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Study of pathogens on automated teller machine in Jeddah, Saudi Arabia

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Abstract

There is no restriction on using automated teller machines (ATMs) and ATMs are prominent habitat for bacterial colonization on key pads and touch screen. The present study focus on the group of bacterial strains present on touch screen and key pad of machines. Following three main highly crowded areas were selected in Jeddah city: industrial area, shopping malls and beach. Swab samples from touch screen and key pad were collected from 15 different ATM machines of each area. Other sources of pathogens in ATM centers were detailed with the study on unclean air condition filters possessing high bacterial load. The results revealed that ATM machines consisted of pathogenic bacterial strains like *Staphylococcus sp*, *Escherichia coli* and *Salmonella sp*. Further investigation on transfer of such pathogen to ATM users was studied in the samples obtained from the fingers of ATM users. Phylogenetic analysis confirmed the presence of bacterial strains such as *Bacillus pumilis*, *Staphylococcus huminis*, *Stenotrophomonas sp*, *Escherichia coli* and *Bacillus sp* on ATM machines (touch screen and key pads) and users hands. The study also revealed the use of hand sanitizers as a control measure to potentially reduce 99% of the bacterial load in ATM users hand.

Key words

Automated Teller Machine, Pathogens, Antiseptic, Sanitization, *Staphylococcus*

Introduction

Microbes can spread various diseases in different ways such as: Direct contact (physical contact-touching, eating contaminated food, inhalation, kissing etc with the infected person) and indirect contact (using objects such as towels, door knob, cup etc handled by the infected person). Normal microbes as well as pathogenic microbes require a particular environment to be in active condition. Most of the ATM centres are air conditioned which accelerates growth of different types of microbes. Thus, ATM centres act as potential habitat for microbes, which also help in spreading different diseases. ATM users are unaware of the presence of pathogenic bacteria's on touch screen and key pads which are capable of spreading different contagious diseases. As ATM is one of the advanced technologies to withdraw money any time, most people irrespective of age bar, prefer using them instead of banks. In Jeddah, within the city limits, every 1 or 2 km (including all the shopping malls) have completely air conditioned ATM centres to meet the needs of increasing population and industrial sectors. Apart from Saudi nationals, expatriates of different nationality are highly populated in Jeddah which makes the region prone for transmission of various contagious diseases. Recent spread of Middle East Respiratory Sydrome (MERS) in Saudi Arabia created mass attraction all over the world. MERS caused by betacoronavirus, spread MERS disease through human to human contact. World Health Organization has recently reported that Saudi Arabia was the worst-affected, accounting for 348 deaths out of 811 confirmed cases for MERS (MOH, 2014).

Previous research on disease transmission by indirect contact was reported by analyzing swab samples from computer keyboards, shopping mall trolleys, elevator buttons and door handles (Schultz *et al.,* 2003, Noskin *et al.,* 1995, Scott *et al.,* 2008, Al-Ghamdi *et al.,* 2011). The reports concluded the presence of pathogenic bacterial strains such as methicillin resistant *Staphylococcus aureus* (MRSA), *Streptococcus*, *Clostridium perfingens*, *Enterococcus* (vancomycin-resistant

Enterococcus faecium) and *Pseudomonas aeruginosa.* The present study details about pathogenic bacterial strains hosted at ATM centres in crowded areas (such as shopping malls, industrial site, beach) in Jeddah, Saudi Arabia. The study also detailed about the importance of using disinfectants to clean hands and cleaning filters of air conditioners in ATM centres as hygienic control measures to prevent harmful pathogens.

Materials and Methods

Sample collection and analysis : Samples were collected twice from different areas of Jeddah namely: Al-Mahajar (Industrial site), Al-Jamia, Tehalia, Al-balad, Sarafiya (Shopping malls) and Abhour (Sea Shore). Sampling sites were selected, based on areas which were heavily crowded. The swab samples were collected from touch screen and metallic key pads, using sterile cotton swabs. Samples collected from hands (fingers) of ATM users were used to identify transmission of bacterial strains. After sample collection, swabs were locked immediately to avoid contamination and brought to laboratory for further analyzes.

