SURVIVAL OF *VIBRIO CHOLERAE* ON DIFFERENT FINGER LOCATIONS OF A VOLUNTEER FOLLOWING ARTIFICIAL INOCULATION

KL Yap and KN Hu

Department of Biomedical Sciences, Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

Abstract. The importance of bacteria-suspending media and fingertip positions on the survival of *Vibrio cholerae* on human fingertips were examined. Vibrios were suspended in phosphate-buffered saline (PBS), PBS with albumin, and PBS with agarose. Each type of preparation was inoculated on the fingerpads, the hyponychia, or the eponychia and lateral nail grooves of the fourth, third and second fingers of a volunteer's hand. The last finger inoculated was immediately washed with PBS and the washing collected for examination ("0 minute" exposure). The third and fourth inoculated fingers were likewise washed for examination 2 and 5 minutes later, respectively. The vibrios obtained from the washings were enumerated by culture. For each of the different groups, which consisted of a different inoculated fingertip position, bacteria-suspending medium and exposure period of 2 or 5 minutes, the proportion of replicate inoculated fingers which retained viable vibrios (isolation rate) and the mean number of surviving vibrios, as a percentage of the inoculated vibrios at "0 minute exposure" (survival rate) were as follows: finger pads: vibrios in PBS, 2 minutes post-inoculation (isolation rate, 25%; mean survival rate, 0.002%); 5 minutes post-inoculation (isolation rate, 0%; mean survival rate, 0%). PBS-albumin: 2 minutes post-inoculation (60%, 0.004%); 5 minutes post-inoculation (40%, 0.03%). PBS-agarose: 2 minutes post-inoculation (100%, 24%); 5 minutes post-inoculation (38%, 0.005%). Lateral nail grooves and eponychia: PBS: 2 minutes post-inoculation (100%, 2.2%); 5 minutes post-inoculation (44%, 0.2%). PBS-agarose: 2 minutes post-inoculation (100%, 32%); 5 minutes post-inoculation (100%, 0.7%). Hyponychia: PBS: 2 minutes post-inoculation (100%, 8%); 5 minutes post-inoculation (100%, 0.2%). PBS-agarose: 2 minutes post-inoculation (100%, 46%); 5 minutes post-inoculation (100%, 8%). The results show that vibrios in moisture-retaining medium (PBS-agarose) and inoculated on a sheltered fingertip locations (hyponychium) have the best survival rates. However, the high survival rate was maintained briefly.

INTRODUCTION

Vibrio cholerae causes cholera and gastroenteritis (Sack *et al*, 2004) and is usually transmitted by consuming contaminated water (Pollitzer, 1959; Colwell, 2004) or food (Rabbani and Greenough III, 1999). Person-to-person transmission of cholera is regarded as uncommon (Mintz *et al*, 1994). However, person-to-person transmission may in fact be common, based on the high rate of secondary infection in households (McCormack *et al*, 1969). This suggests unhygienic practices, including eating with fingers, is more important than contaminated water sources in the transmission of cholera (Singh *et al*, 1995). The hands and fingers may play an important role in the transfer of *V. cholerae* from an infected

Correspondence: Dr KL Yap, Department of Biomedical Sciences, Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300, Kuala Lumpur, Malaysia.
Fax: 603 26929032
E-mail: ykleong@medic.ukm.my

person to another person or an object. Transmission of *V. cholerae*, and other transient bacteria, from one individual to another individual or object via hands and fingers requires the bacteria to survive for at least several minutes on the contaminated hands and fingers (WHO, 2005). It has been reported that *V. cholerae* is able to survive on fingertips for 1 to 2 hours (Public Health Agency Canada, 2001). The fingers have parts that are exposed, such as the finger pads, and others that are sheltered, such as the hyponychia beneath long nails and the lateral nail grooves (Lewin, 1965). Therefore, the sites where the vibrios are deposited may be an important determinant of their survival rate and hence their ability to be transmitted. Earlier studies reported that different types of suspending media effected survival of other types of bacteria (Coates *et al*, 1987; Snelling *et al*, 1991). In natural settings, *V. cholerae* that contaminates fingertips may be suspended in media of different compositions and forms, such as in feces or water used by a person in self-ablution after defecation. Feces containing *V. cholerae* can be in different forms, such as watery fluid which contains protein and high concentrations of sodium, bicarbonate, and potassium, the "rice-water" stool of severe diarrhea, and the more solid feces in infections that are asymptomatic or in mild diarrhea cases (Sack *et al*, 2004). The different compositions and forms of bacteria-suspending media may influence survival of *V. cholerae* on fingertips.

