Hand carriage of aerobic Gram-negative rods by health care personnel

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SUMMARY

A quantitative culture technique (hand washed in a glove containing broth for 30 s) was used to determine the frequency of hand carriage of aerobic Gram-negative rods by various groups of health care workers and 104 control subjects. Overall, 31 % of health care workers carried aerobic Gram-negative rods on their hands compared to 59 % of control subjects ($P < 0.001$). *Enterobacter agglomerans* accounted for 40 % of the isolates, and other *Enterobacter* spp. 7 %. Other organisms included *Acinetobacter calcoaceticus* 21 %, *Serratia* spp. 11 %, *Klebsiella* spp. 10 %, *Moraxella* spp. 3 %, *Pseudomonas* spp. 3 %, *Proteus* spp. 1.5 %, *Escherichia coli* 1 %; *Morganella morganii*, *Citrobacter freundii*, *Aeromonas* sp. and an isolate that was not speciated accounted for 0.5 % each. We conclude that endemic hand carriage of aerobic Gram-negative rods by health care personnel is common, but significantly less than that of control subjects. *Enterobacter agglomerans* is found so frequently on the hands of control subjects that it must be considered part of the normal hand flora.

INTRODUCTION

About 5 % of all patients admitted to hospital develop a nosocomial infection (Eickhoff, 1977) and at least $\frac{2}{3}$ of these infections are caused by Gram-negative rods (Schaffner, 1977). Hand transfer of Gram-negative organisms to patients by health care personnel has been implicated in more than 40 studies on various nosocomial infections (Maki, 1978). Aerobic Gram-negative rods are generally regarded as being transient on the skin (Haverkorn & Michel, 1979a; Nobel & Somerville, 1974) and highly prone to death through desiccation (Haverkorn & Michel, 1979a). *Klebsiella* spp. can survive up to 150 min on the hand (Casewell & Phillips, 1977) and, although *Pseudomonas aeruginosa* survives only a maximum of 11 min (Chadwick, 1973), this time span is certainly sufficient for transfer in a busy hospital environment. Marples & Towers (1979) found that 0.06 % of an inoculum

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of *Staphylococcus saprophyticus* was transferred from one piece of fabric to another by hand contact.

If we are to prevent nosocomial infections, it is essential to delineate the epidemiology of endemic infections caused by aerobic Gram-negative rods. In this study we qualitatively and quantitatively delineated hand carriage of aerobic Gram-negative rods by several groups of health care personnel and control subjects.

MATERIALS AND METHODS

Subjects

Two hundred and fifty-five health care workers were studied: 128 nurses, 40 physicians, 26 porters, 23 respiratory technologists, 16 ward clerks, 10 housekeeping personnel and 12 others. The 104 control subjects were from 3 groups: 62 hospital clerical staff, 18 medical school clerical staff and 24 university students. None of these control groups had any patient contact.

Sampling procedure

After informed consent was obtained, all subjects had their hands examined for the presence of dermatitis and completed a questionnaire. Then the dominant hand was inserted into a sterile plastic glove (Arbrook, Ontario) containing 50 ml of Trypticase Soy Broth (Difco, Detroit, Michigan) and agitated for 30 s. The time of sampling was totally random in relationship to handwashing and patient hand-care activities. The liquid was then poured into a sterile disposable container and taken immediately to the laboratory.

Culture and identification

Two 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 Systems, Cockeysville, Maryland] containing 5% sheep blood) and incubated for 18 h at 37 °C, and identified by using the method of Edwards & Ewing (1972). Because of the large number of *E. agglomerans* isolates in this study we had some of these organisms confirmed as *E. agglomerans* by Ms P. Ewing, Laboratory Centre for Disease Control, Ottawa, Ontario, Canada.

