Typing of Staphylococcus aureus from mobile phones and clinical samples

Article *in* Current Science · April 2006

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Despite the advances in modern medicine, nosocomial infection still poses a risk of increased morbidity and mortality to patients and the hands of healthcare personnel may play an important role in the transmission of hospital-acquired infections (HAIs)[1,2]. The spectrum of nosocomial pathogens has changed over the past decades. Gram-positive cocci such as *Staphylococci* and *Enterococci* have gradually dominated Gram-negative bacilli, and fungal pathogens have gained more importance, particularly in immunocompromised patients. However, the etiological agent of HAI varies from hospital to hospital and in different geographical regions[3].

An important problem that arises in hospitals is the monitoring and detection of nosocomial infections. Highly sensitive, rapid and specific nucleic acid-based assays allow the detection and characterization of microbial DNA or RNA sequences[4], thereby facilitating the study of their transmission patterns. Moreover, the role of the microbiology laboratory in monitoring antimicrobial resistance has gained critical importance due to the alarming frequency of multi-drug resistant pathogens as cause of HAIs.

These days, mobile phones have become an extension of office practice for physicians. Mobile phones, Personal Digital Assistants and wireless computers may transmit more than just information in today's busy hospitals. They may also be involved in the transmission of infections in the healthcare systems. Recent literature search did not yield any reported role of mobile phones in the transmission of hospital infections in India. However, few studies have been reported from Israel and Spain in this regard[5]. With this background, the present study was undertaken to know the role of cell phones in infection transmission in hospital settings.

The study was carried out over a period of two months from September to October 2004, during which random sampling of mobile phones (n = 30) and hands of healthcare professionals (n = 30), attending patients admitted to the medicine and the neonatal ICU at the Kasturbha Medical College, Mangalore was done using sterile cotton swabs by applying an even pressure and rotating the swab without interruption. The samples collected were immediately transported to the microbiology laboratory and inoculated onto Columbia blood agar plates and MacConkeys agar plates (Hi-Media Laboratories, Mumbai). These plates were incubated at 37°C for 48 h. Plates were observed for growth and a Gram smear was performed from different types of colonies. Based on the Gram reaction and colony morphology, catalase and oxidase tests were performed. Species identification was done using biochemical reactions subject to preliminary findings. *Staphylococcus aureus* (n = 15) isolated during the same period from intravenous catheters, pus and blood samples of patients admitted to the medicine and neonatal ICU were also included in the study.

All *S. aureus* isolates were identified by standard biochemical reaction[6]. Gram-positive, catalase-positive cocci were tested for mannitol fermentation on mannitol salt agar and glucose fermentation using oxidative fermentative (OF) medium. Clumping factor was detected using the slide coagulase test and organisms were confirmed as *S. aureus* by the tube coagulase test. All the media and reagents used were procured from Hi-Media Laboratories.

Muller Hinton broth and Muller Hinton agar were used, and antibiotic susceptibility testing was performed by the disk diffusion method[7]. Briefly, biochemically confirmed *S. aureus* isolates (2–3 colonies) were grown in Muller Hinton broth for 6 h at 37°C. Turbidity was adjusted to 0.5 McFarland standard after which Muller Hinton agar plates were seeded with the cultures[8]. Different antibiotic discs like ampicillin, penicillin, erythromycin, lincomycin, netillin, oxacillin, ciprofloxacin and cefoperazone were placed on the inoculated medium. Antibiotic sensitivity plates were incubated at 37°C for 24 h. The zones of clearing around the discs were measured and compared with CLSI standard and interpreted[9] as either sensitive or resistant or intermediate.

DNA was prepared as follows[10,11]. Biochemically confirmed *S. aureus* isolates obtained from mobile phones, the hands of healthcare professionals and clinical samples were grown on tryptic soy agar plates for 24 h at 37°C to obtain isolated colonies. Five colonies were emulsified in 100 µl TRIS EDTA buffer (pH 8.0) and treated with proteinase K (250 µg/ml). This suspension was incubated at 37°C for 1 h and heated for 15 min in a dry bath (Bangalore Genei, Bangalore) at 100°C to inactivate proteinase K. Lysates were centrifuged and 1 µl of the supernatant was used for polymerase chain reaction (PCR). Different dilutions of DNA (24 and 96 ng/reaction) were used to test the reproducibility and eliminate non-specific amplification products from the analysis. Two custom-synthesized decamer random primers R_1 (5′GCGATCCCCA3′) and R_2 (5′CAGCACCCAC3′) procured from Bangalore Genei, Bangalore were used in RAPD reaction[12].

