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Full Length Research Paper

# Isolation and identification of bacteria of public health importance from mobile phones of fish and animal handlers of Kashmir, India

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Mobile phone is an extensively used personal gadget essential in daily life, gradually assuming the status of pathogenic agent of disease transmission. During the present study, 150 swab samples were collected from mobile phones of veterinarians, students (veterinary sciences), laboratory attendants, shepherds, meat and fish handlers of Kashmir valley for isolation, identification of public health significant bacteria and their antibiogram. Out of 150 swab sample examined, 96.66% mobile phones were found to be contaminated with pathogenic bacteria. Mobile phone of animal handlers and veterinary surgeons showed highest and lowest total viable count, respectively. *Escherichia coli, Bacillus cereus, Proteus* spp., *Streptococcus* spp., *Staphylococcus* spp., *Klebsiella* spp. and were frequently isolated from all mobile phone users. Among all the mobile phone users, highest percentage of *E. coli* (72%) and *B. cereus* (84%) was isolated from the fish and meat handlers. All the isolates were highly resistant to Amoxycillin/ Clavulonic acid, Amoxycillin, Ampicillin and Gentamicin. The present study indicates that mobile phones acts as an important source of pathogenic organisms for human and can serve as vehicle for cross-transmission of microbiota.

Key words: Mobile phone, animal handlers, Escherichia coli, antibiogram, resistance.

# INTRODUCTION

India has 929.37 million mobile phone users (TRAI, 2012) and these accounts for 90% of all the telecommunication users. With recent advances in the source of communication, use of mobile phones has become essential in every moment of life. Mobile phone is a widely used personal gadget essential in daily life and is usually kept in close contact with the body. It is used for communication by different group of people in every place or situation including laboratory or clinics. With the invention of 'fully loaded' mobile phones, in addition to the standard voice function, a mobile phone can support many addi-

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tional services such as SMS, email, pocket switching for access to the Internet, and MMS for sending and receiving photos and videos. In fewer than 20 years, mobile phones have gone from being rare and expensive pieces of equipments used primarily by the business elite, to a pervasive low cost personal item. In many countries, mobile phones now outnumber landline telephones with most adults and many children now owning mobile phones. The availability of prepaid services, where the subscriber does not have to commit to a long term contract, has helped fuel for growth of cellular subscribers on a massive scale in India. With high level of mobile phone penetration, a mobile culture has evolved, where the phone becomes a key social tool, and people rely on their mobile phone address book to keep in touch with their family and friends.

Mobile phones serve as clocks, organizers, reminders, calculators etc., depending on the mobile phone accessories. With all the achievements and benefits of the mobile phone, it is easy to overlook the health hazards arising out of the excessive and unusual use of this item. The problems are again aggravated from the fact that many mobile users do not have regard for their personal hygiene. People from many spheres of life are exposed to this risk due to their casualness or lack of knowledge of dos and donts of personal hygiene. They seldom clean their mobiles and often touch them during or after handling of live as well as slaughtered animal and fish without or improper hand washing. The constant handling of the phone by users in all places and occasions makes it open for arrays of microorganisms, making it a harbour and a breeding ground for microbes especially those associated with the skin and working environment. The specific features of mobile phones having depressions at the area of key pad and other places make it a heaven of loads of microorganisms. It can harbour various potential pathogens of zoonotic importance and become a source of infection and a potential health hazard for self and family members (Gurang et al., 2008). From the mobile phones, different microorganisms can spread to the other places and people. It is evident from various research findings that mobile phones can constitute a major health hazard.

Research has shown that the combination of regular handling and the heat generated by the phones creates a prime breeding ground for all sorts of microorganisms that are normally found in our skin and environment. The human body surface is constantly in contact with environmental microorganisms and become readily colonized by certain microbial species (Prescott et al., 2005). The adult human is covered with approximately 2 m<sup>2</sup> of skin, with surface area supporting about 10<sup>12</sup> bacteria (Mackowiak, 1982; Roth and Jenner, 1998). The normal Microbiota is harmless and may be beneficial in their normal location in the host but it can produce disease if introduced into foreign locations or compromise host. Staphylococcus aureus can cause illnesses from pimples and boils to pneumonia and meningitis and is a close relative of methicillin resistant staphylococcus aureus (MRSA).