The objective of this study was to determine the survival rate of *V. cholerae* suspended in different media and inoculated on finger pads, hyponychia, and lateral nail grooves and eponychia of human fingers.

MATERIALS AND METHODS

Vibrio cholerae

The *Vibrio* strain used (O1 serogroup and Inaba serotype) was isolated from a patient at the Universiti Kebangsaan Malaysia Hospital in 2002.

Subject

The fingers of both hands of the main researcher were used in this study. The skin of the fingers and the finger nails were normal and unbroken throughout the study. This study was approved by the relevant review group in the Faculty.

Preparation of *V. cholerae* for experiments

V. cholerae from a stock culture was streaked onto thiosulphate citrate bile salts sucrose (TCBS) agar and incubated at 37ºC overnight. An isolated yellow colony was inoculated into 5 ml of alkaline peptone water and incubated with shaking at 37ºC for about 22 hours. The culture was centrifuged at 8,000*g* for 10 minutes and the pellet resuspended in PBS. Absorbance of the bacterial suspension at 625 nm wavelength was adjusted to a reading of 2.23, which corresponded to about 4 $x10^8$ colony-forming units (CFU)/ml.

Preparation of fingers for experiments

The finger nails were trimmed regularly to a length of 3 mm from the hyponychia. The hands were washed with soap during the first ablution in the morning and not washed again with soap or detergent until after the scheduled experiment. Shortly before the experiment, the hands and fingers were washed once with distilled water and dried with paper towels. Subsequently, the fingers were not allowed to touch any objects before the experiment. The fingers were used again in an experiment only after an interval of at least 24 hours.

Viable counts of *V. cholerae*

V. cholerae was enumerated as CFU by the pour plate method. TCBS agar medium was dissolved in $^9/_{10}$ the final volume of water and maintained as a liquid in a 50ºC water bath. Appropriate dilutions of the bacteria suspensions or washings from the inoculated

fingers were made in PBS. One milliliter from each dilution was transferred into a 9-cm Petri dish and 9 ml of liquid TCBS agar was added and mixed well. After the agar had solidified, the plates were incubated at 37ºC overnight and the number of yellow colonies were counted. When colonies imbedded in the agar were very small, counting was performed after 48 hours of incubation.

Survival of *V. cholerae* on the fingers

The hand was held with the palm facing upwards with the digits extended and abducted. A 5 µl volume of bacteria in PBS (~4 x10^5 or ~4x10^6 CFU) was deposited on the finger pad of the 4th digit and quickly spread to an area of about 6 mm diameter using the side of the micropipette tip used in the inoculation. The procedure was repeated for the 3rd and 2nd digits. Immediately after the 2nd digit was inoculated ("0 minute" exposure), the inoculated surface was washed with 10 ml PBS and the washing collected in a Petri dish held below the finger. The washed finger tip was dipped for a few seconds in 70% ethanol to inactivate any remaining viable vibrios and then dipped into PBS to remove the ethanol. The 3rd and 4th inoculated digits were washed at 2 and 5 minutes after inoculation, respectively. Appropriate dilutions of the washings were made in PBS and viable counts of *V. cholerae* were performed. After each experiment, the entire hand was washed with a bactericidal liquid detergent. In subsequent studies, the bacteria were suspended in PBS containing 4% BSA, and 0.5% dissolved agarose.

In the studies where bacteria was placed on the hyponychia in the subungual position, and in the lateral grooves and eponychia, the sequence of steps were similar to that following inoculation on the finger pads. At least 4 replicate experiments were performed on each fingertip location with a particular bacteria-suspending medium.

Uninoculated fingers were washed with PBS and the washings cultured for the presence of yellow colonies. Since none were detected, all yellow colonies from vibrio-inoculated fingers were counted as *V. cholerae*.