DNA amplification and detection of amplified product were as follows[12,13]. Amplifications were performed in 25 µl reaction mixture consisting of genomic DNA (96 ng/reaction); 1X reaction buffer; 100 µM each of dATP, dCTP, dGTP, and dTTP; 0.2 µM random primer; 2.5 µM $MgCl_2$ and 1U of Taq polymerase. A single primer was used in each reaction. The PCR tubes containing master mix, primer and DNA were amplified in a thermocycler (Bio Rad Inc., USA). PCR reaction was carried out up to 35 cycles. The reaction conditions were: initial denaturation at 94°C for 5 min, denaturation at 94°C for 1 min, annealing at 36°C for 1 min and extension at 72°C for 2 min and the final extension at 72°C for 10 min. The amplified product was resolved by agarose gel electrophoresis using 2% agarose in 1X TAE buffer containing 0.5 µg/ml ethidium bromide. Gels were visualized under UV trans-illuminator and gel pictures were photographed (PhotoCaptMw # 11.01). Banding patterns were determined and arbitrarily numbered.

Of the 30 samples collected from mobile phones and the hands of healthcare professionals, 12 samples from the former (40%) and 12 from the latter (40%) showed growth of *S. aureus*. Other organisms isolated were *S. epidermidis* (two each from hands and mobile phones) and *Bacillus* spp. (one each from hands and mobile phones). Among the swabs collected from mobile phones, 15 did not show any growth whereas 14 hand sam-

Table 1. Antibiotic sensitivity patterns of *Staphylococcus aureus* isolated from different sources

	Mobile phones (n = 12)			Hands (n = 12)			Patient sample (n = 12)		
Antibiotic used	S	R	I	S	R	I	S	R	I
Cefoperazone	7	4	1	2	6	4	7	4	1
Ciprofloxacin	7	5	–	8	3	1	5	7	–
Erythromicin	4	8	–	6	6	–	4	8	–
Lincomycin	9	3	–	10	2	–	8	4	–
Netillin	11	1	–	11	1	–	9	3	–
Methicillin	7	3	2	6	6	–	7	5	–
Penicillin	6	6	–	2	10	–	12	–	–

S, Sensitive; R, Resistant; I, Intermediate.

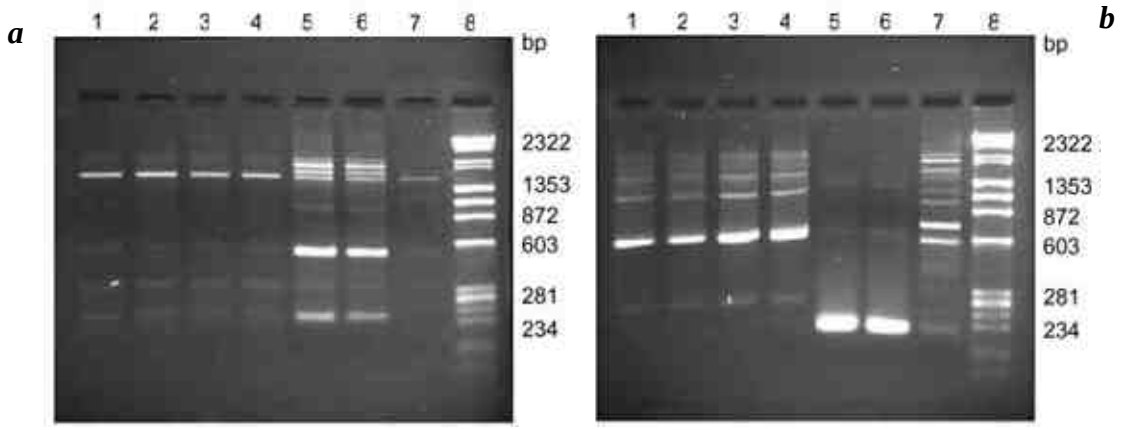

Figure 1. RAPD typing of *Staphylococcus aureus* isolates using (***a***) R1 primer and (***b***) R2 primer. Lanes 1, 2, Isolates from mobile phones; lanes 3, 4, Isolates from hands of healthcare professionals; lanes 5 and 6, Isolates from clinical samples (pus); lane 7, *Staphylococcus aureus* (ATCC 25923), lane 8, Molecular weight marker.

ples were sterile. Among the 15 clinical samples, 12 showed growth of *S. aureus* (80%). Other organisms isolated from clinical samples were *Pseudomonas* spp. (n = 2) and *Acinetobacter* spp. (n = 1). Irrespective of their source, all *S. aureus* isolates showed identical biochemical reaction. They were catalase test-positive, coagulase test-positive, fermented glucose and mannitol.

The antibiotic susceptibility patterns of the isolates from mobile phones, hands of health care professionals and patients are shown in Table 1.

Gel pictures of RAPD are shown in Figure 1 *a* and *b*.