Samples were inoculated into selective media such as Mannitol salt agar (MSA), Salmonella-shigella agar (SS), Eosine metheylene blue (EMB) agar and MacConkey agar to isolate different bacterial strains. The selective media were used to analyse the presence of pathogenic bacterial strains in the collected samples. Nutrient agar was also used to evaluate bacterial cell count on touch screens, key pads and air condition filters.

Phylogenetic analysis : Swab samples were collected and immersed in sterile distilled water. This contributed to a sample mixture from which DNA was extracted to identify bacterial strains present in the sample. Bacterial DNA was extracted using Qiagen DNA extraction kit. Amplification of DNA was performed in Thermal Cycler (Applied Biosystems). PCR was performed with 27F and 1492 as primers (Frank *et al.,* 2008). PCR amplification was performed using PCR supermix from invitrogen (USA) following the protocol detailed by Arulazhagan and Vasudevan (2009). PCR amplified products obtained from extracted DNA were used for DGGE-PCR (Arulazhagan *et al.,* 2010). DGGE PCR was performed with 968 F (with GC clamp) and 1492 R primers (Szekely *et al.,* 2009).

Denaturing agents such as urea and formamide (continous gradient) were used in DGGE. Gel contained 40% polyacrylamide (Nikolausz *et al.,* 2008). Samples mixed with equal volume of loading dye were loaded in the wells. The electrophoresis tank was filled with 7 l of 1X Tris-Acetate-Ethylenediamine tetraaceticacid (TAE) running buffer. DGGE was run at 60ºC in a DCode Universal Mutation Detection System (Bio-Rad, U.S.A.). DGGE was performed at 60 ºC with 130 V for 8 hr. Gel was stained with 250 ml of running buffer and 25 µl 10 mg ml^{-1} of ethidium bromide for 5–15 min. After staining, the gel was transferred into a dish containing 250 ml of 1X running buffer and destained for 5-20 min. The gels were scanned in a GelDoc system (Bio-Rad).

Identified bands were excised from DGGE using sterile blade. The bands were kept in distill water for overnight and a part of it was used as template for PCR. Gel extraction kit was used to purify PCR product, which was further cloned performed using pGEM-T Easy Vector systems II (Promega, USA). ABI-DNA analyser was used to record nucleotide sequence of bacterial DNA (Chromous Biotech, India). BLASTN search confirmed the genus of bacterial strain. Further neighbour hood relationship was obtained by evolutionary analyses using Molecular Evolutionary Genetic Analysis (MEGA v5). Nucleotide sequence from 16S rRNA sequencer was analysed using Basic Local Alignment Search Tool (BLAST) software available in NCBI (National Center for Biotechnology Information). Phylogenetic and molecular evolutionary analyses were carried out using MEGA version 5 software. The partial 16S rRNA sequences were deposited in GenBank under accession numbers from KJ494869 to KJ494873.

Results and Discussion

Samples collected from different ATM centres showed prominent presence of *Staphylococcus* sp. The presence of *Salmonella* and *Shigella* were minimal. However, they constituted more impact on human health as disease causing pathogen. Dominant *Staphylococcus* sp. still persists as a major threat in spreading contagious diseases. The results of the present study with selective media also confirmed the potential domination of pathogenic *S. aureus* at all the sampling sites. *E. coli* stood next to *S. aureus* covering 87% of the industrial sites. As a residential pathogen of skin, *S. aureus* persists in hands due to unhygienic practice or improper washing of hands with handwash solution (Herruzo *et al.,* 2010, Holt *et al.,* 1994; Kampf and Kramer, 2004). *S. aureus* and *Salmonella* are well documented for high pathogenecity and are fatal at extreme conditions (Datta *et al.,* 2009; Mead *et al.,* 1999; FDA 2002). Samples collected from ATM steel pads, touch screen and hands of users were in agreement with the availability of pathogenic bacterial strains. *Shigella* was found in ATMs at shopping malls, which might be either due to handling of pathogen contaminated food items or bare hands of ATM user. *Shigella* causes diarrhoea and dysentery by producing enterotoxin which is predominantly reported in children. *Salmonella* was found in samples collected from shopping malls (SM-8) with food courts (Table 1). This might be due to unhygienic maintenance of food court or due to children prone to use ATMs as toys. *Salmonella* contaminated egg acts as an efficient vehicle for human infection. The infection depends up on handling of eggs during preparation of food in restaurants. Eggs are often used in restaurants in ice creams, salad dressing, mayonnaise and sauce (Braden, 2006). *Salmonella sp.* survive longer period on dry surfaces making its presence significant (Humphrey et al., 1994; Abban and Debrah 2010). The bacterial load in samples from