In each group of inoculated fingers, the isolation rate of vibrios was the proportion of inoculated fingers which retained viable vibrios at a certain period after exposure (2 or 5 minutes). The survival rates (at 2 and 5 minutes) were the mean number of surviving vibrios expressed as a percentage of the inoculated vibrios at "0 minute" exposure. For each group of inoculated fingers, the number of vibrios inoculated on the different replicate fingers (at "0 minute" exposure) was expressed as a variability index obtained by dividing the greatest observed value by the smallest and rounding the figure obtained to a whole number. The variability index of the survival rate of vibrios on the fingers at a specific time after exposure (2 and 5 minutes) was expressed in the same way.

Statistical analysis

Significant differences between the means of the 2 groups was analyzed by an unpaired Student's *t* test for normally distributed data and the Mann-Whitney *U* test for non-normally distributed data using SPSS version 13. The significance level was set at $p< 0.05$.

RESULTS

V. cholerae retained on finger pads after inoculation

Table 1 shows that when *V. cholerae* were suspended in PBS, it was isolated from 1 of 4 finger pads 2 minutes after inoculation, but not from any finger pads 5 minutes after inoculation. When the bacteria-suspending medium was PBS-BSA, vibrios were isolated from 60% and 40% of finger pads at 2 and 5 minutes, respectively. Vibrios suspended in PBS-agarose medium were isolated from all the inoculated finger pads 2 minutes after inoculation and from 33-40% of finger pads 5 minutes

Table 1
Proportions of viable *V. cholerae* retained on finger pads after inoculation with *V. cholerae* suspended in different media.

Bacteria-suspending medium	Detection of *V. cholerae* at different exposure periods (minutes)		
	0	2	5
PBS	4/4[a]	1/4	0/4
	$2.7x10^5 \pm 1.5x10^5$ [b]	2 x10"3 % [c]	0
	$1.4x10^5/4.7x10^5$ [d] (3)[e]	0	
PBS + 4% BSA	5/5	3/5	2/5
	$7.5x10^5 \pm 4.9x10^5$	4 $x10^{-4}$ %[f]	3 $x10^{-2}$ %
	$2.1x10^5/1.5x10^6$ (7)	6 $x10^{-5}$/5 $x10^{-4}$[g] (8)[h]	$1x10^{-2}$/5 $x10^{-2}$ (5)
PBS + 0.5% agarose	3/3	3/3	1/3
	$3.0x10^5 \pm 1.2x10^5$	23.8%[i]	$1x10^{-2}$ %
	1.8 $x10^5/4.2x10^5$ (2)	0.6 /47.8 (75)	0
	5/5	5/5	2/5
	$4.1x10^6 \pm 2.2x10^6$	23.4%[i]	1 $x10^{-4}$ %
	$2.4x10^6/8.0x10^6$ (3)	1 $x10^{-4}$ /57.8 (577,500)	$5x10^{-5}/2x10^{-4}$ (40)

[a]Proportion of inoculated fingertips with *V. cholerae* isolated, [b]mean CFU of vibrios from replicate fingers ± SD, [c]vibrio CFU expressed as % of vibrio CFU at "0 minute" exposure, [d]lowest/highest vibrio CFU from replicate fingers in the group, [e]variability index of vibrios on replicate fingers in the group (The highest CFU value divided by the lowest), [f]mean vibrio CFU expressed as % of vibrio CFU at "0 minute" exposure, [g]lowest/highest vibrio CFU from replicate fingers in the group expressed as % of bacteria at "0 minute" exposure in replicate experiments, [h]variability index of vibrios on replicate fingers in the group (the highest % value divided by the lowest).
[i]Not significantly different

after inoculation with 2 different bacteria doses.

Very low proportions of vibrios were isolated from finger pads 2 minutes after inoculation of vibrios suspended in either PBS or PBS-BSA. However, for vibrios suspended in PBS-agarose, about 25% of inoculated vibrios were isolated from 2 different groups of finger pads inoculated with bacteria doses which differed by 13.5 fold. None to very low proportions of vibrios were isolated from finger pads 5 minutes after inoculation with vibrios suspended in any of the 3 types of media.

The variability indices of vibrios recovered from different fingers immediately after inoculation ("0 minute" exposure) were 7 and below in all 4 groups. After exposure, the variability index of 1 group was also less than 7 (vibrios in PBS + BSA, 5 minutes) but in the 4 other groups they ranged from 40 (high dose vibrios in PBS + agarose after 5 minutes) to nearly 600,000 (high dose vibrios in PBS + agarose after 2 minutes).

