Isolation of Pathogenic Bacteria and Opportunistic Pathogens from Public Telephones

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A survey of 20 randomly selected public telephones in the central business district of Melbourne, Australia, revealed the presence of contaminating pathogenic bacteria and opportunistic pathogens. Bacteria were recovered from all telephones sampled: 40% were contaminated with coagulase-positive Staphylococcus aureus *and 80% with lactose-fermenting and non-fermenting bacteria, indicating possible faecal contamination. Further identification revealed the presence of the following enteric and non-enteric bacteria:* Acinetobacter anitratus, Enterobacter cloacae, Escherichia coli *and* Pantoea agglomerans *(formerly* Enterobacter agglomerans*). A viridans Streptococcus, most probably from the* "S. mitis" *group, was recovered from one of the telephones. All these bacteria are pathogens or opportunistic pathogens. Inoculation of one of the* S. aureus *isolates onto an inert plastic surface and incubation at room temperature indicated that, although a significant reduction in the recovery of viable bacteria was observed after one day, over 10% of bacteria survived for up to seven days. This was reduced to less than 5% survival after 14 days. Our results suggest that telephone surfaces may serve as potential reservoirs for the transmission of disease-causing bacteria.*

Key Words: *Telephones, Bacterial Contamination, Disease Transmission, Fomites*

Numerous studies have demonstrated the survival and persistence of bacteria and viruses on various environmental surfaces (Abad, Pinto & Bosch 1994; Bean et al. 1982; Cozanitis, Falsey & Walsh 1993; Davies et al. 2000; Grant & Makela 1978; Musa, Desai & Casewell 1990; Noskin et al. 1995; Rafferty & Pancoast 1984; Rogers et al. 2000). These fomites can act as transmission vehicles for pathogens and can be the cause of nosocomial infections in hospitals and intensive care units (Cozanitis, Grant & Makela 1978; Davies et al. 2000; Noskin et al. 1995). Personal contact, especially through contaminated hands, and aerosols are also known to be important in the transmission dynamics of many diseases (Maki 1989). Many studies have investigated disease transmission in hospital settings and day care centres, although common domestic surfaces are also known to be the source of potential pathogens. Such studies have focussed on hospital settings primarily because of the concern of opportunistic nosocomial infections especially in immune compromised individuals (Quinn 1998; Rogers et al. 2000).

There have been few studies of the role of telephones, a common item in commercial, domestic and health care environments, as fomites. The majority of these studies have also focussed on hospital settings. One study found 7% of hospital telephones sampled contained potentially pathogenic bacteria including *Klebsiella, Enterobacter, Pseudomonas and Aeromonas* (Rafferty & Pancoast 1984) while potentially pathogenic bacteria have been recovered from telephones in an intensive

care unit (Cozanitis, Grant & Makela 1978). Another study demonstrated that vancomycin-resistant enterococci (VRE) experimentally contaminated onto fingertips and environmental surfaces commonly encountered in the health care setting (including telephones) survived for at least 60 minutes (Noskin et al. 1995). This suggested that environmental surfaces could serve as potential reservoirs for transmission of VRE. Telephones can also be a source of contamination with pathogenic viruses (Butz et al. 1993). The potential importance of telephones in the transmission of infectious diseases was recently demonstrated by Rusin et al. (2000). This study showed that the transfer efficiency of bacteria and viruses from a telephone receiver to the hand (39% and 66%) was greater than the transfer efficiency from a faucet handle (28% and 34%).

Given the potential for telephones to act as vehicles for disease transmission, we have investigated the presence and nature of bacteria contaminating public telephone surfaces, specifically the hand contact area and the mouthpiece. We have investigated the occurrence of common pathogens normally present on human hands and faces, and looked for evidence of faecal contamination.

