. In that study, one-third of the health care workers and two-thirds of the control subjects had aerobic Gram-negative rods isolated from single handwash cultures (Adams & Marrie, 1982).

Gram-negative bacilli are considered to be transient organisms on the skin (Buxton *et al.* 1978) and routine handwashing is usually sufficient to remove them (Ayliffe, 1980). However, Casewell *et al.* (1977) demonstrated hand carriage of an epidemic strain of *Klebsiella aerogenes* by a nurse for a period of 62 days. Some individuals, especially those with hand dermatitis, may persistently carry Gram-negative organisms on their hands despite vigorous handwashing (Knittle, Eitzman & Baer, 1975; Parry *et al.* 1980). Such individuals may represent reservoirs for the spread of nosocomial infections (Meers, Foster & Churcher, 1978).

The objective of this study was to determine whether or not hand carriage of aerobic Gram-negative rods is transitory.

MATERIALS AND METHODS

Subjects

Seventeen subjects participated in this study. All the health care worker groups were employed at the Victoria General Hospital, Halifax, Nova Scotia, and included a group of four nurses from a surgical intensive care unit, four nurses from a medical ward and four subjects (three nurses, one ward aide) with hand dermatitis. The five control subjects were medical school secretaries; none had any patient contact. All subjects were female. Since there was no correlation between age and hand carriage of Gram-negative rods in our previous study, no attempt was made to match subjects according to age. With the exception of the dermatitis group, all subjects were selected randomly. The group with hand dermatitis consisted of volunteers who responded to our request in the hospital newsletter. All subjects had their hands examined by one of us (T.J.M.). All five subjects with dermatitis had redness and scaling of their hands. None had an exudative dermatitis.

Sampling protocol

These studies were performed between July and November 1980. Each subject's dominant hand was sampled twice per working day, and most samples were taken approximately 4 h apart (range = 2–8 h). Occasionally it was only possible to sample subjects once per day. As in our previous study, sampling of hands was done at a random point in time with respect to the activities of the worker (i.e. patient care, and time from last handwash). Each subject was told at the commencement of the study to try not to let the experiment influence her normal handwashing behaviour.

Handwash procedure

Each subject was sampled by placing her dominant hand in a disposable plastic glove (Arbrook, Ontario) containing 50 ml of Trypticase Soy Broth (Difco, Detroit, Michigan) and the hand was vigorously agitated for 30 s. The handwash fluid was then transferred to a sterile disposable container and immediately transported to the laboratory and held at 4 °C until the pour plates were ready.

Culture and identification

Five ml of handwash fluid was transferred to 20 ml of molten MacConkey agar (Difco, Detroit, Michigan) in a petri dish, mixed thoroughly by gently swirling the dish, allowed to harden and incubated for 18 h at 37 °C. In addition, 0·01 and 0·001 ml aliquots were plated onto MacConkey agar plates using calibrated loops. Colony counts were performed and each morphologically distinct colony type was streaked onto blood agar (Trypticase Soy Agar [BBL Microbiology System, Cockeysville, Md.] containing 5% sheep blood) and incubated for 18 h at 37 °C. Enterobacteriaceae were identified by the method of Edwards & Ewing (1972), oxidase-positive organisms by the API 20E system (Analytab Products, Inc., Plainview, N.Y.) and oxidase-negative non-glucose fermenters by the criteria of Gilardi (1978).

Typing studies

Klebsiella pneumoniae and *Klebsiella oxytoca* isolates were capsular serotyped by Dr R. P. Rennie of the Henderson General Hospital in Hamilton, Ontario, by the method of Rennie & Duncan (1974). *Pseudomonas aeroginosa* isolates were pyocin typed by Ms Pauline Ewan of the Laboratory Centre for Disease Control, Ottawa, Ontario. *Enterobacter agglomerans* isolates were biotyped in our laboratory using the method of Ewing & Fife (1972).

Data analysis

Data were analysed for statistical significance by using the Chi-square test.

RESULTS

The rate of hand carriage of aerobic Gram-negative rods by all four study groups is shown in Table 1. There was no difference in the rate of hand carriage of aerobic Gram-negative rods between medical ward and surgical intensive care unit nurses. Health care workers with hand dermatitis had a significantly higher carriage rate of aerobic Gram-negative rods than the above two groups. Control subjects had the highest carriage rate (Table 1). In addition to these intergroup differences, large variations in individual hand carriage rates were noted. Specifically, while the mean number of surgical intensive care unit group samples positive for aerobic Gram-negative rods was 36·6%, the number of samples positive per subject ranged from 23·5% to 73·9%. Similarly, the ranges for the medical ward subjects were 16·7% to 83·3%, dermatitis group 17·4% to 100% and control group 60% to 88·9%.

