CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction

Microorganisms are found everywhere and constitute a major part of every ecosystem. In the environment, they live either freely or as parasites (Sleigh and Timbury, 1998). Human hands usually harbor microorganisms as part of body normal flora as well as transient microbes contacted from the environment (Lindberg *et al.*, 2004; Oranusi S et al., 2013). In some cases, they live as transient contaminants in fomites or hands where they constitute a major health hazards as sources of community and hospital acquired infections (Pittet *et al.*, 1999).

Beside the day to day interaction of people, which constitute one way of spreading disease, the major source of spread of community acquired infections are fomites (Prescott *et al.*,1993). Indeed, fomites when in constant contact with humans or natural habitats of pathogenic organism constitute a major source of spread of infectious diseases (Osterholm *et al.*, 1995). Such fomites include door handles of conveniences, showers, toilet seats and sinks, lockers, chairs, tables especially those found in public offices, hospitals, hotels, restaurants and restrooms(Bright *et al.*, 2010). Although it is accepted that the infection risk in general community is less than that associated with patients in hospital, the increasing incidence of epidemic outbreaks of certain diseases and its rate of spread from one community to the other has become a major public health concern (Scott *et al.*, 1982).

Hand washing is thought to be effective for the prevention of transmission of diarrhea pathogens. However, it's not conclusive that hand washing with soap is more effective at reducing contamination with bacteria associated with diarrhea than using water only (Burton *et al.*, 2011). Many authors considered that hand washing is normal practice thought to be effective for the prevention of disease, keeping hands clean and improving the hand hygiene is one of the most important steps taken to avoid getting sick and spreading germs to others (Chinakwe *et al.*,2012).

However, some materials e.g. brass, copper and silver are slowly poisonous to many microbes. The exact mechanism is not known, but is commonly thought to be via the oligodynamic effect, or by some other electrostatic effect(Gregor *et al.*, 2011).

In the university community, students have access to service offices regularly for different purposes. Given that the door handles are not routinely disinfected, the opportunity for the transmission of contaminating microorganisms is increasing day after day.

1.2. Rationale

Lack of knowledge about source of bacteria, make people believe that microbes are only present in research laboratories or in hospital and clinics and thus they have a misleading feeling of security in other places. Researchers considered that 80% of infections are spread through hands contact with hands or other objects (Al-Ghamdi *et al.*,2011). Hand washing is normal practice thought to be effective for the prevention of disease (van Tonder, 2007; Chinakwe *et al.*, 2012).

Door handles of offices were investigated for bacterial contamination elsewhere (Nworie *et al*., 2012). To our knowledge, there have been no published data available on the contamination of door handles of the service offices among Sudanese Universities. This study is expected to increase the awareness of people about a source of bacterial contamination.

1. 3. Objectives

1. 3. 1. General objective

To assess Gram-positive bacteria in door handles of service offices at some universities in Khartoum State.

1. 3. 2. Specific objectives

a. To determine the presence of bacterial contamination on the door handles.
b. To estimate the load of bacterial contamination in the door handles.
c. To isolate Gram-positive bacteria from the door handles.
d. To identify specific infectious species that contaminates the door handles.

CHAPTER TWO

LITERATURE REVIEW

2.1. Definition of door handles

A door handle is an attached mechanism used to open or close a door. Its location on the door may vary between few inches or centimeters away from the edge of the door to the exact center of the door, depending on the local culture, decorative style or owner preference. The term door handle tends to refer to round operating mechanisms. Door handles and door knobs are the same exact thing (http//en.wikipedia.org/wiki/Door handle).

2.2. History of door handles

Door comes from the old German word *thurh* which meant opening. It is well-known historical fact that the Romans invented the door, but the Creek being more technologically advanced and invented the handle to be able to pull the door safely and effortlessly (http://uncyclopedia.wikia.com/wiki/ Door_ handle).

2.3. Bacterial contamination

Microorganisms that cause infections can be found in any environment including soil, air, water and food as well as on environmental surfaces or objects. The infection can spread to humans in different ways; directly or indirectly via inanimate objects called vectors (Neely and Sittig,2002).The presence of the pathogenic bacteria on environmental surfaces such as door handles poses a potential risk to vulnerable, immune-compromised individuals. It has been shown that hard, non-porous surfaces,

such as door handles, have the highest bacterial transfer rates to hands (Rusin, et al., 2002). Microorganisms are found everywhere and constitute a major part of every ecosystem. In these environments; they live either freely or as a parasite (Sleigh and Timbury, 1998).In some cases, they live as transient contaminants in fomites or hands where they constitute a major health hazards as sources of community and hospital acquired infections (Pittet *et al*., 1999). Although it's accepted that the infection risk in general community is less than that associated with patient in hospital, the yearly increase in food poisoning cases in which household outbreaks are a major factor, requires an assessment of the probable causes and sources (Scott *et al*., 1982).

Beside the day to day interaction of people, which constitute one way of spreading disease, the major source of spread of community acquired infections are fomites (Prescott *et al.*,1993). Fomites when in constant contact with humans or natural habitats of pathogenic organism constitute a major source of spread of infectious diseases (Osterholm *et al*., 1995).Such Fomites include door handles of conveniences, showers, toilet seats and sinks, lockers, chairs, tables especially those found in public offices, hospitals, hotels, restaurants and restrooms(Bright *et al*.,2010).