Methods

Study design and sampling

Twenty (of the approximately 120) public telephones in the central business district of Melbourne, Australia, were selected by random sampling for investigation. All sampling was carried out within a two-hour time period of a normal business day. Two samples were obtained from each telephone, one from the hand contact area and one from the mouthpiece. Sampling was carried out by swabbing the area with a sterile swab moistened with sterile saline. The tip of the swab was broken, transferred to a sterile tube containing 3 ml of Nutrient Broth (NB) (Oxoid Ltd., Basingstoke, UK) and transported to the laboratory on ice. The NB culture was incubated at 37°C for 24 hours to enrich for any bacteria present.

Microbiological media and procedures

All media used in this study were from Oxoid Ltd. and were prepared according to the manufacturer's instructions. The media used were Baird-Parker Medium (BPM), Eosin-Methylene Blue Agar (EMB), Mannitol Salt Agar (MSA) and MacConkey Agar No. 2 (MAC2). After enrichment, all samples were inoculated onto EMB (for detection of *E. coli*), MAC2 (for detection of enteric bacteria and enterococci) and MSA (for detection of staphylococci). Presumptive coagulase-positive staphylococci were sub-cultured onto BPM. All media was incubated at 37°C for 24-48 hours.

Biochemical and confirmatory tests

The Gram reactions and cellular morphology of all isolates were determined. Presumptive *Staphylococcus aureus* isolates were tested for catalase activity, from colonies grown on BPM, using 3% hydrogen peroxide on a glass slide and observing for vigorous bubbling (Koneman et al. 1997). The Staphytect Plus (Oxoid Ltd.) latex slide agglutination test was used to confirm coagulase-positive staphylococci. The presence of the cytochrome enzyme oxidase was determined using Oxidase Identification Sticks (Oxoid Ltd.). Oxidase-negative bacteria were identified using the Microbact™ 24E computer aided bacterial identification system (Medvet Science, Adelaide, Australia). The Streptococcal Grouping Kit (Oxoid Ltd.) latex agglutination test was used to classify presumptive streptococci into groups A, B, C, F or G. Further identification of streptococci was carried out by testing for growth in NB plus 6.5% (w/v) NaCl and for hydrolysis of aesculin in NB containing 0.1% (w/v) aesculin. The type of haemolysis on horse blood agar (HBA) was also determined.

Bacterial survival experiments

To investigate the survival of *S. aureus* on telephone surfaces, 10µl of an overnight NB culture of one of the *S. aureus* isolates was centrifuged and the cell pellet resuspended in 1 ml of sterile saline. Samples (10µl) were inoculated onto a sterile inert plastic surface (to simulate a telephone hand contact area) and allowed to dry. Sterile swabs were used to sample each area after incubation for 0, 1, 2, 7 and 14 days at room temperature (under sterile conditions). Swabs were plated onto NA plates and incubated at 37°C overnight. The procedure was carried out in triplicate.

Results

The findings of the bacteriological survey of 20 public telephones are presented in Table 1. Overall, pathogenic bacteria or opportunistic pathogens were recovered from 13 (65%) of the telephones. For three of the telephones, bacteria were recovered from both the hand contact area and from the mouthpiece. For the others, six had bacteria recovered only from the hand contact area and four only from the mouthpiece. Bacteria were recovered on MSA from all telephones, however, only data for confirmed *S. aureus* (Gram positive cocci, catalase-positive, coagulase-positive) is presented in the table. Bacteria other than confirmed *S. aureus* were presumed to be coagulase-negative non-pathogenic staphylococci. *S. aureus* was recovered from eight telephones (40%), with three of these having the organism present on both the hand contact area and the mouthpiece.

Sixteen (80%) of the telephones were contaminated with bacteria which were isolated on MAC2. The majority of these fermented lactose, indicating the presence of enteric bacteria and possible faecal contamination. A selection of lactose-fermenting and non-fermenting, oxidase-negative isolates were sub-cultured and identified using the Microbact™ 24E system as *A. anitratus*, *E. cloacae*, *E. coli* and *P. agglomerans* (Table 1). The presence of *E.coli* was also indicated by the recovery of characteristic colonies on EMB and confirmed by Gram reaction and microscopic examination.