The main reservoir of *S. aureus* is the hand from where it is introduced into food during handling and preparation (Hui et al., 2001). Hand serves as a major vehicle of transmission of various microbes including the Enterobacteriaceae group (Kapdi et al., 2008). They stressed that the microbial population of the hand is extremely complex and variable, consisting of gram positive organisms like *S. aureus* and gram-negative organism like *Pseudomonas aeruginosa*, which may survive for sufficient period of time on the hand and may thus serve either as a reservoir or shelter of infection.

S. aureus, Enterococcus feacalis, P. aeruginosa, Escherichia coli, Klebsiella spp., Serratia spp., Proteus vulgaris and Bacillus spp. were frequently isolated from the mobile phone of health workers (in turkey), marketers, food vendors, lecturer, students etc (in Nigeria) (Akinyemi et al., 2009; Kilic et al., 2009; Famurewa and David, 2009).

Communicable diseases like diarrhoea (23%), respiratory diseases (19%) and urinary tract infection most commonly caused by *E. coli, Klebsiella* spp. and *Staphylococcus* spp. are the main disease burden in Kashmir (Ahmad, 2008; Kadri et al., 2004). Cell phone of doctors and other health care workers carry nosocomial pathogens which cause every form of skin infections, diarrhoea, pneumonia and meningitides (Butz et al., 1993).

According to the WHO, anti-microbial resistance is one of the world's most serious public health problems. The economic condition of the people of Kashmir valley is far better than any part of India and 4% of people living below poverty line in Srinagar (Waldman, 2009). At the same time, unrestricted availability of cheap Chinese made mobile phones in the valley a child's toy. Total numbers of mobile phone users in Jammu and Kashmir is nearly 27 lakhs as on May, 2012. Then also, many are not satisfied with one mobile phone and are used to keep at least two in their pockets.

Till date, there is no report on the rate of bacterial contamination from mobile phones of veterinarians, students, laboratory attendants, animals and fish handlers of Kashmir valley. In the light of these, this study was carried out to know the rate of bacterial contamination, isolate the microbes from mobile phones and their antibiogram.

## MATERIALS AND METHODS

#### Sample collection

A total of 150 swab samples, 25 samples from each group were collected randomly from the mobile phones of laboratory attendant, animal handlers, meat handlers, fish handlers, veterinary surgeon and students of Veterinary Science of SKUAST-K using sterile cotton swab sticks. The sample was collected in duplicate by rotating the moist cotton swab stick with normal saline over the surface of the both sides of mobile phones.

#### **Microbiological study**

Nutrient broth, nutrient agar, plate count agar, MacConky agar, 5% sheep blood agar, Eosin Methylene Blue agar, Mannitol-egg yolk-polymyxin-B agar and Muller Hinhton agar were used in the present study for isolation of the microbes. The classical culture techniques were used as standard practices in detection of the pathogens and our previous bacterial isolates from different sources were used as standard to confirm the microbes.

#### Total viable count

Total viable count was done as per the guidelines of American Public Health Association (1992) using pour plate method. Swab samples were initially soaked properly in 1 ml of 0.1% peptone water and after that to get 1:10 dilution, 9 ml of 0.1% peptone water were mixed properly. Thereafter, 10 fold serial dilutions were made up to 6<sup>th</sup> dilution. 1 ml each from the 10<sup>4</sup> and 10<sup>5</sup> dilutions was inoculated in the pre-sterilized Petri plate. About 15 ml of sterilized nutrient agar maintained at about  $45 \pm 1^{\circ}$ C was then poured in each Petri plate and were mixed uniformly and allowed to solidify. The plates were incubated at 37°C for 24 h. Average count of the colonies between 30 to 300 in the 10<sup>4</sup> and 10<sup>5</sup> dilutions was multiplied by the dilution factor to get the total viable bacterial count of the sample.

#### Isolation and identification

One set of the swab stick were then soaked in nutrient broth and incubated aerobically at 37°C for 24 h for the growth of microorganisms. After that each sample was streaked on the nutrient agar plate and MacConky agar plate for growing of non-fastidious organisms and to differentiate between lactose fermenters and non lactose fermenters, respectively. Then the streaked nutrient agar and MacConky agar plates were incubated at 37°C for 24 h for the growth of microorganisms. The microbes were identified by colony characteristic, Gram's staining, differential and selective media and by appropriate conventional biochemical tests (Cowan and Steel, 1993).

