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Identification of Bacterial Isolates from Hand Dryers of Malls Toilets in the City of Baghdad and Detection of their Virulence Factors

Basam Basim Mohammed[1]

[1]Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq.
E-mail: BsmBasimMo@uomustansiriyah.edu.iq

ABSTRACT

Background: High incidence of microbial transmission occurred in public places which are usually occupied by many individuals such as, hospitals, clinics, restaurants, fitness clubs, supermarkets and malls. Bacterial communities colonize all surfaces and items that existed within these places more than other locations used by limited numbers of people. One of these items is the hand dryer that is considered an essential device of modern toilets in public buildings. Objective: This study aimed to isolate and identify different bacterial species from the hand dryers in ladies' toilets located at a shopping mall in the city of Baghdad. Also aimed to investigate several virulence factors produced by bacterial species isolated in this research such as biofilm formation, hemolysin, and protease. Methods: Thirty-five bacterial species were isolated by the exposure of nutrient agar plates to the air emitted from three different types of hand dryers located in mall toilets. These isolates were identified based on cultural, morphological characteristics and the Vitek test. Only thirteen isolates were investigated for the production of several virulent factors including, biofilm formation, hemolysin and protease. A total of eleven bacterial species were distinguished in this study. Four different species were recognized as Acinetobacter baumannii, Kocuria kristinae, Enterococcus faecalis and Enterococcus faecium. At least one virulence factor was detected in each of these species. Conclusions: Isolation of several bacterial species from hand dryers indicates that these devices are contaminated. As well, several virulence factors were found in these species. Therefore, hand dryers have to be located outside the toilet area to avoid contaminating it from the air of the toilet room, toilet items, and surfaces.

KEYWORDS

Hand Air Dryers, Shopping Mall, Toilet Room, Virulence Factors, Contamination.

Introduction

Restroom areas have a higher number of microbes than other rooms in the home. The frequent defecation of toilet users in the home increases the incidence of microbes spread around the toilet environment. Therefore, other toilet items could be contaminated with large numbers of fecal bacteria such as Enterobacteria. Public toilets are usually used by many individuals with various health situations. Thus, these toilets are crowded with hundreds of Iraqis who visit malls during weekends, holidays, and events. In shopping malls, toilets are the most contaminated places with a wide variety of bacteria and the risk of contamination may be twice as high as home toilets (Sinclair *et al*, 2010; Barker and Jones, 2005).

Numerous studies described toilets as the highest contaminated locations although the absence of a nutrients source obviously. Likewise, all items located inside the toilet might be subjected to contamination with fecal microbes, regardless of the direct contact by the toilet user. People who frequently use the toilet pay less attention to the handling of toilet items. Hand dryers could be also contaminated by the toilet surrounding .Drying the hands is an important practice of good hygiene after hand washing since it can stop microbial transmission by removing the wetness from hands (Frank Ngonda, 2017; Shawk and Nawas, 2018).

According to a study published by Luz del *et al.* (2018) who confirmed that wet hands are more likely to pick up bacteria by the direct contact with contaminated objects than dry hands (Luz del Carmen *et al*, 2018). Before 1921, cloth and paper towels were commonly used for hand drying. However, with the development of technology, towels were replaced by blowing air devices which were called the “electric towels”. Using this technology allowed the complete drying of hands in a few seconds (Fig.1) and decreased the burden of waste disposal of paper towels (Paul *et al*, 2016).

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Fig. 1. Lady hands using air dryer in public toilet

Bacterial cells count inside the hand's dryer was often two to four times less than that on other toilet surfaces, such as basins, toilet seats, toilet lids, floor, walls, doorknobs, handles, and soap dispensers. In addition, a large number of microbes could be transmitted by toilet users from outside to the items that existed in the toilet, and vice versa. (Sinclair *et al*, 2010; Mutters and Warnes, 2019).