Table 1. Pathogenic and opportunistic pathogenic bacteria isolated from public telephones (hand contact area and mouthpiece)

Telephone	Hand contact (A) Mouthpiece (B)	Bacteria isolated[1]
1	A	*S. aureus*
	B	*S. aureus*
2	A	-
	B	-
3	A	*E. coli, P. agglomerans*
	B	-
4	A	-
	B	-
5	A	-
	B	*E. cloacae*
6	A	-
	B	-
7	A	*P. agglomerans*
	B	-
8	A	-
	B	-
9	A	*A. anitratus*
	B	-
10	A	*S. aureus*
	B	-
11	A	*S. aureus*
	B	-
12	A	*S. aureus, P. agglomerans*
	B	*S. aureus, E. coli, P. agglomerans*
13	A	*S. aureus*
	B	-
14	A	-
	B	*A. anitratus, E. coli*
15	A	-
	B	-
16	A	-
	B	-
17	A	-
	B	*S. aureus*
18	A	*S. aureus E. coli*
	B	*S. aureus, A. anitratus,* viridans streptococci
19	A	-
	B	-
20	A	-
	B	*S. aureus*

[1] *A. anitratus = Acinetobacter anitratus, E. cloacae = Enterobacter cloacae, E.coli = Escherichia coli, P. agglomerans = Pantoea agglomerans, S.aureus = Staphylococcus aureus.*

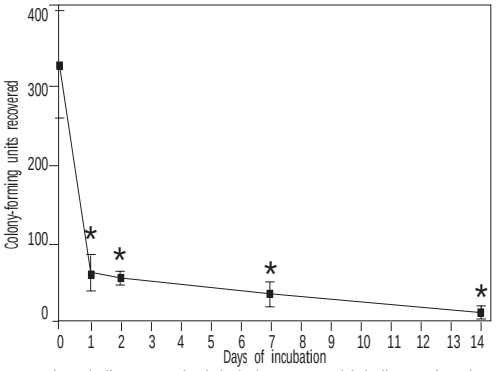
Table 2. Survival of* S. aureus *isolate on inert plastic surface

Length of incubation (days)	CFU recovered (Mean ± SD)	Percent survival
0	327± 67	100.0
1	59 ± 19	18.0
2	58 ± 8	17.7
7	36 ± 15	11.0
14	14 ± 7	4.3

One isolate from MAC2 was further examined because of its characteristic growth that suggested the presence of enterococci, hence faecal contamination. However, the isolate was not identified as group D, exhibited a-haemolysis on HBA, did not hydrolyse aesculin and did not grow in NB + 7.5% (w/v) NaCl. On the basis of these characteristics, the isolate was identified as a member of the *"S. mitis"* group of viridans streptococci (Koneman et al. 1997).

The potential for telephones to act as a vehicle for the transmission of pathogenic bacteria is dependent on the survival of such bacteria on a nutritionally inert surface. The survival of *S. aureus* on the surface of a telephone was investigated as described (see Methods). The number of colony-forming units (CFU) recovered at each sample time are shown in Figure 1. The survival of *S. aureus* was determined from the percentage of CFU recovered after 1, 2, 7 and 14 days relative to the 0 time point. Table 2 shows that there was a significant reduction in viable bacteria after one day, however, over 10% of bacteria survived for up to seven days. After 14 days, the survival rate was reduced to 4.3%.