#### Antibiotic sensitivity test

Fresh culture was used for antibiotic sensitivity test (AST). Colonies were transferred into 5 ml of Tryptone Soya broth and was incubated at 37°C for 5 h. After that a sterile cotton swab was dipped into the sterilized inoculum and the soaked swab was rotated firmly against the upper inside wall of the tube to remove the excess fluid. The entire surface of the agar plate was spread properly with the swab. Then the inoculum was allowed to dry for 10 min with the lid in place. The predetermined battery of antimicrobial discs was dispensed aseptically onto the surface of the inoculated agar plate. The selection of these antibiotics was done according to the antibiotics frequently used in Kashmir. Each disc was pressed down to ensure complete contact with the agar surface and was incubated at 37°C for 24 h.

## RESULTS

In all, 150 swab samples of mobile phone from laboratory attendant, animal handlers, meat handlers, fish handlers, veterinary surgeon and students of Veterinary Science were randomly examined and 96.66% mobile phones were found contaminated with microbiota. The highest total viable count (TVC) was observed in animal handlers  $(228.08 \pm 7.04 \times 10^4)$  and lowest in veterinary surgeons  $(153.56 \pm 14.25 \times 10^4)$  mobile phones (Table 1). The research findings indicated that Streptococcus spp., Staphylococcus Bacillus cereus and spp., Enterobacteraece group of bacteria particularly Klebsiella spp., Proteus spp. and E. coli were the main isolates frequently associated with the mobile phones of

laboratory attendants, animal handlers, meat handlers, fish handlers, veterinary surgeons and students of veterinary science. Gram positive cocci, *Streptococcus* and *Staphylococcus* spp. were identified based on morphological characteristics and biochemical tests. The highest prevalence of *Streptococcus* spp. was observed in laboratory attendants (64%) and lowest in animal handlers (40%) mobile phones. *Staphylococcus* spp. was frequently isolated from all groups of mobile phone handlers and the highest rate of contamination was recorded in animal and fish handlers (84%), followed by meat handlers (76%), laboratory attendants (72%), veterinary surgeons (68%) and students (60%) mobile phones (Table 1).

Pink (lactose-positive) and cream/off-white (lactosenegative) colonies were observed in MacConkey agar. After that the pink colony was streaked on EMB agar and was incubated at  $37^{\circ}$ C for 24 h. The organisms in EMB agar plate showed clear metallic shine which indicated the presence of *E. coli* in the sample. Biochemical tests (IMVIC) revealed that the gram negative coccobacilli was motile, positive for indole and methyl red, negative for Voges proskauer, citrate utilization, urease production, H<sub>2</sub>S production which was specific for *E. coli*. Among all

the mobile phone users, highest contamination of *E. coli* was isolated from fish handlers (72%) and lowest from veterinary surgeons (4%) mobile phone. Second highest contamination of *E. coli* was found in the mobile phone of animal handlers (64%) (Table 1). Few lactose fermentive colonies did not produce any metallic shine in EMB agar plate but these organism was non motile, negative for indole production, methyl red reaction and  $H_2S$  production but positive for Voges proskauer, citrate utilization and urease production which was specific for *Klebsiella* spp.

Klebsiella spp. was isolated from all groups of mobile phone handlers with a highest prevalence in fish handlers (60%) and lowest in veterinary surgeons (24%) mobile phone (Table 1). Gram negative, motile, non lactose fermentive bacteria was positive for indole production, methyl red reaction,  $H_{2}S$  production and urease production and negative for Voges proskauer and citrate utilization which was typical for Proteus spp. 44% animal handlers, 40% laboratory attendants and 12% veterinary Surgeons' phones were carrying Proteus spp. (Table 1). On the basis of Gram staining, morphological characteristics and motility tests, the Bacillus spp. suspected colonies were streaked on mannitol-egg yolk-polymyxin-B agar and incubated at 37°C for 24 h. The distinct flat pink colonies with serrated borders, surrounded by a zone of Lecithinovitellin reaction were taken as B. cereus. Biochemical tests revealed that the isolates were positive for Catalase reaction, citratate utilization, nitrate reduc-tion, glucose fermentation, Voges-proskauer test and negative for indole, mannitol and arabinose, which is a characteristic for B. cereus.