Table 1. *Rate of hand carriage of aerobic Gram-negative rods by three groups of health care workers and one group of control subjects who were sampled repeatedly over a period of three to six weeks*

Study group (no. of subjects)	No. samples/ group	Mean no. samples/ subject	Mean no. (%) samples positive for aerobic Gram-negative rods	
Surgical intensive care unit nurses (4)	101	25·2	37 (36·6)	$P > 0.05$ NS
Medical ward nurses (4)	93	23·2	36 (38·7)	$P < 0.05$
Health care workers with hand dermatitis (4)	79	19·8	44 (55·7)	$P < 0.001$
Control subjects (5)	129	25·8	92 (71·3)	$P < 0.05$

$P < 0.001$ $P < 0.05$ $P < 0.001$ $P < 0.05$

Table 2. *Aerobic Gram-negative rods isolated from the hands of study group*

Organism	No. (%) of total	No. (%) handwash samples with this organism			
		S.I.C.U.	Med. ward	Control subjects	Dermatitis
*Enterobacter agglomerans**	87 (28·8)	15 (14·9)	7 (7·5)	59 (45·7)	6 (7·6)
E. aerogenes	5 (1·7)	—	2 (2·2)	1 (0·8)	2 (2·5)
E. cloacae	4 (1·3)	1 (1·0)	—	2 (1·6)	1 (1·3)
E. gergoviae	3 (1·0)	—	—	3 (2·3)	—
E. hafnia	1 (0·3)	—	—	—	1 (1·3)
Acinetobacter anitratus†	47 (15·5)	7 (6·9)	5 (5·4)	27 (20·9)	8 (10·1)
A. lwoffi	13 (4·3)	—	3 (3·2)	5 (3·9)	5 (6·3)
Klebsiella pneumoniae‡	41 (13·6)	2 (2·0)	22 (23·7)	8 (6·2)	9 (11·4)
K. oxytoca	15 (5·0)	8 (7·9)	—	—	7 (8·9)
K. ozonae	3 (1·0)	—	2 (2·2)	1 (0·8)	—
Escherichia coli	5 (1·7)	1 (1·0)	—	2 (1·6)	2 (2·5)
Citrobacter freundii	1 (0·3)	1 (1·0)	—	—	—
Serratia liquefaciens	12 (4·0)	3 (3·0)	2 (2·2)	—	7 (8·9)
S. rubideae	7 (2·3)	1 (1·0)	2 (2·2)	—	4 (5·1)
Proteus mirabilis	5 (1·7)	4 (4·0)	—	—	1 (1·3)
Pseudomonas aeruginosa§	21 (6·9)	5 (5·0)	1 (1·1)	—	15 (19·0)
P. fluorescens-putida	8 (2·6)	1 (1·0)	—	6 (4·7)	3 (3·8)
P. stutgeri	1 (0·3)	—	—	1 (0·8)	—
Moraxella spp.	5 (1·7)	—	—	5 (3·9)	—
Unidientified	17 (5·6)	1 (1·0)	—	9 (7·0)	7 (8·9)

Statistical analysis of rate of carriage of:

* *E. agglomerans:* controls > S.I.C.U., medical ward, dermatitis subjects $P < 0{\cdot}001$; all others not significant.

† *A. antiratus*: controls > dermatitis subjects, $P < 0{\cdot}05$; > medical wards, S.I.C.U. $P < 0{\cdot}01$; all others not significant.

‡ *K. pneumoniae*: medical ward > S.I.C.U., controls, $P < 0{\cdot}001$ > dermatitis subjects, $P < 0{\cdot}05$; dermatitis subjects > S.I.C.U. $P < 0{\cdot}01$; all others not significant.

§ *P. aeruginosa:* dermatitis subjects > S.I.C.U. $P < 0{\cdot}01$ > medical ward, $P < 0{\cdot}001$ > controls.

There were 301 aerobic Gram-negative rod isolates from 402 handwash samples (Table 2). The most common isolates were *Enterobacter agglomerans* (28·8 % of the total), followed by *Acinetobacter calcoaeceticus* var. *anitratus* (15·5 %), *Klebsiella pneumoniae* (13 %) and *Pseudomonas aeruginosa* (6·9 %). Table 2 also shows the distribution of the various species of aerobic Gram-negative rods as a percentage of the total number of isolates from each group. Control subjects carried significantly more *E. agglomerans* and *A. calcoaceticus* var. *anitratus* than any other study group. Medical ward subjects carried more *K. pneumonia* than all other groups, while subjects with hand dermatitis carried more *Ps. aeruginosa* than any other group.