RESULTS

Table 1 shows the rate of hand carriage of aerobic Gram-negative rods by eight groups of health care workers and three groups of control subjects. Overall, 31.3% of the 255 health care workers carried aerobic Gram-negative rods on their hands compared with 58.7% of 104 control subjects ($P < 0.001$). Among the health care

Table 1. Hand carriage of aerobic Gram-negative rods by health care workers and control subjects

Group	No. sampled	M/F		Mean age (yrs)	No. (%) with AGNR	Percentage of subjects who washed hands with chlor- hexidine
Health care workers (overall)	255	58	197	29.7	80 (31.3)*	73.3
Nurses	128	1	127	29.7	37 (28.9)	89.8
Physicians	40	35	5	29.8	12 (30.0)	72.5
Porters	26	11	15	26.4	10 (38.5)	50.0
Respiratory technologists	23	8	15	28.2	7 (30.4)	87.0
Ward clerks	16	0	16	36.8	6 (37.5)	87.5
Housekeeping staff	10	2	8	36.3	2 (20.0)	50.0
Other†	12	1	11	24.3	6 (50.0)	25.0
Control subjects (overall)	104	26	78	27.9	61 (58.7)*	1.0
Hospital clerical staff	62	6	56	27.7	23 (37.1)	0
University students	24	16	8	19.7	22 (91.7)	0
Medical school clerical staff	18	4	14	39.8	16 (88.9)	5.5

* $\chi^2 = 23.0$, $P < 0.001$.

† Includes 7 X-ray technicians, 2 dieticians, and 1 each of: audiologist; physiotherapist; EKG technician.

workers, rates of hand carriage of these organisms ranged from 28.9 % of the nurses to 50 % for 'other'. Hand carriage of aerobic Gram-negative rods by control subjects ranged from 37.1 % of hospital clerical staff to 91.7 % of the university students. Differences in hand carriage rates of aerobic Gram-negative rods were not related to age or sex. The mean age of the medical school clerical staff control group was 39.8 years and that of the university student control group was 19.7 years. Both groups had similar hand carriage rates (88.9 % and 91.7 % respectively). Although 127/128 nurses sampled were female and 35/40 physicians sampled were male, their hand carriage rates (28.9 % and 30 % respectively) were nearly identical. Handwashing practices did affect hand carriage of aerobic Gram-negative rods. Many (73.3 %) health care personnel regularly washed their hands with 2 % chlorhexidine digluconate (Hibitane®), while only 1.0 % of the controls used this skin antiseptic. Health care workers who used chlorhexidine carried aerobic Gram-negative rods less frequently than health care workers who did not use chlorhexidine ($P < 0.05$). We have cultured aliquots of the chlorhexidine used in our hospital and have found them to be sterile.

One hundred and eighty aerobic Gram-negative rods were isolated from 359 subjects (Table 2). *Enterobacter agglomerans* accounted for 39.8 % of the isolates, *Acinetobacter calcoaceticus* accounted for 21 %.

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 health care workers and control subjects. *Enterobacter agglomerans* ($P < 0.001$), *Acinetobacter calcoaceticus*

Table 2. *Aerobic Gram-negative rods isolated from the hands of health care workers and control subjects*