In Muslim cultures, people usually use their hands to clean the anal area with water after the defecation process as a habit. Thus, the contamination rate of toilet environment, tools, and surfaces within these cultures might be higher than in other cultures. Other factors like the time length for handwashing and the quality of soap used, could also have an essential impact on hand cleaning (Ofonime and Obio, 2018; Snelling *et al*, 2010). This study aimed to identify the bacterial species isolated from hand dryers and to explore their ability to cause a public health risk through the detection of their virulence factors.

Materials and Methods

Sites Selection of Sampling

The air of hand dryers was collected from three different ladies toilets of shopping malls in the city of Baghdad - Iraq, during December 2019.

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Microbiological Sampling

Collection of Samples

Plastic Petric plates filled with nutrient agar which faced with the air current of hand dryers that generated for 30 seconds, the culture medium who positioned 10 cm from the end of the dryer nozzle (Paul *et al*, 2016) (Fig.2); Plates were covered, placed in sterile plastic bags, and transported to the lab were analyze in the laboratory of the department of science, AL-Mustansiriyah University, where they were incubated overnight at 37°C.

Fig. 2. During air sampling of the dryer using an open agar plate

Identification of isolates

The bacterial colonies were differentiated depending on color, size, margin, pigmentation, surface texture and elevation, each different colony was considered as a different bacterial species; transferred to new nutrient agar plates then cultured on blood agar, mannitol salt agar, MacConkey agar, SS agar as described by (Barrow and Feltham**,** 1993). After 24 h each isolate was characterized macroscopically. The final identification of the bacteria by the staining procedure in test Gram positive and Gram negative bacteria isolates was also assessed using a VITEK 2 Automated Microbiology System.

Culture Media for Screening of Some Virulence Factors

Skim milk agar: To detect proteolytic activity, we used the medium described by (Siddalingeshwara *et al*., 2010). This medium contained of sterilized nutrient agar with 1 % skimmed milk and 1 % casein. After incubation, A clear zones around bacterial growth indicated the presence of protease.

Congo red agar: Isolates were tested for biofilm formation was agreeing to (Neihaya Zaki *et al*., 2017) by inoculating bacteria on the agar of congo red which containing (18.5 g/500 ml) brain Heart Infusion broth, (25g/500 ml) sucrose,

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(7.5 g/500 ml) agar, and (0.4g/ 500 ml) Congo red dye. Slime producing isolates presented black colonies while non - producing isolates developed red colonie.

Blood agar: The hemolytic activity of bacteria was assessed on blood agar plates by Added 5ml of sterilized sheep blood to 100 ml of Mueller-Hinton agar or trypticase soy agar that cooled to 60ºC after autoclave, hemolysis was determined by observation of the zone around colonies after incubation overnight at 37ºC (Rasha Al-Oqaili, 2018).

Result

This research, which focused on identify the microbial contamination and detect of some virulence factors of bacteria isolated from three different types of hand dryers in malls. There was a total of 35 isolates for 11 different bacterial species obtained from the air dryer are shown in (Fig 3 & Table 1). Among the bacteria isolated from this study: Staphylococcus epidermidis, Staphylococcus aureus (Fig.4), Escherichia coli, Pseudomonas aeruginosa (Fig.5), Salmonella (Fig.6), Bacillus sp and Clostridium limosum. Of all diagnosed bacteria, the number of Gram-positive bacteria was higher than Gram-negative bacteria Table 2. To detect the production of biofilm, hemolysin and protease enzyme; was selected 13 frequent bacterial isolates of Acinetobacter baumannii (Fig.7), Kocuria kristinae (Fig.8), Enterococcus faecalis (Fig.9), and Enterococcus faecium (Fig.10), Table 3 showed some properties of bacterial isolates which selected to detect some virulence factor. Additionally, nutrient agar plates that been exposed to air emitted from a hand dryer appeared fungal growth.