Table 1 : Pathogens in ATMs at shopping malls

Sample ID	Shopping malls (SM)							
	Staphylococcus		*E.coli*		*Salmonella*		*Shigella*	
	S1	S2	S1	S2	S1	S2	S1	S2
SM-1	+	+	+	+	-	-	-	-
SM-2	+	+	-	+	-	-	-	-
SM-3	+	+	+	+	-	-	-	-
SM-4	+	+	+	+	-	-	-	-
SM-5	+	+	-	-	-	-	-	-
SM-6	+	+	-	-	-	-	-	-
SM-7	-	-	+	+	-	-	-	-
SM-8	+	-	+	+	+	-	-	-
SM-9	+	+	+	-	-	-	-	-
SM-10	+	+	-	-	-	-	-	-
SM-11	+	+	-	-	-	-	-	-
SM-12	+	+	+	+	-	-	+	-
SM-13	+	+	+	+	-	-	-	-
SM-14	+	+	+	+	-	-	-	-
SM-15	-	-	+	+	-	-	-	-

S1- First sample collection 1; S2- Second sample collection

ATMs near beach possessed limited number of cell count as compared to samples from other two sites (Fig. 1). The bacterial load increased every day at industrial site and malls (ranged from 10^4 to 10^7 cfu ml^{-1}), but in beach area the increase in bacterial load was only observed during the weekends. Bacterial cell count and bacterial strains indicated the possibility of cross-contamination of fingers, when ATMs were used with foodborne pathogens (*Bacillus, Salmonella* and *Shigella*). The results were also in agreement with the previous study on ATMs and other electronic devices used around the world (Abban and Debrah, 2011; Ashgar and El-Said, 2012; Josaline *et al.,* 2014).

Among the samples collected from three different areas, samples collected from industrial area topped the list of bacterial contamination, followed by shopping mall and beach area samples. In Jeddah, people from different nations working in different industrial sectors were prone to many type of bacterial strains (Table 2). Samples collected from beach showed 80% presence of *S. aureus* and 40% *E. coli* (Table 3). Thus, samples collected even after two month interval almost possessed the same result (Table 1 to 3). The infections caused by these kinds of bacterial strains depended on immunity of the person (Scott and Bloomfield, 1990; Foster, 2002). Using ATM without washing or sanitizing hands, ATM users' activities such as rubbing of hands, handling food and shaking hands may spread different diseases through ATM borne pathogens.

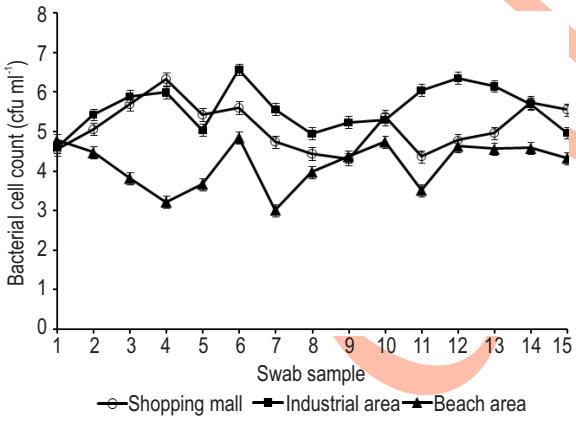

Fig. 1 : Bacterial cell count in ATMs from different areas

The present study is reliable as the presence of pathogenic bacterial strains on ATM machines were detailed using consistent methods. Pathogen prone unclean ATMs act as hub to spread diseases to the users. The temperature and moisture content in air conditioned room facilitates growth of pathogens on ATM machines. The research study was mainly carried out to spread awareness about disease causing pathogenic bacterial strains on ATM machines to public and scientific community.

Apart from bacterial strains isolated by specific media, the study holds interest on other pathogenic bacterial strains present in the samples. A mixture of three ATM samples (including number pads, touch screen and hands of the users) for each area as a representative was used for this study. DNA samples were isolated from the sample mixture and DGGE profile showed five bands from the sample (Fig. 2). Individual bacterial DNA was sequenced after DGGE using 16s rRNA sequencing technique.