Fig. 1 shows the frequency distribution of the number of species of aerobic Gram-negative rods per handwash sample for each of the four study groups. None of the handwash cultures for the surgical intensive care unit, medical ward and control subject groups contained more than two species; however, subjects with

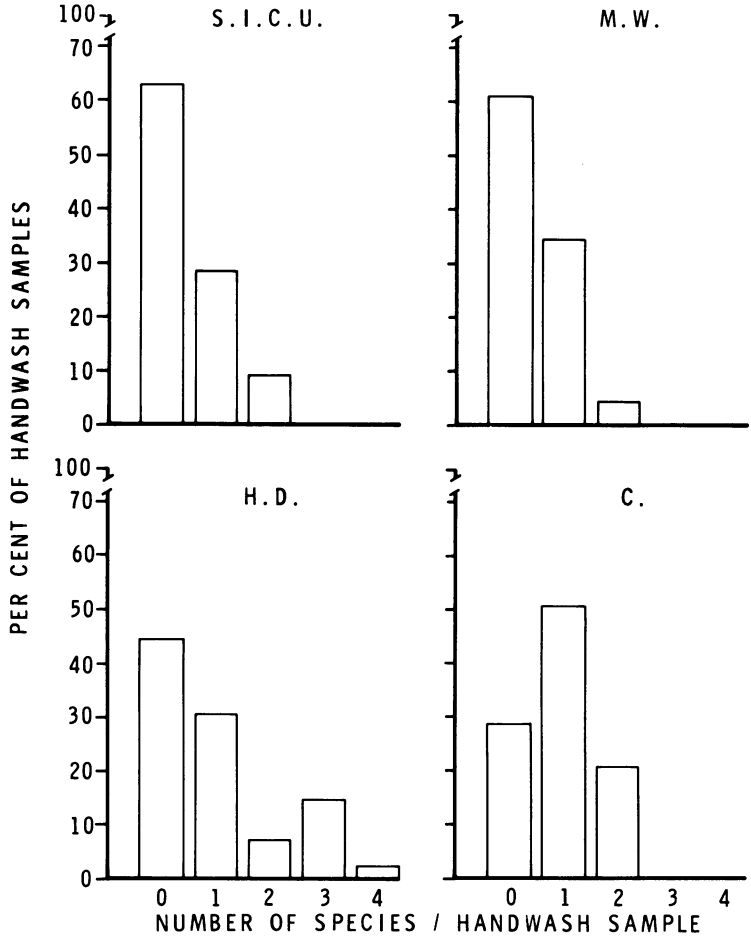

Fig. 1. Distribution of number of species of aerobic Gram-negative rods per handwash sample as a percentage of the total number of handwash cultures for four surgical intensive care unit nurses (S.I.C.U.); four medical ward nurses (M.W.); four health care workers with hand dermatitis (H.D.) and five secretary control subjects (C).

hand dermatitis had up to four species of aerobic Gram-negative rods isolated from a single handwash culture.

Fig. 2 shows the frequency distribution of the counts of aerobic Gram-negative rods isolated from the hands of each study group. In general, the numbers of organisms carried were low – 91 % of the surgical intensive care unit subjects, 82 % of the medical ward subjects, 71 % of the dermatitic subjects and 80 % of the control subjects carried less than 1000 Gram-negative bacilli/hand. Some surgical intensive care unit and medical ward subjects carried up to 10^6 organisms/hand, and control subjects > 10^6 organisms/hand; dermatitis subjects, however, carried up to 10^5 organisms/hand. Surgical intensive care unit, medical ward and control