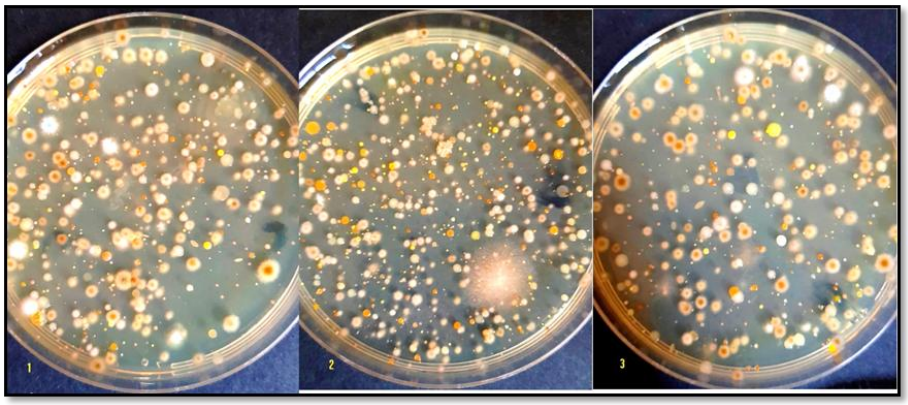

Fig. 3. The colonies of microorganisms on a nutrient agar after overnight incubation when exposed to air of hand dryers

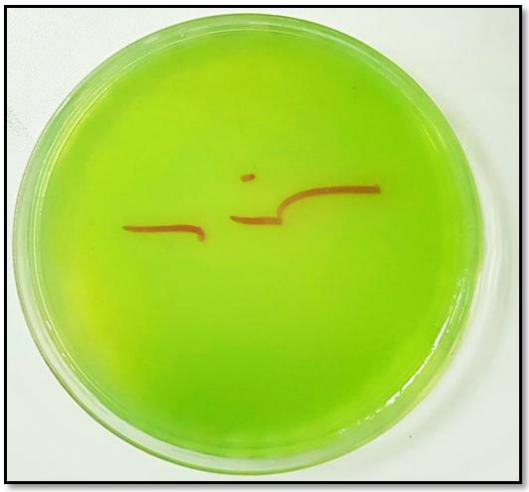

Fig. 4. Staphylococcus aureus mannitol salt agar; **Fig. 5.** Pseudomonas aeruginosa on nutrient agar

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Fig. 6. Salmonella on SS agar

Fig. 7. Acinetobacter baumannii on MacConkey agar. **Fig. 8.** Kocuria kristinae on blood agar

Fig. 9. Enterococcus faecalis on MacConkey agar. **Fig. 10.** Enterococcus faecium on MacConkey agar.

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Table 1. The bacteria isolates from all hand dryers of the ladies' toilets included in this study

Bacteria	Number of isolates
Staphylococcus epidermidis	5
Staphylococcus aureus	2
Escherichia coli	6
Pseudomonas aeruginosa	3
Enterococcus faecalis	5
Enterococcus faecium	3
Bacillus sp	2
Clostridium limosum	2
Salmonella sp	2
Kocuria kristinae	2
Acinetobacter baumannii	3

Table 2. Distribution of Isolates According to Gram's stained

Isolates	Number of isolates	Percentage (%)
Gram positive	21	63.6 %
Gram Negative	14	36.3%

Table 3. Some properties of selected bacterial isolates from the hand dryer

Bacterial isolated	Natural habitat	Some characteristics of bacteria
Enterococcus faecalis	It can be commensal in the gastrointestinal tract of humans and animals. Also, able survive in aquatic environments for longer periods of time than other intestinal microorganisms.	are facultative anaerobe, lactose fermented, Gram-positive that occur either singly or arranged in pairs or short chains. Non- spore-forming.
Enterococcus faecium	Are survives in aquatic environments. Also, they are commensal in the gastrointestinal tract of humans and animals.	are facultative anaerobe, lactose fermented, Gram-positive, non-sporulating.
Kocuria kristinae	It inhabits humans skin and oral mucosa and has also been found in the urinary tract of patients with UTI.	Is Gram-positive, is a member of the Micrococcaceae family, a facultative anaerobic bacterium that occurs in tetrads and growth on blood agar to produce pale cream colonies.
Acinetobacter baumannii	Commonly isolated from aquatic environments and soil. also, it colonizes the skin and mucous membranes.	is a Gram-negative bacillus that is aerobic, non-lactose fermented, pleomorphic and non-motile.