	No. (%) of health care workers who carried this organism	No. (%) of control subjects who carried this organism	No. (%) of total isolates
<i>Enterobacter agglomerans</i>	33 (12.9)*	39 (37.5)*	72 (39.8)
<i>E. aerogenes</i>	8 (2.9)	1 (1.0)	9 (5.0)
<i>E. cloacae</i>	2 (0.7)	0	2 (1.1)
<i>E. hafnia</i>	0	2 (1.9)	2 (1.1)
<i>Klebsiella pneumoniae</i>	8 (2.9)	2 (1.9)	10 (5.6)
<i>K. oxytoca</i>	6 (2.2)	0	6 (3.3)
<i>K. ozaenae</i>	0	1 (1.0)	1 (0.6)
<i>Escherichia coli</i>	2 (0.7)	0	2 (1.1)
<i>Serratia liquefaciens</i>	9 (3.5)	4 (3.8)	13 (7.2)
<i>S. marcescens</i>	3 (1.2)	1 (1.0)	4 (2.2)
<i>S. rubideae</i>	0	3 (2.9)	3 (1.7)
<i>Citrobacter freundii</i>	1 (0.4)	0	1 (0.6)
<i>Acinetobacter calcoaceticus</i> var <i>lwoffi</i>	12 (4.3)	6 (5.8)	18 (10.0)
<i>Acinetobacter calcoaceticus</i> var. <i>anitratatus</i>	9 (3.2)†	11 (10.6)†	20 (11.0)
<i>Proteus mirabilis</i>	2 (0.7)	0	2 (1.1)
<i>P. rettgeri</i>	1 (0.4)	0	1 (0.6)
<i>Morganella morganii</i>	0	1 (1.0)	1 (0.6)
<i>Pseudomonas aeruginosa</i>	3 (1.0)	0	3 (1.7)
<i>Pseudomonas</i> spp.	2 (0.7)	1 (1.0)	3 (1.7)
<i>Aeromonas</i> sp.	1 (0.4)	0	1 (0.6)
<i>Moraxella</i> spp.	0‡	5 (4.8)‡	5 (2.8)
Not speciated	1 (0.4)	0	1 (0.6)

* $\chi^2 = 27.8$, $P < 0.001$ † $\chi^2 = 7.0$, $P < 0.01$ ‡ $\chi^2 = 12.4$, $P < 0.001$

var. *anitratatus* ($P < 0.01$), and *Moraxella* spp. ($P < 0.001$) were isolated significantly more frequently from the control group.

The number of aerobic Gram-negative rods on the hands of health care workers and control subjects is shown in Fig. 1. In general, the number of Gram-negative rods carried was low – 72 % of health care workers and 82 % of controls who carried aerobic Gram-negative rods had less than 1000 per hand. Some health care workers carried more than 10^6 organisms, while some control subjects carried 10^4 – 10^5 aerobic Gram-negative rods per hand. One of the 3 health care workers who carried more than 10^6 aerobic Gram-negative rods had hand dermatitis. Control subjects who carried aerobic Gram-negative rods on their hands most frequently had between 10^2 and 10^3 organisms per hand, while health care workers most frequently carried 25 to 10^2 organisms per hand.

Fig. 1. Distribution of aerobic Gram-negative rod counts per hand as a percentage of the total number of handwash cultures for health care workers and control groups.

^a Limits of detection = 25 aerobic Gram-negative rods per hand.

DISCUSSION

The three methods most commonly used to evaluate the bacterial flora of the hands are the swab, contact plate and gloved hand or sterile bag methods. The advantages and disadvantages of each are discussed by Larson, Strom & Evans (1980).

In this study we used the gloved-hand technique to enable us to sample the entire surface of the hand. Only the dominant hand of each subject was sampled because this hand is often washed less thoroughly (Taylor, 1978).

In studies which delineated hand flora by the gloved-hand technique various wash solutions have been used including water (Salzman, Clark & Klemm, 1968), 0.9% NaCl (Haverkorn & Michel, 1979b), Brain Heart Infusion (BHI) broth (Buxton *et al.* 1978) and Ringer's Solution (Casewell & Phillips, 1977). We used BHI broth in our preliminary trials but switched to using trypticase soy broth for our study because subjects objected to the smell of the BHI broth.

The normal bacterial flora of the hand includes *Staphylococcus epidermidis*, micrococci and diphtheroids (Evans *et al.* 1950 and Marples, M. J., 1969). Although these organisms form the major component of the normal skin flora, they are unusual causes of nosocomial infections (Schaffner, 1977).