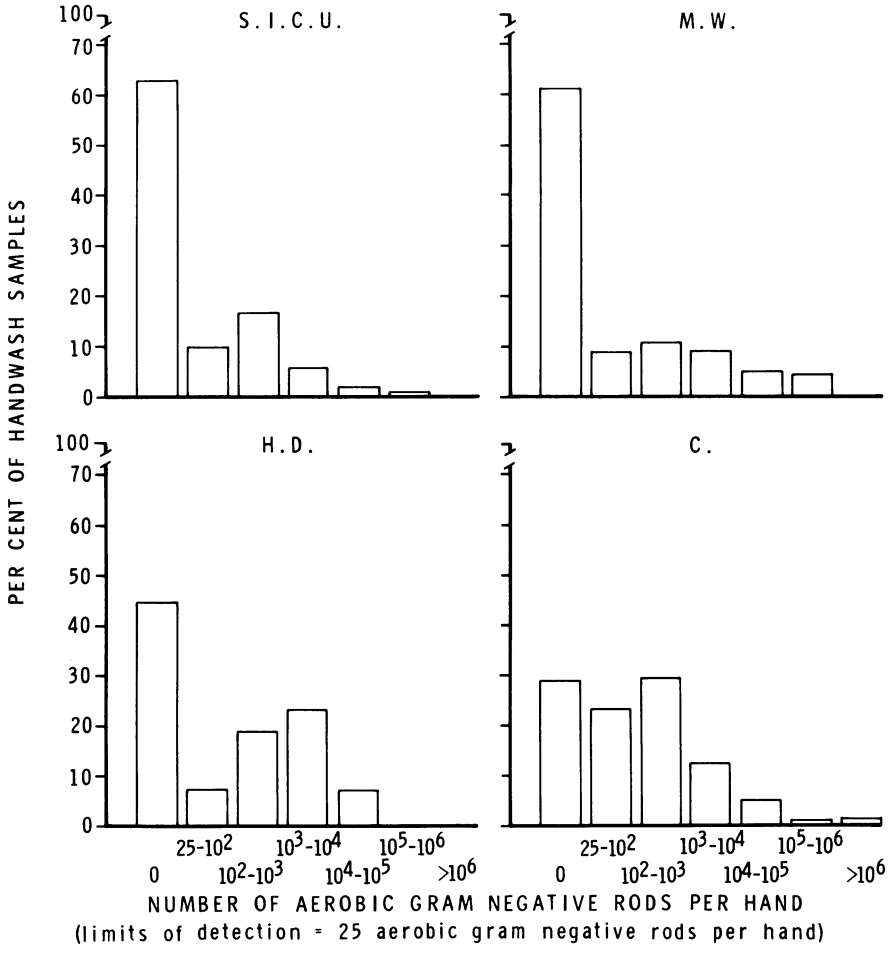

Fig. 2. Distribution of aerobic Gram-negative rod counts per hand as a percentage of the total number of handwash cultures for four surgical intensive care unit nurses (S.I.C.U.); four medical ward nurses (M.W.); four health care workers with hand dermatitis (H.D.) and five secretary control subjects (C).

subjects most frequently carried 10^2–10^3 aerobic Gram-negative rods/hand, while subjects with dermatitis most frequently carried between 10^3 and 10^4 organisms/hand.

Typing studies

For this study we defined continuous carriage of aerobic Gram-negative rods by each of our subjects as the isolation of an organism of the same serotype, pyocin type or biotype from more than two handwash samples.

Forty-one isolates of *K. pneumoniae* and *K. oxytoca* were capsular serotyped. Ninety per cent (37/41) were typable. Thirteen capsular types were identified. Nine of these capsular types were isolated only once (Fig. 3). All seven isolates of