Figure 1: Recovery of* S. aureus *isolate from an inocluated plastic surface incubated at room temperature



Error bars indicate standard deviation. An asterisk indicates that the difference between the time point and the 0 time point is statisitclly significant ($p<0.05$; paired t-test)

Discussion

Our survey of bacterial contamination of 20 randomly selected public telephones indicated that 65% were contaminated with pathogenic bacteria or organisms that are able to act as opportunistic pathogens. Forty percent of the telephones were contaminated with *S. aureus*, an established human pathogen commonly found in the nasal cavity and in nasal secretions, which is the causative agent of a variety of skin infections and diseases (Noble 1998) and an important food-borne pathogen (Dinges, Orwin & Schlievert 2000). A viridans group Streptococcus was recovered from one telephone. The characteristics of this isolate suggested that it belonged to the *"S. mitis"* group, which is also part of the normal upper respiratory tract flora and has been associated with bacterial endocarditis (Koneman et al. 1997).

A number of opportunistic pathogens were recovered from eight telephones. The role of *E. coli* as an opportunistic pathogen, in particular in urinary tract infections (UTI), is well established (Faro & Fenner 1998). The presence of this bacterium is also an indicator of faecal contamination. Other enteric and non-enteric bacteria were isolated, including *Acinetobacter anitratus*, *Enterobacter cloacae* and P*antoea agglomerans*. Members of the genus *Acinetobacter* are implicated in various nosocomial infections, particularly bacteraemia, secondary meningitis and UTI (Towner 1997). *E. cloacae* and *P. agglomerans* (formerly *E. agglomerans*) are found in water, sewage, soil and vegetables and as part of the commensal enteric flora. They have been associated with a variety of opportunistic infections of the urinary and respiratory tracts and can cause septicemia and meningitis (Koneman et al. 1997; Maki et al. 1976).

Although this study has demonstrated that public telephones are contaminated with a variety of bacteria, there is no direct evidence that the use of public telephones is an infection hazard. However, given that

telephones have recently been shown to be an efficient transfer vehicle for bacteria and viruses (Rusin et al. 2000), it is conceivable that the contaminated surfaces may serve as potential reservoirs for transmission of pathogenic microorganisms. In addition, we have demonstrated that *S. aureus* is capable of survival on an inert plastic surface for up to 14 days. It is therefore conceivable that potentially pathogenic bacteria can survive on nutritionally inert surfaces for extended time periods. If the surface was also contaminated with organic matter (e.g., blood or mucous in a hospital environment), the potential for survival would increase. Further studies could therefore involve survival studies of *S. aureus* and other bacteria under different incubation conditions (temperature and presence of organic matter). Similarly, sampling of telephones could be carried out over a wider time interval to investigate whether seasonal variation contributes to the survival and variety of recovered species.

Another limitation of the present study is that this survey was not quantitative and, therefore, did not yield information about the numbers of bacteria present and whether they posed an infection hazard. However, given that evidence of faecal contamination was obtained and large numbers of pathogens can be shed in the stool of an individual, it is possible that small amounts of contamination indicate a potential health risk. It might, therefore, be informative to carry out direct sampling and plating procedures from telephone surfaces to yield quantitative data about the level of bacterial contamination.

Conclusion

The typical design of a telephone and the normal usage practices are conducive to the transfer of infectious agents. There is considerable opportunity for contamination given the contact with hands, face and lips. In this study, telephone users were observed coughing, sneezing and laughing into the mouthpiece thereby facilitating the contamination of telephone surfaces by nasal and oral aerosols. In addition, it is not common practice for users to wash their hands before or after telephone use. For these reasons, this study could be extended to include a survey of viral pathogens present on telephone surfaces. This could be achieved by subjecting swabbed material to polymerase chain reaction analysis for specific viruses or by attempting to culture viruses.

The present study may act to increase public awareness of the importance of good hygiene practices after any activity, which has the potential to transfer infectious agents, such as the use of public telephones. Reducing the microbiological hazards associated with contaminated surfaces is particularly important in environments where susceptible individuals are likely to be found, such as hospitals. The results of similar and wider studies may help to identify and reduce the hazards of common sources of bacterial contamination in domestic environments.

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