Table 4 showed that Isolates 1, 3 and 5 of Enterococcus faecalis had the ability to form strong biofilms Fig. 11, while the isolates 2, 3 and 4 were protease producers. All the five isolates of this species presented Beta-hemolysis. Two isolates of Enterococcus faecium from the total of three were positive for Biofilm formation (Fig.11). Two isolates showed α-hemolysis excluding one was γ -hemolysis, whereas only two were unable to produce protease. All isolates of Acinetobacter baumannii and Kocuria kristinae, showed the ability to form biofilms (Fig. 12). All the three isolates of Acinetobacter baumannii produced beta-hemolysis, while none of them was protease producer (Fig. 13). With respect to Kocuria kristinae, the two isolates had the ability to produce protease (Fig.14), while only one isolate showed hemolysis on blood agar. Three different types of hemolysis in (Fig. 15,16)

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Fig. 11. Strong biofilm formation on Congo agar of faecalis & Enterococcus faecium

Fig. 12. Weak biofilm formation on Congo agar of Enterococcus Kocuria kristinae & Acinetobacter baumannii

Fig .13. Enterococcus faecium had strong - protease producing, While Kocuria kristinae had weak – protease producing

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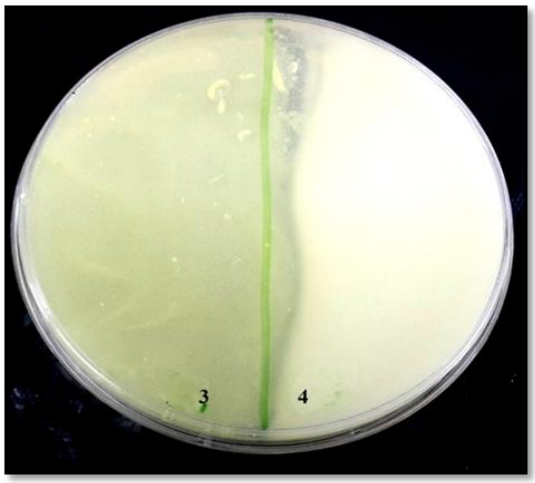

Fig .14. Enterococcus faecalis had strong - producing of protease, While Acinetobacter baumannii was non-protease producing

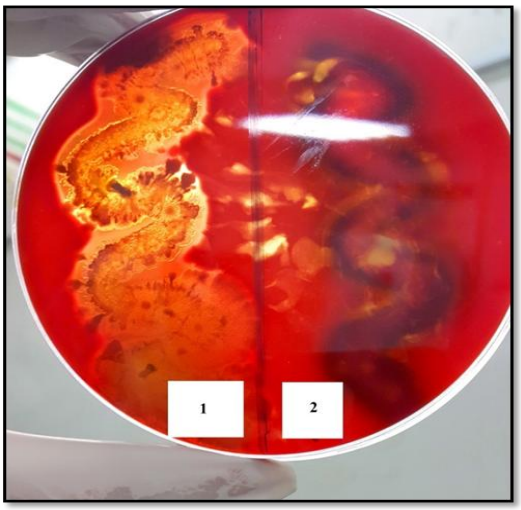

Fig. 15. B-hemolytic of Enterococcus faecalis & α-Green of hemolytic Enterococcus faecium

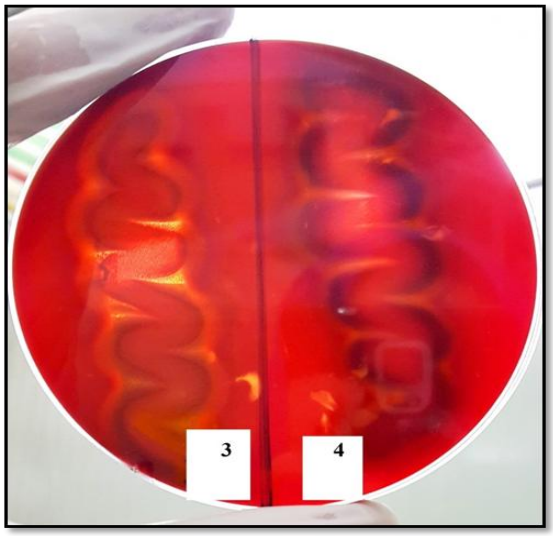

Fig. 16. α-Green hemolytic & γ-hemolytic of Kocuria kristinae

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Table 4. Type of virulence factors detected in selected bacterial isolates