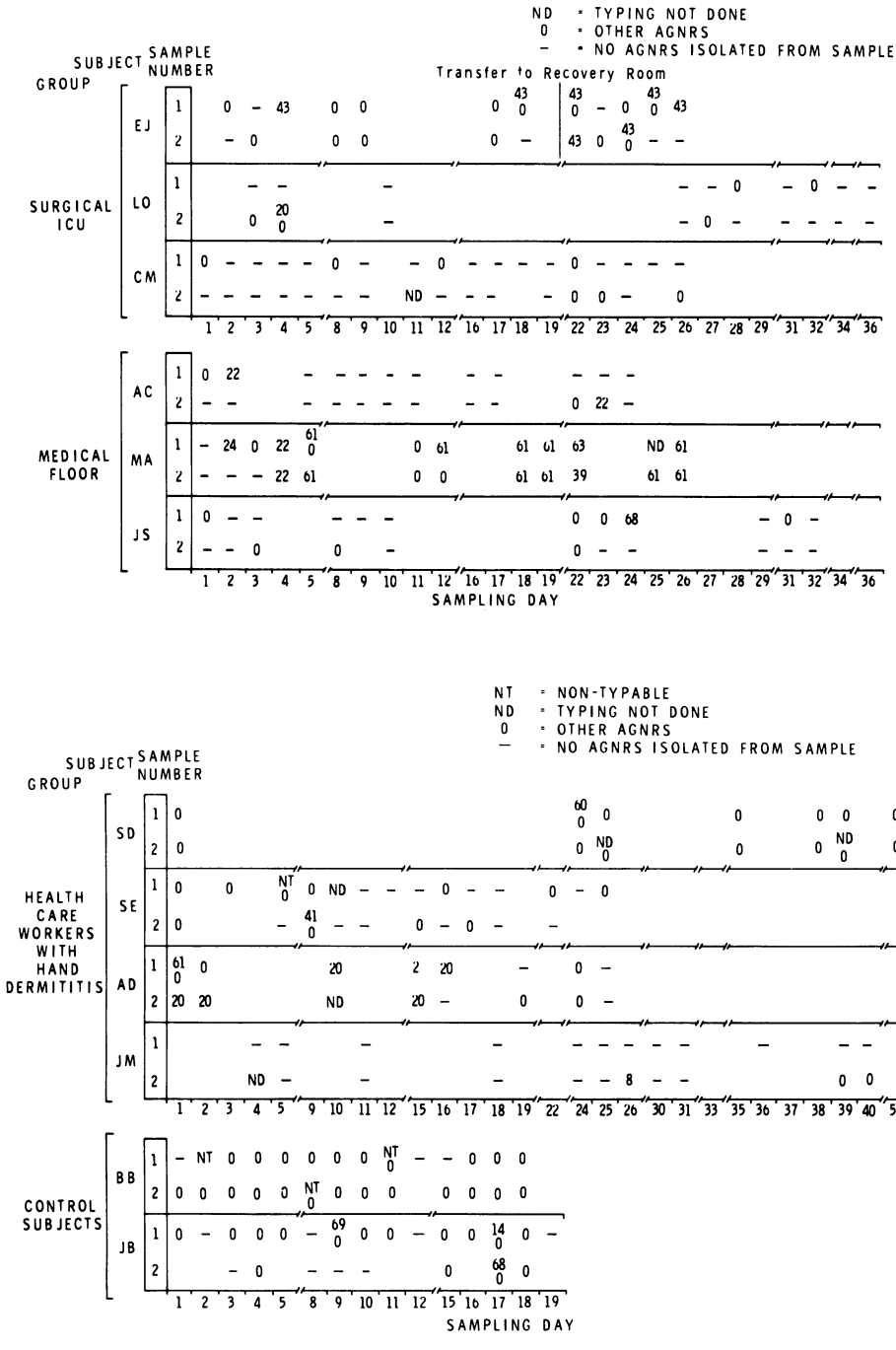

Fig. 3. Capsular serotypes of *Klebsiella pneumoniae* and *Klebsiella oxytoca* isolates.

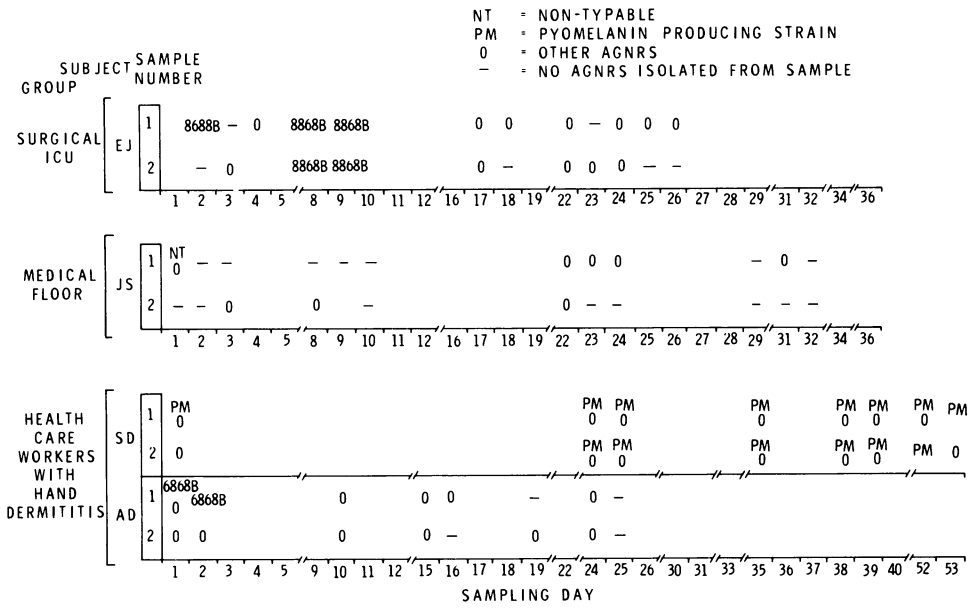

Fig. 4. Pyocin types of *Pseudomonas aeruginosa* isolates.