Table 2 : Pathogens in ATMs at industrial sites

Sample ID	Industrial sites (IS)							
	Staphylococcus		*E.coli*		*Salmonella*		*Shigella*	
	S1	S2	S1	S2	S1	S2	S1	S2
IS-1	+	+	+	+	-	-	+	-
IS-2	+	+	+	+	-	-	+	-
IS-3	+	+	+	+	-	-	-	-
IS-4	+	+	+	+	-	-	-	-
IS-5	+	+	-	-	-	-	-	-
IS-6	+	+	+	+	-	-	-	-
IS-7	+	+	+	+	-	-	-	-
IS-8	-	-	+	+	-	-	-	-
IS-9	+	+	-	-	-	-	-	-
IS-10	+	+	+	+	-	-	-	-
IS-11	+	+	+	+	-	-	-	-
IS-12	+	+	+	+	-	-	-	-
IS-13	+	+	+	+	-	-	-	-
IS-14	+	+	+	+	-	-	-	-
IS-15	+	+	+	+	-	-	-	-

Table 3 : Pathogens in ATMs at beach area

Sample ID	Beach area (BA)							
	Staphylococcus		*E.coli*		*Salmonella*		*Shigella*	
	S1	S2	S1	S2	S1	S2	S1	S2
BA-1	+	+	-	-	-	-	-	-
BA-2	+	+	-	-	-	-	-	-
BA-3	+	+	-	-	-	-	-	-
BA-4	+	+	+	+	-	-	-	-
BA-5	-	-	+	+	-	-	-	-
BA-6	-	+	-	-	-	-	-	-
BA-7	+	+	+	+	-	-	-	-
BA-8	+	+	-	-	-	-	-	-
BA-9	+	+	-	-	-	-	-	-
BA-10	+	+	+	+	-	-	-	-
BA-11	+	+	-	-	-	-	-	-
BA-12	+	+	-	+	-	-	-	-
BA-13	-	-	+	+	-	-	-	-
BA-14	+	+	+	+	-	-	-	-
BA-15	+	+	-	-	-	-	-	-

The results showed the presence of different bacterial strains such as: *Escherichia coli* strain ATM1 (KJ494869), *Stenotrophomonas sp* strain ATM2 (KJ494870), *Staphylococcus huminis* strain ATM3 (KJ494871), *Bacillus pumilis* strain ATM4 (KJ494872) and *Bacillus sp* strain ATM5 (KJ494873). Among the strains, *Staphylococcus sp Bacillus sp* and *E. coli* were found to dominate bacterial profile in all the samples. The phylogenetic representation was drawn based on the nucleotide sequence that showed distant relationship between the strains (Fig. 3).

The present study recorded *Staphylococcus* strain to be the commonly present species on all the three surfaces (Key pad, Touch screen, Hands of user). The presence of *Staphylococcus* on fingers might be expected if washing was not done thoroughly since it is a well known resident microflora of skin (Holt *et al.,* 1994; Foster, 2002). The main source of ATM machine contamination and disease transmission is due to anthropogenic activities (Saroja *et al*., 2013). To find other sources of infection, the research turned towards analysis of filters in air-conditioners. The results showed that filters were highly contaminated with dust and high bacterial load (Table 4). The bacterial load in industrial and beach area was found to be in the range of 10^4 to 10^6 cfu ml^{-1} and most of the shopping malls possessed centralized air conditioners. Handling of food items inside ATM centres and air-conditioners

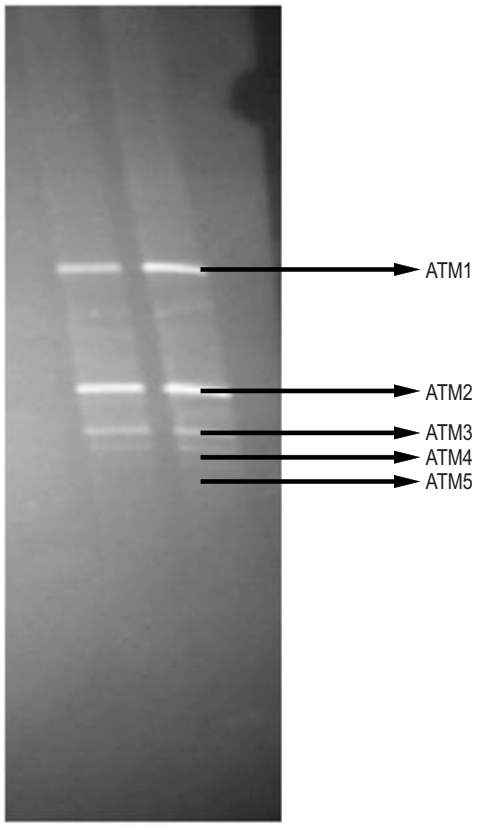

Fig. 2 : DGGE profile picture representing the bands of the bacterial strains in the ATM samples