All 3 types of bacteria-suspending media inoculated on the fingers became dry at about the same time after inoculation. Inoculum containing PBS-BSA and PBS-agarose formed visible films over the inoculated area.

V. cholerae retained on lateral nail grooves and eponychia after inoculation

Table 2 shows when the lateral nail grooves and eponychia were inoculated with vibrios suspended in PBS, vibrios were isolated from all fingertips of 2 groups at 2 minutes; the

Table 2
Proportions of viable *V. cholerae* retained on lateral nail grooves and eponychia after inoculation with *V. cholerae* suspended in different media.

Bacteria-suspending medium	Detection of *V. cholerae* at different exposure periods (minute)		
	0	2	5
PBS	5/5[a]	5/5	3/5
	$4.3 \times 10^4 \pm 4.0 \times 10^4$ [b]	3.9% [c,h]	0.4%
	$1.1 \times 10^4 / 8.7 \times 10^4$ [d] (8)[e]	$3 \times 10^{-2} / 15.1$[f] (504)[g]	0.1/0.8 (6)
	4/4	4/4	1/4
PBS	$1.8 \times 10^6 \pm 1.7 \times 0^6$	0.4%[h]	2×10^{-3} %
	$3.6 \times 10^5 / 3.6 \times 10^6$ (10)	$5 \times 10^{-2} / 0.9$ (19)	0
PBS + 0.5% agarose	5/5	5/5	5/5
	$5.6 \times 0^5 \pm 2.6 \times 0^5$	32.3%[i]	0.7%[i]
	$2.5 \times 10^5 / 9.3 \times 10^5$ (4)	2.1/88.3 (42)	$1 \times 10^{-2} / 2.6$ (261)

[a]Proportion of inoculated fingertips with *V. cholerae* isolated, [b]mean CFU of vibrios from replicate fingers ± SD, [c]mean vibrio CFU expressed as % of vibrio CFU at "0 minute" exposure, [d]lowest/highest vibrio CFU from replicate fingers in the group, [e]variability index of vibrios on replicate fingers in the group (the highest CFU value divided by the lowest), [f]lowest/highest vibrio numbers from replicate fingers in the group expressed as % of bacteria number at "0 minute" exposure in replicate experiments, [g]variability index of vibrios on replicate fingers in the group (the highest % value divided by the lowest).
[h]Not significantly different
[i]Significantly different (t = 2.765, df = 8, p = 0.024)

fingertips were inoculated with bacterial doses which differed by 42 fold. At 5 minutes, vibrios were isolated from 60% and 25% of fingers inoculated with lower and higher doses of bacteria, respectively. Vibrios suspended in PBS-agarose were isolated from all fingers at 2 and 5 minutes after inoculation.

Small proportions of vibrios in PBS were isolated from the inoculated sites at 2 minutes. The proportions isolated were reduced by a further 10 fold or more by 5 minutes. The proportions of viable vibrios recovered from the fingers inoculated with 2 different doses of vibrios suspended in PBS were not significantly different at either 2 or 5 minutes. The proportion of vibrios in PBS-agarose isolated from the fingers at 2 minutes was relatively high but the figure at 5 minutes was significantly lower by 46 fold at 5 minutes (t = 2.765, df = 8, p = 0.024). The proportion of vibrios suspended in PBS-agarose isolated from the fingers at 2 minutes was significantly higher than the mean figures from the 2 different doses of *V. cholerae* suspended in PBS (2.15%, averages of 39% and 0.4%) at the corresponding time (Mann-Whitney test, z = -2.6000, p <0.05).

The variability among the indices of vibrios recovered from fingers immediately after inoculation ("0 minute" exposure) in the 3 groups was 10 or below. After 2 and 5 minutes, the variability index of 1 group was below 10 (low dose vibrios in PBS at 5 minutes) but in 4 other groups the variability indices ranged from 19 (high dose vibrios in PBS at 2 minutes) to 504 (low dose vibrios in PBS at 2 minutes).

V. cholerae retained on hyponychia after inoculation

Table 3 shows that *V. cholerae* was isolated from all inoculated fingers at 2 and 5 minutes when the vibrios were suspended in

Table 3
Proportions of viable *V. cholerae* retained on hyponychia after inoculation with *V. cholerae* suspended in different media.