*B. cereus* was predominantly present in the mobile

phone of meat handlers (84%) followed by animal handlers (80%), fish handlers (60%), laboratory attendants (48%), veterinary surgeons (20%) and students (12%) mobile phones (Table 1). Antibiotic sensitivity test results revealed that Streptococcus spp., Staphylococcus spp., E. coli and Proteus spp isolated from different animal and fish handlers mobile phone were highly sensitive to Ciprofloxacin, Erythromycin, Amikacin, Chloramphenicol and Enrofloxacin and moderately sensitive to Sulphamethoxazole/ Trimethoprim, Streptomycin and Tetracycline. Klebsiella spp and B. cereus were also highly sensitive to Ciprofloxacin, Amikacin, Chloramphenicol, Enrofloxacin and moderately sensitive to Erythromycin, Sulphamethoxazole/ Trimethoprim, Streptomycin and Tetracycline. All the isolates were highly resistant to Amoxycillin/ Clavulonic acid. Amoxycillin, Ampicillin and Gentamicin (Table 2).

# DISCUSSION

The research findings indicate that Streptococcus spp., Staphylococcus spp., B. cereus and Enterobacteraece group of bacteria particularly Klebsiella spp, Proteus spp. and E. coli were the main isolates frequently associated with the mobile phones of laboratory attendants, animal handlers, meat handlers, fish handlers, veterinary surgeons and students of veterinary sciences. The overall contamination of mobile phone was 96.66%. Highest total viable count was observed in animal handlers and lowest in veterinary surgeons mobile phone. Although, TVC is not immediately harmful, increased levels indicate poor personal hygiene. The results are in accordance with the finding of Bhat et al. (2011) who observed that 99% of the phones of healthcare workers were contaminated with pathogenic micro-organisms and multi drug resistant bacteria. Mobile phone of healthcare workers in Turkey and India were contaminated with microorganism with a prevalence of 95.5 and 91.60%, respectively (Ulger et al., 2009; Jayalakshmi et al., 2008). In Nigeria, the public phones were frequently contaminated with S. aureus, B. substilis and Enterobacter aerogenes and the mean bacterial viable count of these public phones was 4.93\*10<sup>6</sup> CFU/g on nutrient agar, 2.12\*10<sup>6</sup> CFU/g on MacConkey agar and 3.22\*10<sup>6</sup> CFU/g on Mannitol salt agar (Ekrakene and Igeleke, 2007).

Streptococcus spp., Staphylococcus spp., B. cereus, Klebsiella spp., Proteus spp. and E. coli were frequently isolated from the fish handlers, meat handlers, animal handlers, laboratory attendants, veterinary surgeons and students mobile phone (Table 1). The higher prevalence of microbiota in the mobile phones of fish handlers, animal handlers and meat handlers could be attributed to the poor hygienic and sanitary practices associated with the low level of education. The reasons of contamination of mobile phones mainly due to selected groups of people do not wash their hands properly after handling fish, meat and animals. Moreover, the water used for washing of hands itself may be a source of E. coli and other Enterobacteriaceae group of organism (Rather, 2009). In animal handlers, close proximity of men and animals results in rapid transmission of microbes from animal to handlers. Fish of different water bodies and raw meat of Kashmir were found mostly contaminated with E. Proteus spp., Klebsila spp., coli, В. cereus, Streptococcus spp. and Staphylococcus spp (Bhat, 2011) and these organisms were transmitted to the mobile phone through handlers.

E. coli has been the predominant organism isolated from urinary tract infection in Kashmir without much change over the last so many decades (Kadri et al., 2004). Even in well-educated families, householders do not wash hands after going to the toilet or before eating or drinking (Ahmad, 2008) which is the predominant cause of mobile phone contamination. The research findings indicated that the mobile phones acts as an important source of pathogenic organisms for human and can serve as vehicle for cross-transmission of microbiota. Our findings are well corroborating with the observation of other workers. Healthcare workers mobile phones in Turkey were contaminated with Staphylococcus epidermidus, Staphylococcus aureus, Bacillus spp. and E. coli (Kilic et al., 2009; Ulger et al., 2009). 20% of the mobile phones tested harboured pathogenic microorganisms especially Acinetobacter wolffii (53.6%), MRSA (10.3%), Pseudomonas spp. (8%) in three hospitals of Israel and one hospital of New York (Goldblatt et al., 2007). Karabay et al. (2007) also isolated E. coli, Bacillus spp. and coagulase negative Staphylococcus from mobile phone of health care staffs.