without proper maintenance also results in delivering infectious disease causing pathogens along with the money from ATM.

To prevent transmission of pathogenic bacterial strains, the present study recommends public awareness and hygienic practices as main control measure. ATM centres should be maintained in sanitized condition and users should wash their hands after using ATM machine. Consumer awareness is of paramount importance in handling food material, especially ready-to-eat foods, as previous studies have provided insight into the inadequacies in general public's knowledge with regard to food handling principles (De Jong *et al.,* 2008; Van Asselt *et al.,* 2009). To control transmission of infectious disease causing pathogens, two hand wash solutions (Dettol, Lifebuoy) and a hand sanitizer (Septogel) were tested. Swab samples were collected from ATM users' hand, before and after washing with disinfectants. Bacteriological analysis after the use of antiseptic was performed in all the three areas and 15 samples/area were collected and their average bacterial load is given in Table 5. Negligible amount of antiseptic resistance strains persisted even after using the sanitizers. This has to be taken into account to keep environment as pathogen free zone. The previous report on *Staphylococcus aureus* proved to possess antiseptic resistant gene in MRSA (Methicillin Resistant *Staphylococcus aureus*) isolated from patient with impetigo and Staphylococcal scaled skin syndrome (Noguchi *et al.,* 2006). Antiseptic resistance in *S. aureus* is caused by proton motive force-dependent multidrug efflux (Grinius and Goldberg, 1994; Finn *et al.,* 2013). Joshaline *et al.* (2014) reported a study on biofilm on ATM machines and the antibiotic resistance pattern developed by bacterial isolates. These kind of strains can be avoided by good hygiene through public education in relation to hand washing, food preparation and avoiding sharing toilet articles as control measures. Alcohol based disinfectants were eminent in reducing bacterial strains prone to cause clinical infections (Herruzo *et al.,* 2010). In the present study, all the three sanitization solutions showed almost above 97% reduction of bacterial load.

Table 4 : Bacterial load in air condition filters

Shopping malls	Cell count (cfu ml^{-1})	Industrial sites	Cell count (cfu ml^{-1})	Beach area	Cell count (cfu ml^{-1})
SM-1	$5x10^6$	IS-1	$7x10^6$	BA-1	75
SM-2	CN	IS-2	$10x10^5$	BA-2	1x102
SM-3	CN	IS-3	$4x10^6$	BA-3	$6x10^6$
SM-4	$6x10^4$	IS-4	$2x10^4$	BA-4	$3x10^5$
SM-5	CN	IS-5	$3x10^3$	BA-5	$8x10^4$
SM-6	CN	IS-6	$7x10^4$	BA-6	$6x10^2$
SM-7	CN	IS-7	$12x10^4$	BA-7	$5x10^6$
SM-8	CN	IS-8	$14x10^5$	BA-8	$9x10^7$
SM-9	CN	IS-9	$6x10^6$	BA-9	$6x10^4$
SM-10	$4x10^5$	IS-10	$13x10^5$	BA-10	$11x10^6$
SM-11	CN	IS-11	$4x10^6$	BA-11	$5x10^3$
SM-12	$2x10^6$	IS-12	$2x10^6$	BA-12	$2x10^2$
SM-13	$7x10^3$	IS-13	$9x10^7$	BA-13	$4x10^3$
SM-14	CN	IS-14	$11x10^5$	BA-14	$7x10^5$
SM-15	CN	IS-15	$8x10^6$	BA-15	$12x10^4$

CN- Centralized air condition (data not available)