K. pneumoniae from the hands of surgical intensive care unit subject E. J. were type 43. This subject was transferred from the surgical intensive care unit to a new work area (recovery room) on sampling day 22, yet she continued to carry type 43. Medical ward nurse A. C. had type 22 isolated twice. Medical ward nurse M. A. had *K. oxytoca* isolated from 16 of the 21 handwash cultures. Ten of these isolates were type 61 and two were type 22. One of the four dermatitis subjects had type 20 isolated from her hands five times. One control subject (B.B.) had *K. pneumoniae* isolated from her hand three times. Although these three could not be capsular typed, they were the same biotype and shared the same antibiotic sensitivity pattern (data not shown). Thus, four of 11 subjects from whom *Klebsiella* was isolated demonstrated continuous carriage.

Twenty-one *Ps. aeruginosa* isolates were pyocin typed (Fig. 4). Five isolates of *Ps. aeruginosa* were obtained from nurse E. J. over an eight-day period; the last four of these were of the same pyocin type (8868B). Both *Ps. aeruginosa* isolates from the dermatitis subject A.D., collected on consecutive sampling days, were pyocin type 6868B. Although nurse S.D. in the dermatitis group had *Ps. aeruginosa* isolated from her hand 13 times, all were pyomelanin-producing isolates and were non-pyocin typable. We considered the pyomelanin-producing isolates to be the same and, therefore, two of the three subjects from whom *Ps. aeruginosa* was isolated carried this organism continuously.

Fig. 5 shows the biotypes of *E. agglomerans* isolated in this study. Four of the five control subjects continuously carried the same biotype of *E. agglomerans*.

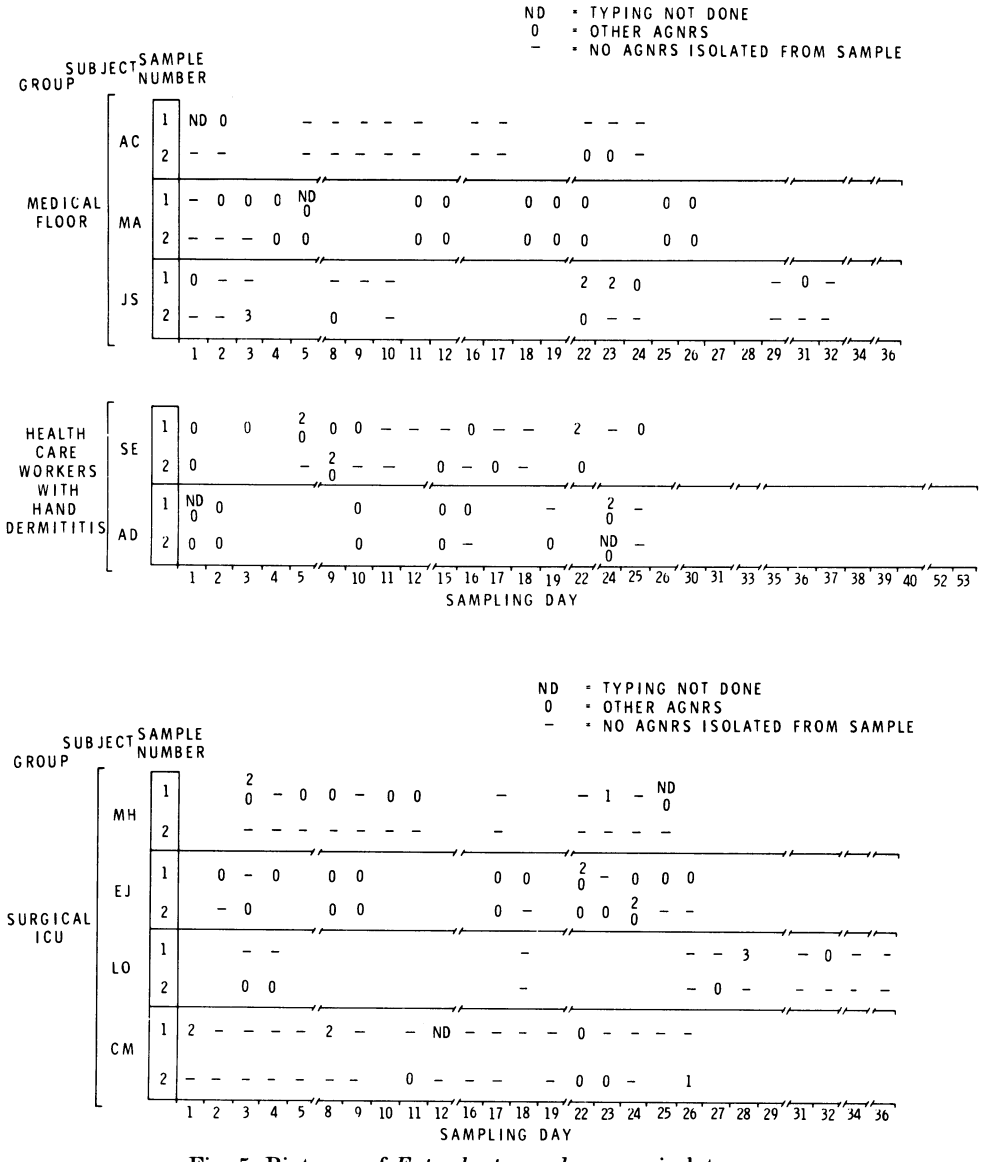

Fig. 5. Biotypes of *Enterobacter agglomerans* isolates.