70% of the mobile phones brought to the operation theatre in three different hospitals in Sri Lankan were contaminated with human pathogenic bacteria mainly MRSA which accounted for 15% of mobile phone contaminating organisms (Gunasekara et al., 2009). According to Akinyemi et al. (2009), mobile phone served as vehicles for transmission of both hospital and community acquired bacterial diseases. They observed that the mobile phone of marketers, food vendors, lecturers, students, public servants and hospital workers were contaminated with S. aureus, Enterococcus feacalis, P. aeruginosa, E. coli and Klebsiella spp. Rusin et al. (2002) documented hand-to-mouth transfer of microbes after handling contaminated fomites during casual activities. During every phone call the mobile phones come into close contact with strongly contaminated human body areas with hands to hands and hands to other areas (mouth, nose and ears). Srikanth et al. (2008) isolated S. aureus, MRSA, E. coli, P. aeruginosa and K. pneumoniae and Coagulase negative staphylococci from mobile phones of health care workers in Sri Ramachandra Medical College, Chennai, India. Mobile phones had 18 times more bacteria than toilet handles (Andrew, 2010) and 16% of mobile phones were contami-

Profession of mobile phone possessor	No. of sample screened	No. of positive <sup>_</sup> sample	TVC count (mean ± SE)		% of isolated bacteria					
			x 10 <sup>4</sup>	x 10⁵	Streptococcus spp. (%)	Staphylococcus spp. (%)	E. coli (%)	Klebsiella spp. (%)	Proteus spp. (%)	B. cereus (%)
Laboratory attendants	25	25	195.56±8.17	136.4 ±6.42	64	72	12	36	40	48
Animal handlers (Shepherd)	25	25	228.08±7.04	165.92±6.65	40	84	64	48	44	80
Meat handlers	25	25	216±8.50	154.36±4.96	56	76	36	40	28	84
Fish handlers	25	25	224.32±9.08	162.24±5.47	44	84	72	60	36	60
Students (Veterinary Science)	25	23	176.92±12.20	121.68±8.55	48	60	8	36	20	12
Veterinary Surgeon	25	21	153.56±14.25	104.92±10.15	56	68	4	24	12	20

**Table 1.** Total viable count and prevalence of isolated bacteria from different mobile phone holder.

Table 2. Antibiotic sensitivity patterns of the isolated bacteria from mobile phone of fish and animal handler.

Name of different isolates	No. of total isolates	Name of antibiotic discs (mcg/disc)							
		Ciprofloxacin (30)	Erythromycin (15)	Amikacin (30)	Chloramphenicol (30)	Enrofloxacin (10)	Sulphamethoxazole/ Trimethoprim (30)		
Streptococcus spp.	77	75 (97.40%)	77 (100%)	77 (100%)	65 (84.41%)	71 (92.21%)	43 (55.84%)		
Staphylococcus spp.	111	109 (98.20%)	110 (99.10%)	105 (94.59%)	111 (100%) 102 (91.89		61 (54.95%)		
E. coli	49	46 (93.88%)	45 (91.84%)	45 (91.84%)	42 (85.71%)	38 (77.55%)	26 (53.06%)		
Klebsiella spp.	61	59 (96.72%)	35 (57.38%)	57 (93.44%)	57 (93.44%)	45 (73.77%)	15 (24.59%)		
Proteus spp.	45	43 (95.55%)	32 (71.11%)	43 (95.55%)	40 (88.89%)	39 (86.67%)	20 (44.44%)		
Bacillus cereus	76	76 (100%)	26 (34.21%)	73 (96.05%)	67 (88.16%)	39 (51.31%)	27 (35.53%)		
Name of different isolates	77	Streptomycin (30)	Tetracycline (30)	Amoxycillin/ Clavulonic acid (30)	Amoxycillin (30)	Ampicillin (10)	Gentamicin (10)		
Streptococcus spp.	111	38 (49.35%)	40 (51.95%)	12 (15.58%)	5 (6.49%)	3 (3.90%)	0		
Staphylococcus spp.	49	47 (42.34%)	56 (50.45%)	18 (16.22%)	6 (5.40%)	4 (3.60%)	0		
E. coli	61	9 (18.37%)	21 (42.86%)	6 (12.24%)	3 (6.12%)	4 (8.16%)	4 (8.16%)		
<i>Klebsiella</i> spp.	45	14 (22.95%)	25 (40.98%)	9 (14.75%)	5 (8.20%)	0	0		
Proteus spp.	76	19 (42.22%)	24 (53.33%)	7 (15.55%)	2 (4.44%)	0	0		
Bacillus cereus	77	23 (30.26%)	36 (47.37%)	11 (14.47%)	0	0	0		