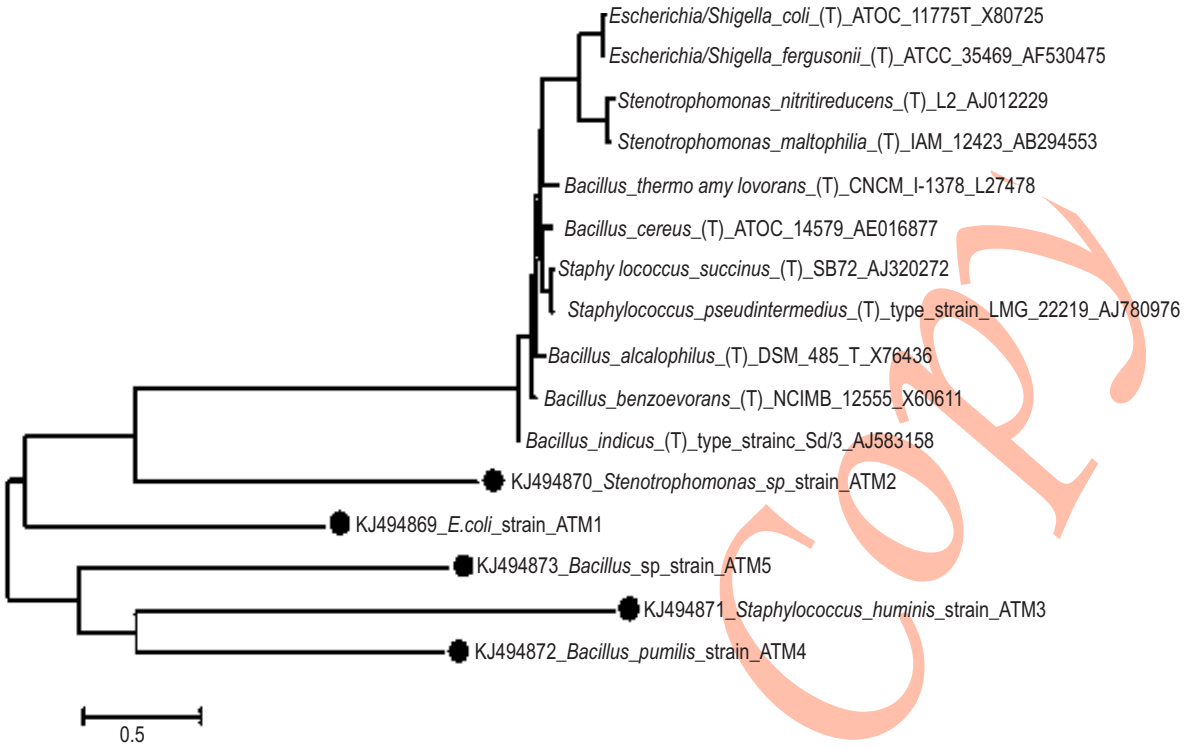

Fig. 3 : Phylogenetic tree analysis of bacterial species present in ATM samples and their closely related bacteria obtained from BLAST search by Neighbour Joining Method. Numbers at nodes are bootstrapping values. Scale bar represents 0.5 substitutions per nucleotide position.

Table 5 : Control of pathogens by different sanitizers

Sample detail	Hand wash	Bacterial cell count (cfu ml^{-1}) before wash	Bacterial cell count (cfu ml^{-1}) after wash	Antimicrobial activity (%)
Industrial	SEPTOGEL®	16x10^3	42	99.7
	Lifebuoy nature	15 x10^3	67	99.5
	Dettol	16 x10^3	85	99.4
Shopping malls	SEPTOGEL®	8 x10^3	40	99.5
	Lifebuoy nature	7 x10^3	50	99.3
	Dettol	7 x10^3	65	99.0
Beach	SEPTOGEL®	140	2	98.5
	Lifebuoy nature	147	3	97.9
	Dettol	128	3	97.6

Among the three antiseptic, septogel was found to be eminent in reducing bacterial load in all the three areas. Recently, spread of MERS has made a huge impact in Saudi Arabia. To avoid such contagious disease, frequent cleaning and replacement of filters in air conditioners is required. Thus, article details the importance of asepsis to avoid different disease causing pathogens in ATM. The study also details about highly polluted filters in air conditioners possessing high bacterial load and recommendation on replacement or cleaning of air condition filters at appropriate time intervals. Conducting awareness campaign among public may add more value on the importance of hygienic practice and knowledge on the impact of disease causing pathogens.

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