DISCUSSION

In this study we used Casewell & Phillips' (1977) gloved-hand sampling technique to determine the hand carriage of aerobic Gram-negative rods by health care personnel and control subjects over a period of several weeks. The gloved-hand technique, while technically complicated, is sensitive and permits the sampling of the entire hand surface (Larson, Strom & Evans, 1980).

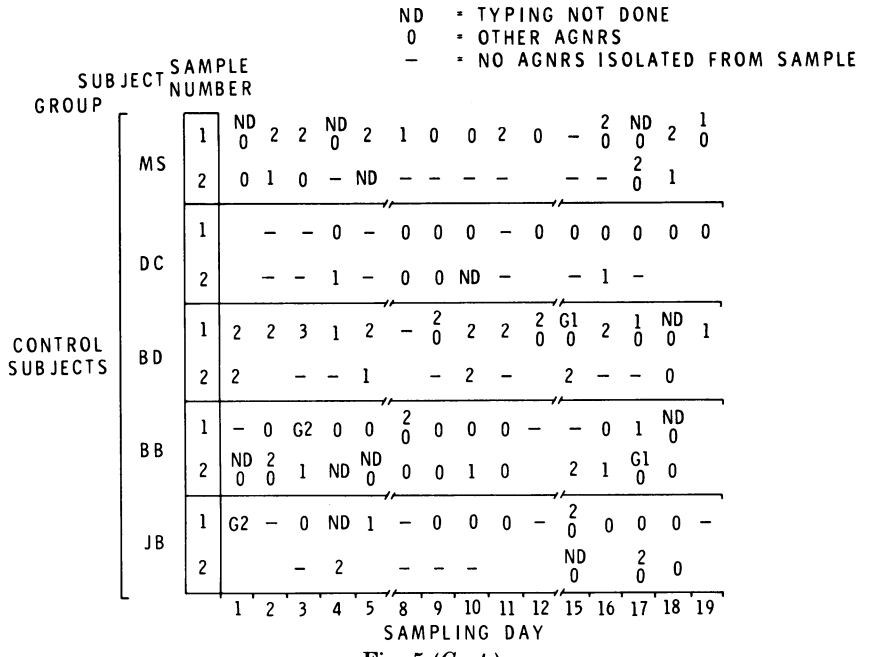

Fig. 5 (*Cont.*)

Our data show that control subjects carried significantly more aerobic Gram-negative rods than subjects with hand dermatitis who, in turn, carried significantly more aerobic Gram-negative rods than both surgical intensive care unit and medical ward nurses. Qualitative differences were also evident in that control subjects carried *E. agglomerans* and *A. calcoaceticus* var. *anitratus* more frequently than the other groups. Hand dermatitis subjects carried more *Ps aeruginosa* and the medical ward nurses carried more *K. pneumoniae* than the other groups.

Few studies have investigated endemic hand carriage of aerobic Gram-negative rods by health care workers; of these studies only two involved multiple samples. Maki (1978) states that all health care personnel carry Gram-negative bacilli on their hands at some time, and our data support this statement. Salzman *et al.* (1968) used a handwash method to sample 28 health care workers' hands two to four times each over several months; 61·5 % contained coliforms. In a study of an outbreak of nosocomial infection due to aminoglycoside-resistant Gram-negative rods, Knittle, Eitzman & Baer (1975) found that 86·1 % of 151 handwash cultures from 13 nurses over a three-month period were positive for Gram-negative bacilli.

With the exception of *Acinetobacter* (Taplin, Rebell & Zaias, 1963; Ramphal & Kluge, 1979), Gram-negative bacilli are generally regarded as transient organisms on the skin (Knittle, Eitzman & Baer, 1975). Although the importance of routine handwashing for the removal of transient contamination has been emphasized, both the frequency (Albert & Condie, 1981) and the thoroughness (Taylor, 1978) of handwashing by health care workers are often inadequate.