nated with *E. coli* and 1 in 6 cell phones were contaminated with faecal matter in U.K. (Sora, 2011). However, information on microbial contamination of mobile phones of animal and fish handlers in Kashmir valley or India is not available

so no comparison could be possible with the findings of the present study.

Most of the mobile users frequently used their mobile phones during working in laboratories, clinics and also during handling of fish, animals and meat. The microbes isolated from the present study have been shown to survive on inanimate surfaces for months. Therefore, the personal item like mobile phone used by animal handlers, meat handlers, fish handlers, laboratory attendants, veterinary surgeon and students can be a continuous source of transmission of microbes, in the absence of regular surface disinfection practices.

All the isolates were highly resistant to Amoxycillin/ Clavulonic acid, Amoxycillin, Ampicillin and Gentamicin (Table 2). The antibiotic resistance of the isolated bacteria was due to indiscriminant use (overuse, misuse, prolonged use, self medication etc.) of antibiotic in animals, fish farm as well as in humans who generally do not complete the dose of antibiotics prescribed by physician. The results of the present studies are in accordance with the findings of Bhat et al. (2011) who isolated Methicillin-resistant and Methicillin-sensitive *S. aureus*, *E. coli*, *K. pneumoniae*, *Acinetobacter*, *E. faecalis* and *P. aeruginosa*.

MRSA and E. coli were mainly isolated from cell phones of healthcare personnels performing surgeries or handling acutely ill patients (Arora et al., 2009). Healthcare workers mobile phones in Turkey carried ceftazidime resistant Gram negative isolates and half of S. aureus isolates were resistant to methicillin (Ulger et al., 2009). Cephalothin (6.7%) and amoxicillin (25%) resistant S. epidermidis was isolated from Health Care Worker's mobile phones in three teaching hospitals in Kerman, Iran (Sepehri et al., 2009). In Kashmir, majority of the uropathogens like E. coli, K. pneumoniae, P. aeruginosa, E. faecalis and S. aureus had already developed resistance to most commonly used drugs including norfloxacin, co-trimoxazoile and ampicillin (Kadri et al., 2004). Multi-drug resistant communityacquired pneumonia and typhoid are common in the valley (Ahmad, 2008). Drug prescribing in Kashmir has taken shape of a racket where every stake holder, except the patient is benefited (Gaash, 2008).

# Conclusion

The overall inference of the results is that the mobile phones, though, are made to make communication easy and accessible by almost all, are gradually assuming the status of pathogenic agent of disease transmission. Even, it could be a vehicle for the transmission of biological weapon of mass destruction if proper care is not been taken. Due to low awareness among the general people, like lab attendant, fish handlers and also other animal handlers, personal items like mobile phone is infrequently disinfected but extensively used every where even in sterile environment. Currently, there are no restrictions for bringing or using the mobile phone within the laboratories, clinics or other places. Although, it seems impossible, in the light of all these findings, we should be aware of limiting the mobile phone usage as it has high risk for spreading infections. According to these results, it is obvious that, the training of laboratory attendant, meat handlers, students, animal and fish handler about strict infection control procedure, hand hygiene, environmental disinfection, and eventually, optimum disinfection methods are of great importance. The present studies emphasize the importance of increased personal hygiene and surface disinfection of mobile phone of animal handlers, fish handlers, meat handlers, laboratory attendant, veterinary surgeons and students of veterinary science. Regular surface disinfection of personal items can contribute to the reduction of transmitssion of pathogenic microorganism in the healthy family members.

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