Bacterial isolates	Biofilm formation	Hemolysin	Protease
Enterococcus faecalis1	++	**β-hemolysis**	-
Enterococcus faecalis2	+	**β-hemolysis**	+
Enterococcus faecalis3	++	**β-hemolysis**	+
Enterococcus faecalis4	+	**β-hemolysis**	++
Enterococcus faecalis5	++	**β-hemolysis**	-
Enterococcus faecium1	++	**α-hemolysis**	-
Enterococcus faecium2	+	**γ -hemolysis**	++
Enterococcus faecium3	++	**α-hemolysis**	+
Acinetobacter baumannii1	++	**β-hemolysis**	-
Acinetobacter baumannii2	++	**β-hemolysis**	-
Acinetobacter baumannii3	++	**β-hemolysis**	-
Kocuria kristinae1	++	**α-hemolysis**	+
Kocuria kristinae2	++	**γ-hemolysis**	+

-: Non-production/ lysis, + : weak production/ lysis, ++ : Strong production/lysis, β: Complete hemolytic, α: Green hemolytic, γ: Non-hemolytic

Discussion

A variety of bacterial species were detected in the hand dryer suggesting that this device acts as a reservoir for the accumulated microorganisms (Lorna *et al*,2019) due to the high rate of individuals who used the toilet. Public toilet users, including healthy, not healthy or asymptomatic individuals could contribute to the spread of microorganisms around the toilet, especially drug-resistant bacteria (Lorna *et al*, 2019). The transmission of specific bacterial species is due to several activities carried out by users in the toilet. For example, Staphylococcus epidermidis and Staphylococcus aureus might be resulted from shed skin microbiota or could be released from the respiratory tract by coughing, sneezing, discharged sputum. Furthermore, the spread of Escherichia coli probably associated with feces due to defecation process. Both urinary and respiratory tract infections could be the cause of Pseudomonas aeruginosa existence. Moreover, Bacillus sp and Clostridium limosum were also found in the toilet because of the toilet user footwear which may spread soil on the toilet floor. Detection of Salmonella and Staphylococcus could be related to persons who work in the mall restaurants, who may transmit these bacteria by their hands from foods such as raw meats and eggs. In addition, Salmonella and E. coli may result from diarrhea or vomiting of infected people who had gastroenteritis. Besides, rubbish bins placed in the toilet were also considered as sources for the spread of more microorganisms. These species may danger to those individuals with open wounds. (Snelling *et al*, 2010; Surfaces *et al*, 2011).

Previous studies reported that microorganisms can persist for a long time on toilet surfaces and items due to several factors, including warm and wet environment, poor hygiene measures used, and inadequate ventilation. Toilets occupied by ladies are usually more crowded than gents toilet for different reasons women are often accompany with their children or friends. They may also spend a long time checking and adjusting their appearance. Thus, the contamination in ladies' toilet is higher than gents toilet indicating the presence of many types of microorganisms in the hand dryer of ladie's toilet (Al-Harbi *et al*, 2017).

After each toilet flushes, new microbial aerosols were sprayed away from toilet seats into the air to a distance up to $6m^2$ and via an air distribution system as fans in the toilet; the bioaerosols can dispersal throughout the area resulting in contamination of the toilet environment, and the air blowing from hand dryer, as well (Sinclair *et al*, 2010; Huang *et al*, 2012).