Price (1938), however, states that Gram-negative organisms may deviate from

the behaviour of typical transient hand flora. Polk & Lopez (1972), Bruun & Solberg (1973), Knittle, Eitzman & Baer (1975), French *et al.* (1980) and Parry *et al.* (1980) have concluded from their studies that Gram-negative organisms may colonize the hands of some individuals and become part of their resident hand flora, thereby serving as a reservoir for nosocomial infections rather than a passive vehicle for nosocomial transmission. Such persistent hand carriage has been frequently associated with the presence of hand dermatitis. Buxton *et al.* (1978) traced an outbreak of respiratory infections due to *Acinetobacter* to the colonized dermatitic hands of a respiratory therapist. Parry *et al.* (1980) linked a nursery outbreak of *Citrobacter* to a nurse with a 'marked dermatitis'.

In our study, the dermatitis group carried aerobic Gram-negative rods significantly more often than health care groups with normal skin. Individual subjects with dermatitis showed a wide range in hand carriage patterns from a low of 17% by one subject to 100% by another. Maki (1978) also noted a large amount of variation in individual hand carriage patterns Both Maki (1978) and McBride *et al.* (1972) attributed these differences to differences in handwash techniques and environment.

The demonstration of prolonged carriage of a single strain of a species of aerobic Gram-negative rods requires a method for typing the bacterial isolates. Capsular serotyping of our *Klebsiella* isolates revealed that several of our subjects showed prolonged carriage of a single strain. Knittle, Eitzman & Baer (1975) found that 12/12 of the *Klebsiella* handwash isolates from one nurse, and 10/12 of the *Klebsiella* isolates from another nurse, were of the same capsular serotype. We also found evidence of prolonged single-strain carriage of *Ps. aeruginosa*. Knittle, Eitzman & Baer (1975) found that 25/151 cultures from 13 nurses grew *Ps. aeruginosa*; however, they did not type their isolates. There are no reports of prolonged hand carriage of *E. agglomerans* in the literature. Our biotyping data for this organism suggest possible prolonged carriage, but no conclusion can be drawn from these data since only four biochemical reactions are used to place an isolate in one of 11 biotypes. Clearly, an alternative method for typing *E. agglomerans* is needed.

The prolonged simultaneous hand carriage of three species of aerobic Gram-negative rods has been reported (Knittle, Eitzman & Baer, 1975). Casewell & Phillips (1977) have shown that more than one strain of a single species of aerobic Gram-negative rods may be present on a hand simultaneously. Ørskov (1955) stresses that several colonies, grown directly from the handwash, should be typed in order to determine if more than one strain is present. We picked only one of each morphologically identical colony type from the MacConkey plates. Our data then err on the side of underestimating continuous carriage. Using a variety of typing methods, and our definition of continuous carriage, we showed that four of 11 subjects who had *Klebsiella* isolated and typed carried this organism continuously. Similarly, two of the three subjects who carried *Ps. aeruginosa* and five of 12 from whom *E. agglomerans* was isolated carried these organisms continuously. Four of the five control subjects demonstrated continuous carriage of *E. agglomerans*.

The other question from our data is the role of exposure to infected patients in continuous hand carriage of aerobic Gram-negative rods. We did not follow the patients who were cared for by these nurses, but several observations are important. Nurse E.J. continued to carry *Klebsiella* capsular type 43 following her transfer to the recovery room. Subject M.A. continued to carry *Klebsiella* type 61 over a 19-day period which included six off-duty days. Finally, one of the control subjects, B.B., a secretary, carried the same biotype of *Klebsiella*. Similarly, two of the three subjects who carried *Ps. aeruginosa* and five of 12 from whom *E. agglomerans* was isolated carried these organisms continously. Four of the five control subjects demonstrated continuous carriage of *E. agglomerans*. Similarly, S.D., a nurse with hand dermatitis, carried a pyomelanin-producing strain of *Ps. aeruginosa* for 53 days. She worked in the cardiovascular intensive care unit, an area with a short patient stay. Lastly, the continuous carriage of *E. agglomerans* among the control subjects who had no exposure to patients suggests that other factors are operative.

Further studies are needed to determine why some subjects continuously carry aerobic Gram-negative rods and others do not. Our data suggest that hand factors should be examined next, e.g. differential adhesion, and survival of various species of aerobic Gram-negative rods on hands.

We thank Dr R. P. Rennie of the Henderson General Hospital in Hamilton, Ontario for capsular serotyping the *Klebsiella* isolates, and Ms Pauline Ewan of the Laboratory Centre for Disease Control in Ottawa, Ontario, for pyocin typing the *Pseudomonas aeruginosa* isolates. Dr Chris Field of the Department of Mathematics, Dalhousie University, helped with the data analysis. Finally, we are most grateful to the volunteers who participated in this study.

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