Bacteria-suspending medium	Detection of *V. cholerae* at different exposure periods (minute)		
	0	2	5
PBS	5/5[a]	5/5	5/5
	$1.3x10^5 \pm 9.4x10^4$ [b]	8.4% [c]	0.2%
	$3.9x10^4/2.7x10^5$ [d] (7)[e]	0.3/34.1[f] (131)[g]	$1 x10^{-3}$/0.8 (750)
PBS + 0.5% agarose	3/3	3/3	3/3
	$3.6x10^5 \pm 1.8x10^5$	53.1%[h]	10.8%[h]
	$1.1x10^5/5.4x10^5$ (5)	12.1/96.3 (8)	1.9/27.7 (15)
	4/4	4/4	4/4
	$3.6x10^6 \pm 2.6x10^6$	38.7%[h]	4.6%[h]
	$6.5x10^5/1.7x10^6$ (3)	18.5/62.5 (3)	0.1/6.7 (67)

[a]Proportion of inoculated fingertips with *V. cholerae* isolated, [b]mean CFU of vibrios from replicate fingers ± SD, [c]mean vibrio CFU expressed as % of vibrio CFU at "0 minute" exposure, [d]lowest/highest vibrio CFU from replicate fingers in the group, [e]variability index of vibrios on replicate fingers in the group (the highest CFU value divided by the lowest), [f]lowest/highest vibrio numbers from replicate fingers in the group expressed as % of bacteria at "0 minute" exposure in replicate experiments, [g]variability index of vibrios on replicate fingers in the group (the highest % value divided by the lowest).
[h]Not significantly different.

PBS or PBS-agarose and inoculated on the hyponychia beneath the finger nails.

For *V. cholerae* suspended in PBS, the mean number of vibrios isolated from fingers at 2 minutes was 8% the number at "0 minute" exposure. Five minutes after exposure, the figure was reduced 44 fold. For vibrios suspended in PBS-agarose, a relatively high proportion of vibrios was isolated at 2 minutes from 2 groups of fingers inoculated with vibrios doses which differed by about 10 fold. At 5 minutes, the proportions of vibrios isolated were reduced by about 5 and 9 fold at 2 minutes after inoculation with low and high doses, respectively. The proportions of viable vibrios recovered from the fingers inoculated with the 2 different doses of vibrios suspended in PBS-agarose were not significantly different at either 2 or 5 minutes. The average of the proportions of vibrios recovered from the 2 different doses at 5 minutes (7.7%) was significantly lower than at 2 minutes (45.9%) (t = 3.575, df = 12, p = 0.004). Both these figures were significantly higher than the corresponding figures from vibrios suspended in PBS (2 minutes: t = -2.950, df = 10, p = 0.015; 5 minutes: t = -2.631, df = 10, p = 0.025).

The variability indices of vibrios recovered from different fingers immediately after inoculation ("0 minute" exposure) were 7 or below for all 3 groups. After exposure, the variability indices of 2 groups were also 7 or below (low and high vibrios doses suspended in PBS + agarose at 2 minutes) but in 3 other groups they ranged from 15 (low dose vibrios in PBS+agarose at 5 minutes) to 750 (vibrios in PBS at 5 minutes).

DISCUSSION

This study established the presence of viable *V. cholerae* on fingertips was influenced by the site of the finger where the vibrios were inoculated and the medium in which the vibrios

were suspended.

V. cholerae survival on fingertips was evaluated by obtaining the proportion of inoculated fingers which retained viable bacteria and the mean proportion of viable bacteria remaining on vibrio-positive fingers at 2 and 5 minutes after inoculation. The latter was expressed as a percentage of the inoculum on the fingers at "0 minute" exposure. This is a more accurate indicator of transmission potential since the presence of viable bacteria below the infectious dose does not lead to transmission (Hornick *et al*, 1971). Using both criteria, the hyponychia provided the best location for survival followed by the eponychia and lateral nail grooves, and the finger pads. The differences in the ability of the 3 sites to sustain bacteria became more apparent at longer periods after inoculation.