It is interesting to note that mobile phones of a few healthcare professionals in our hospital harbour bacteria like *S. aureus*. A similar study at Soroka hospital in Israel found that 12% of cell phones belonging to doctors and nurses carried *Acinetobacter baumanii*, a drug-resistant bacteria that can be lethal to critically ill patients. Hence, the use of cell phones in patient-care areas has been banned in that hospital[5]. In another study, a variety of bacteria were found on electrocardiogram (ECG/EKG) wires used to monitor ICU patients[14]. The researcher recommended developing effective disinfection procedures for EKG wires or using wireless or disposable wires, as they come in direct contact with ICU patients. In the present study, it has been observed that 25% of *S. aureus* isolated from mobile phones, 50% of *S. aureus* from the hands of the healthcare professionals and 41.7% of the isolates from patients were methicillin-resistant. Reported carriage rates[15] in injection drug users, persons with insulin-dependent diabetes, patients with dermatologic conditions, patients with long-term indwelling intravascular catheters and healthcare workers are 25 to 50%. Our results are in agreement with the reported results.

Two *S. aureus* isolates from two of the hands of healthcare professionals and mobile phones showed identical antibiotic sensitivity pattern being resistant to penicillin, ampicillin, lincomycin, erythromycin and methicillin. Two clinical isolates of *S. aureus* were resistant to penicillin, ampicillin and methicillin, thereby showing similar antibiotic sensitivity patterns probably indicating their origin from the same source. Hence to confirm the validity of these findings, a more discriminating genetic typing method like RAPD was used.

RAPD analysis of genomic DNA of *S. aureus* strains (n = 36) using random primers R1 and R2 yielded 34 different profiles or banding patterns with each primer. Two isolates of *S. aureus* from mobile phones and two from the hands of healthcare professionals showed similar profile in major bands (distinctly visible bands with sizes ranging between 234 and 2322 bp). This shows genetic relatedness among the isolates from mobile phones and hands. Two clinical isolates of *S. aureus* showed identical pattern with respect to each other. However, these profiles were totally different in the isolates from mobile phones and hands (Figure 1 *a* and *b*), which indicates genetic polymorphism or differences. We also observed that when RAPD reactions were performed with the same preparation of

genomic DNA, using the same brand and the same lot of enzyme, the banding pattern was reproducible and specific for each strain. Earlier studies have shown RAPD to be an effective method for epidemiological investigation of outbreaks by demonstrating possible relationship between host origin, mutation and genetic variation among *S. aureus*[10,16,17].

Our results indicate that mobile phones may get contaminated through the hands. Hence, these mobile phones when used carelessly in the ICU or surgical wards may act as a source of MRSA to patients. Moreover, these contaminated mobile phones and the hands of the healthcare professionals may also pose a danger in the spread of infection to the community. In our set-up, though *S. aureus* was isolated from clinical samples, mobile phones and hands of healthcare professionals, the clinical isolates did not show any genetic relatedness to those from mobile phones and hands. These findings clearly explain that, in spite of colonization of hands and mobile phones by *S. aureus*, they are not transferred to critically ill patients by our healthcare professionals, probably because they are compliant and followed strong and stringent hand-washing procedures prior to patient examination. However, reported growth of organisms from hand samples of healthcare professionals (12 out of 30) could be due to relaxed hand washing practice after patient examination. Nevertheless, further studies involving more number of samples and better discriminating molecular techniques are needed to substantiate the role of mobile phones in the transmission of infection to critically ill patients in the hospitals.

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ACKNOWLEDGEMENT. A.K. thanks Indian Council of Medical Research, New Delhi for the award of Short-term Research Studentship 2004 (two months) for this study.

Received 5 October 2005; revised accepted 30 January 2006

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Sighting of an albino bat in a colony of cave-dwelling microchiropteran, *Hipposideros diadema nicobarensis* at the Nicobar Islands

The bodies of majority of the species of bat are covered with grey, black or brown fur. A few individuals within the same species are relatively more colourful than their conspecifics. For example, in colonies of hipposiderid bats *Hipposideros speoris* and *H. fulvus* among the brown-furred individuals, a few of them have yellowish-orange fur (Marimuthu, pers. obs.). In the short-nosed fruit bat *Cynopterus sphinx*, harem males are more brightly coloured than non-harem males and females (Marimuthu, pers. obs.). The common names of a few species of bat mainly indicate the colours of their bodies or wing membranes. For example, painted bat *Kerivoula picta*, yellow-winged bat *Lavia frons*, grey bat *Myotis grisescens*, red bat *Lasiurus borealis*, yellow bat *L. ega*, big brown bat *Eptesicus fuscus*, little brown bat *Myotis lucifugus*, white bat *Ectophylla alba*, and straw-coloured fruit bat *Eidolon helvum*. In the midst of such a variety of colourful bat species, rare observations of albino individuals within colonies have also been recorded. Among the 1001 species of bat occurring in the world[1], complete albinism has been recorded in a total of at least 64 individuals from 38 species, with one to five individuals in each[2]. We describe here an albino individual hipposiderid bat, *Hipposideros diadema nicobarensis* Geoffroy, 1813 that we have sighted during our recent survey at the Andaman and Nicobar Islands.

The Andaman and Nicobar archipelagoes sprawl in a crescent from south, off the Myanmar coast near Sumatra, situ-