Very few studies have investigated hand carriage of aerobic Gram-negative rods by control (i.e. non-health care workers) subjects. Larson, Strom & Evans (1980), using the sterile bag technique with quarter-strength Ringer's solution containing antiseptic neutralizers, isolated *Acinetobacter* from the hands of one of their control subjects. McBride *et al.* (1972, 1974) used the swab technique with buffered saline in studies comparing the flora of nurses' hands with that of control subjects. They did not speciate their isolates but reported that about 27 % of their control subjects carried glucose non-fermenting Gram-negative rods compared to 9 % of nurses. Of the control subjects 4 % had Enterobacteriaceae on their hands compared to 1.5 % of nurses. There are many reports of hand carriage of aerobic Gram-negative rods by health care workers; however, the vast majority of these have been carried out during the investigation of outbreaks of nosocomial infection (Buxton *et al.* 1978; Knittle, Eitzman & Baer, 1975; Lowbury & Fox, 1954; Mayhall *et al.* 1979; Parry *et al.* 1980; Selwyn, 1965; Shulman, Terry & Hough, 1971). In the studies carried out in the endemic situation (Ayliffe *et al.* 1979; Brown & Baublis, 1977; Bruun & Solberg, 1973; Casewell & Phillips, 1977; Larson, Strom & Evans, 1980; McBride *et al.* 1972, 1974; Polk & Lopex, 1972; Salzman, Clark & Klemm, 1968) sampling techniques have varied and often speciation of the bacteria isolated was not performed. Hence, it is difficult to compare our data on endemic hand carriage rates with that of others.

Enterobacter agglomerans, an uncommon clinical isolate in our hospital, was recovered from 37.5 % of the control subjects and from 12.9 % of the health care workers. This high carriage rate of *E. agglomerans*, especially by the control subjects, suggests that this organism is part of the normal hand flora. Taylor, Kropp & Molina (1978) isolated this organism from the skin of 17 % of their control subjects. *E. agglomerans*, a plant pathogen, is prevalent in the general environment, but is of low pathogenicity to man (Geere, 1977), with most infections occurring as a result of contamination of wounds sustained outdoors (Pien *et al.* 1972). However, contamination of commercially prepared intravenous fluids by *E. agglomerans* with a resultant 10 % mortality rate has been reported (Maki, 1976). About 25 % of these wound infections were nosocomial. In addition, these authors noted a 'striking frequency' of isolation during the months of June to October. During this study and another, both of which were carried out from October 1979 to November 1980, no seasonal variation in hand carriage of *E. agglomerans* was noted.

The higher incidence of *A. calcoaceticus* on the hands of control subjects was not surprising, since this organism is considered part of the normal skin flora. Ramphal & Kluge (1979), and Taplin, Rebell & Zaias (1963) reported that up to 25 % of non-hospitalized individuals have *A. calcoaceticus* on their skin, while Buxton *et al.* (1978) noted that *A. calcoaceticus* can be isolated from the hands of 33 % of hospital personnel.

A. calcoaceticus is increasing in importance as a nosocomial pathogen (CDC, 1979; Glew, Moellering & Kunz, 1977; Ramphal & Kluge, 1979; Rosenthal, 1978), and in some instances hand carriage of this organism by health care personnel, especially those with hand dermatitis, has resulted in nosocomial infection (Buxton *et al.*

1978). French *et al.* (1980), while investigating an outbreak of *A. calcoaceticus* var. *anitratus* in a urological ward, found that between 6 c.f.u. and 5×10^4 c.f.u. of the epidemic strain were transferred from patient's skin to staff hands. Two of the microbiologists investigating this outbreak were found to carry *A. calcoaceticus* var. *anitratus* on their hands, and in one of them this organism became part of the resident skin flora.

Moraxella was isolated from control subjects only. This organism is not recognized as part of the normal skin flora. Van Bijsterveld (1972) failed to isolate *Moraxella* from the skin, but did isolate it from 13 % of nose swabs of non-hospital personnel. *Moraxella* has been isolated from eye, genito-urinary and respiratory tract infections (Rosenthal, 1978; van Bijsterveld, 1972).

From this study it is evident that there is differential hand carriage of aerobic Gram-negative rods. The factors responsible for this are probably multiple and include both host and organism attributes. The elucidation of these factors should lead to a better understanding and control of some nosocomial infections.

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