The different levels of protection provided by the 3 different locations were likely related to their different abilities to prevent or delay dehydration of *V. cholerae,* which is very sensitive to drying (WHO, 1993), and also in providing shelter from sunlight which damages the bacteria (Pan American Health Organization, 1991). Of the 3 finger positions studied, the hyponychium in the subungual position was the most sheltered location. The fingernails which extended 3 mm beyond the hyponychia were a major contributor to the sheltered position of the hyponychia in this study. The most exposed fingertip position was the finger pad. The level of cover provided by the lateral nail grooves and eponychium was between that of the finger pad and hyponychium; the intimate contact between the surface of the lateral nail fold and the nail produced the sheltered lateral nail groove but the eponychium was exposed.

In the natural setting, *V. cholerae* may be suspended in media of different forms and compositions. Liquid media include water which have relatively low concentrations of salts and suspended particles, and "rice-water" stool, which contains protein, mucous flecks and high concentrations of certain electrolytes (Sack *et al*, 2004). In less severe infections feces may be semi-solid or solid (Sack *et al*, 2004). In addition to form, each vibrio-suspending medium probably has a different composition. Previous studies reported that the compositions of the bacteria-suspending medium influenced bacteria survival and media of more complex compositions allowed better survival of bacteria on fingertips. Thus campylobacters suspended in chicken or horse blood survived longer on fingertips than when the bacteria were suspended in 0.1% peptone solution (Coates *et al*, 1987). *Listeria monocytogenes* suspended in milk survived longer than when suspended in saline (Snelling *et al*, 1991). This study also revealed that the *V. cholerae* milieu was an important factor in determining the survival rate of the bacterium on fingertips. *V. cholerae* suspended in a viscous PBS-agarose medium has higher survival rates on all fingertip locations compared to vibrios suspended in PBS alone. PBS is a simple inorganic liquid not harmful to the bacterium. The presence of a soluble protein (BSA) in PBS did improve survival on finger pads by increasing the proportion of inoculated fingers which retained viable bacteria. However, the proportions of surviving bacteria remained very low. PBS-agarose and PBS-BSA formed visible films over the inoculated areas. This may have the effect of sealing inoculated bacteria inside the many minute cracks, crevices, and hollows found on normal skin (Nobel, 1992). However, BSA-agarose film has a much greater anti-desiccation ability because it is hydrated and probably thicker. The ability to better resist desiccation is due to the presence of water molecules bound inside the double helices of agarose, while the thickness of the film comes from aggregation of these helices (Arnot *et al*, 1974).

The range in the numbers of vibrios recovered from inoculated fingers in the 10

treatment groups was smaller immediately after inoculation than by 2 and 5 minutes after exposure. The variability indices (a measure of range) in 60% of the 10 groups were 5 or below while the indices for the remaining groups did not exceed 10. In contrast, 75% of the 16 groups exposed for 2 or 5 minutes had variability indices which ranged widely from 15 to nearly 600,000. The variations in vibrios numbers recovered from the different fingertips immediately after inoculation were probably caused by unintentional differences in the inoculum sizes used in the different experiments. This occurred because the number of viable bacteria in an inoculum could only be estimated. In addition, unintentional variations may have occurred during washing of the vibrios from the different fingers. Two to 5 minutes after exposure, the wide range in survival rates of the vibrios on different fingertips for most of the groups suggests an additional factor was involved. The vibrios on the fingers probably became dry and inactive at different rates due to surface differences in the skin of the different fingers or day-to-day variations in the skin surface of the same finger of the voluenteer. Thus factors relating to the surface features of the fingers may also affect the survival rate of *V. cholerae* in addition to the type of bacteria-suspending medium and the fingertip site where the vibrios were inoculated.

The finding that high concentrations of *V. cholerae* remained viable for relatively short periods on fingertips even under the best combination of location and bacteria-suspending medium indicates a very narrow temporal window for transmission, which would be consistent with the current view that direct person-to-person transmission is rare (Mintz *et al*, 1994). However, when a number of fingers are contaminated, the bacterial numbers on the contaminated fingers may reach an infectious dose for direct person-to-person transmission in some situations where the infectious dose is lower (Hornick *et al*, 1971, Sacks Jr *et al*, 1972), or it increases the chances of the bacterium surviving and replicating when transferred to a suitable object which supports replication, such as food. The probability of these scenarios is increased when the fingers are contaminated by high doses of vibrios since more viable vibrios would be present even though this study revealed that larger doses did not lead to a higher proportion of vibrios surviving.