It was stated by Lorna *et al.* 2019, that microbial species found on toilet surfaces were similar to those isolated from the blowing air of hand dryer. Furthermore, difficulty of sterilization the dryer internal parts may result in increased microbial load, and establishment of bacterial spores. Colonization of a large number of microbes had a major effect on the development of microbial virulence. Several factors also affect bacterial transmission from the dryer to the user hands, including type of the electric dryer, exposure period to the blowing air, frequent use of the dryer, type of bacterial species and number of diffused bacterial cells. It was reported that electric hot air dryers can disperse bacteria into a distance up to one meter (about 3 feet), while jet air dryers can spread them up to two meters (around

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6 feet).

Virulence factors of thirteen isolates were investigated in the present study such as biofilm formation, hemolysin and protease production. At least one virulence factor was detected in each bacterial isolate. These factors are responsible for the severity of infections including those which affect individuals with weak immune system such as infants, and elderly people. Moreover, multiple virulence factors of a single bacterial species could have a further influence on the survival and persistence of that species. For instance, most microorganisms are able to live in significant populations for weeks or months by the formation of biofilms; Another study mentioned that some bacterial species can survive for several years on inanimate objects, especially in aquatic environments. In addition, surface bacterial structures, such as pili are short organelles which assist the adherence and colonization of bacteria to environmental surfaces or host cells (Axel Kramer and Ojan Assadian,2014) .Hazards associated with bacteria in virulence factors even if the low number of microbes can causing disorders may affect immunocompromised persons (Noor Al-Marzoog and Amal Hameed,2018).

E. faecium and E. faecalis may be spread after the flush latrines to other items of the toilet. Also these two species could be transferred to toilet surfaces by the users when hand washing was not performed properly (Timur *et al*, 2006). The existence of Acinetobacter baumannii and Kocuria kristinae in the hand dryer or on other toilet surfaces resulted from secretions of the user respiratory tract (Azam *et al*, 2012; Kandi et al, 2016). They could be also transmitted from mucosa of the digestive and urinary tracts of infected or even healthy people. Furthermore, these two bacterial species might be spread as a result of skin shedding of toilet users (Mao Chen *et al*, 2015). Acinetobacter baumannii could be transferred to the toilet by the user shoes (Karolien *et al*, 2004) the natural habitat of these bacteria present in Table 3. All of these bacteria circulate in a closed area and are picked up by the hand dryer.

Both bacteria and fungi have direct or indirect influences on each other through several interactions, including reproduction, nutrition, and pathogenicity. These interactions are considered as mutualism rather than antagonism. Fungal factors can influence bacterial growth or control bacterial survival. Therefore, the presence of fungi with bacteria in a single environment like the hand dryer may lead to the stimulation of genes encoding for bacterial virulence factors. Moreover, detergents used in toilets that lack the bactericidal activity, could develop more virulent or new bacterial strains with novel combinations of virulence factor genes. Similarly, competitions among different bacteria genera enhance the production of virulence factors and increase mutability to survive. Bacteria may have a large number of virulence factors, however, one of these factors can allow the bacteria to cause the disease.(Aurelie Deveau *et al*,2018; Anton *et al*, 2010)

Conclusion

Results reported in this research showed that all hand air dryers placed in three ladies toilets in three malls are contaminated. Also, the use of toilet items should be carefully handled. Several microbial groups have been detected inside the electric dryer indicating that the process of drying hands can cause contamination of the user's hands resulted in further spread of microbes to other toilet surfaces and objects or even to other parts of the building. The use of hand dryers by many people could be the reason for the cross-contamination among individuals.

Recommendations

The electric hand dryer is unsuitable for drying inside the toilet environment and use paper towels is the most hygienic way to complete the cleanness procedures, high-efficiency air filters could be attached to the dryers; which will help to capture most of the microbes before the air exit to the hands. The selection of hand dryers supplied with ultraviolet lights would also assist in the suppression of microbial species transmitted by using the dryer. Hand dryers should be located outside the toilet area to prevent microbial transmission from the toilet zone and other toilet surfaces. Hand dryers could be also connected to the external environment to obtain pure air with fewer microbes.

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