A limitation of this study was the use of one volunteer; since skin characteristics of each individual have been reported to determine the survival rate of transient microorganisms (Seligmann and Rosenbluth, 1975). The use of more volunteers would have given a more representative picture of *V. cholerae* survival on human fingers, although there are considerable difficulties in getting volunteers for such a study.

In conclusion, the location of the fingertip contaminated with *V. cholerae* and the type of medium the bacteria were suspended in were important determinants of *V. cholerae* survival on human fingers. However, for all vibrio-suspending media and fingertip locations, survival rates of vibrios on the fingers was only maintained briefly. A combination of bacteria-suspending medium that retains moisture (PBS-agarose) and a sheltered position on the fingertips (hyponychia with extended fingernails) provided the best survival rates for *V. cholerae* on the fingers.

ACKNOWLEDGEMENTS

This study was supported financially by the Department of Biomedical Science, Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia.

REFERENCES

Arnot SA, Fulmer WE, Scott IC, *et al*. The agarose double helix and its function in agarose gel

structure. *J Mol Biol* 1974; 90: 269-84.

Coates D, Hutchinson DN, Bolton FJ. Survival of thermophilic campylobacter on fingertips and their elimination by washing and disinfection. *Epidemiol Infect* 1987; 99: 265-74.

Colwell RR. Infectious disease and environment: cholera as a paradigm for waterborne disease. *Int Microbiol* 2004; 7: 285-9.

Hornick RB, Music SI, Wenzel R, *et al*. The Broad Street pump revisited: response of volunteers to ingested cholera vibrios. *Bull NY Acad Med* 1971; 47: 1181-91.

Lewin K. The normal finger nail. *Br J Dermatol* 1965; 77: 421-30.

McCormack WM, Mosley WH, Fahimuddin M, Benenson AS. Endemic cholera in rural East Pakistan. *Am J Epidemiol* 1969; 89: 393-404.

Mintz ED, Popovic T, Blake PA. Transmission *of V. cholerae* O1. In: Wachsmuth IK, Blake PA, Olsvik O, eds. *Vibrio cholerae* and cholera: molecular to global perspectives. Chapter 22 Washington DC: ASM Press, 1994: 345-56.

Nobel WC. The skin microflora and microbial skin disease. Cambridge: Cambridge University Press, 1992.

Pan American Health Organization. Cholera in the Americas. *Bull Pan Am Health Organ* 1991; 25: 267-73.

Pollitzer R. Cholera*. World Health Organ Monogr Ser* 1959; 43.

Public Health Agency Canada. Material safety data sheet - Infectious substances section I - infectious agent. Name: *Vibrio cholerae,* serogroup O1*,* serogoup O139 (Bengal). 2001. [Cited 2007 May 29]. Available from: URL: http://www.phac-aspc.gc.ca/msds-ftss/msds164e.html

Rabbani GH, Greenough III WB. Food as a vehicle of transmission of cholera. *J Diarrheal Dis Res* 1999; 17: 1-9.

Sack DA, Sack BR, Nair GB, Siddique AK. Seminar Cholera. *Lancet* 2004; 363: 223-33.

Sack Jr GH, Pierce NF, Hennessey KN, Mitra RC, Sack RB, Mazumder, DN. Gastric acidity in cholera and noncholera diarrhea. *Bull World Health Organ* 1972; 47: 31-6.

Seligmann R, Rosenbluth S. Comparison of bacterial flora on hands of personnel engaged in non-food and food industries: A study of transient and resident bacteria. *J Milk Food Technol* 1975; 38: 673-7.

Snelling AM, Kerr KG, Heritage J. The survival of *Listeria monocytogenes* on fingertips and factors affecting elimination of the organism by hand washing and disinfection. *J Food Protect* 1991; 54: 343-8.

Singh J, Bora D, Sharma RS, Khanna KK, Verghese T. Epidemiology of cholera in Delhi–1992. *J Trop Pediatr* 1995; 41: 139-42.

WHO. Guidelines for cholera control. Geneva: WHO, 1993.

WHO. Guidelines on hand hygiene in health care (advanced draft). Geneva: WHO, 2005.