2.4. Previous studies

Nwori *et al*. (2012) investigated 180 door handles/knobs of public conveniences of selected public offices, motor parks and markets in Abuja Metropolis, Nigeria. They found that 156 (86.7%) were positive for bacterial growth. The bacteria were *Staphylococcus. aureus* (30.1%), *Klebsiella . pneumoniae*(25.7%), *Escherichia coli* (15.6%), *Entero.bacter* spp.(11.2%), *Citrobacter* spp.(7.1%), *Pseudomonas aeruginosa* (5.9%) while *Proteus* species had the least prevalence,(4.5%).

Kamiya *el al.,* (2002) investigated the contamination of room door handles by methicillin-sensitive/ methicillin-resistant *Staphylococcus. aureus* in wards of university hospital. They reported 53 (27%) of 196 rooms were contaminated by methicillin-sensitive *Staphylococcus.aureus* and/or methicillin-resistant *Staphylococcus aureus.*

Study done by Duszak R .JR ,et al.(2014) was quantifying and characterizing bacterial contamination of radiologist work stations, all samples from radiologist computer work stations and rest room sites were contaminated with bacteria; mean colony count was 14.8+/-16.0(range 1-36) from rest room door knobs . All work station sites 64.3% (9 of 14) grew *Staphylococcus aureus* and 21.4% (3 of 14) grew enteric organisms. Bacterial contamination of microphones and computer mice is common, with colonization was greater than nearby restroom toilet seats and doorknobs. Simple, rapid, and inexpensive disinfection techniques nearly completely eradicated workstation bacterial contamination.

Sabra (2013) carried out a study on public female restrooms at Taif, Kingdom of Saudi Arabia, in order to determine the locality of contamination and bacterial loads. She found that out of 260 specimens investigated 187(71.9%) were positive growth. The predominant positive was toilet handles (91.3%), followed by room door handles 59(73.8%), and room sink 38(63.3%). Isolated bacteria arranged according their percentage, *Staphylococcus. aureus* 40.6%, *Escherichia. Coli* 22.5%, *Bacillus* spp. and *Klebsiella. pneumoniae* 21.4%, *Enterococcus. faecalis* 13.4%, *Citrobacter* spp. 9.6%, *Pseudomonas. aeruginosa* 8.6% and *Proteus .mirabilis* 7%.

Another study was carried out by Lamrechts and his co-workers (2014) to evaluate the efficacy of hand washing practices and sanitation before commencing work among food handlers in the convenient food industry in Gauteng, South Africa. They reported that the most prevalent organisms in hands were *Staphylococcus. aureus* and *Escherichia. coli*. The study proved that hand hygiene is unsatisfactory and may have serious implications for public health. This therefore underlined the importance of further training to improve handlers' knowledge of good hand washing practices.

Moayad and his colleagues (2011) in a conference poster hypothesized that door handles may aid in the spread of microbes between individuals and that they may be a reservoir of microbial contamination. They assessed the Gram-negative bacteria that were found on door handles. They found that a larger percentage of the bacteria sampled from the door handles were Gram negative but equal to the number of Gram-positive. The common prevalence of Gram-positive bacterium, *Staphylococcus.aureus*, can be attributed to the presence of that found on the skin.

Hedieh *et al*. (2012) conducted a study to determine whether microbial contamination of door handles in two busy intensive care units and one high dependency unit was related to their design, location , and usage. They found a significant correlation between the frequency of movement through a door and the degree to which it was contaminated. Furthermore, the doors location, design and mode of use all influenced contamination. When compared to push plate designs, pull handles revealed on average a fivefold higher level of contamination; lever handles, however, displayed the highest levels of bacterial contamination when adjusted for frequency of use. They

also observed differences in contamination levels at doors between clinical areas, particularly between the operating theaters and one of the Intensive Care Units.

Mendes and Lynch (1976) made a survey of bacterial flora present at various positions in 130 males and females washrooms and toilets. The results are used to assess priorities for disinfection. Some surfaces (cubical door locks and handles and flush handles) were rarely contaminated. These surfaces are normally dry and bacteria cannot be expected to survive well. In contrast, the inside handles of the entrance door and tap handles showed an alarming degree of contamination, No doubt moisture from the hands aids bacterial survival. The most important areas are the inside handles of the entrance door; these provide routes for cross-infection via the body and hands. Of moderate priority importance are flush handles, cubical door handles and locks, under the flushing rim, and water in the pedestal. Fecal bacteria occur in large numbers on surfaces which users of washrooms and toilets readily contact. They recommended daily cleaning and disinfection in conjunction with regular hygiene service to reduce cross-infection risks in washrooms and toilets.

Axel *et al*. (2006) summarized data on the persistence of different nosocomial pathogens on inanimate surfaces. They found that most Gram-positive bacteria, such as *Enterococcus* .spp, *Staphylococcus. aureus* (including MRSA) and *Streptococcus. Pyogenes* survive for months on dry surfaces. Many Gram-negative species, such as *Acinetobacter* spp. ,Escherichia. *coli*, *Klebsiella* .spp.,

Pseudomonas. aeruginosa , *Serratia. marcescens*, or *Shigella*. spp, can also survive for months'. A few others, such as *Bordetella* . *pertussis*,

*Haemophilus. influenza*e, *Proteus .vulgaris*, or *vibrio. cholera*, persist only for days. The most common nosocomial pathogens may well survive or persist on surfaces for months and can thereby be continuous source of contamination and transmission if no regular preventive surface disinfection is performed.

Aminu et al. (2014) conducted a study to determine the anti biogram of bacteria isolated from fomites in teaching hospital in Nigeria. 35 samples were used for the study. Twenty three (65.7%) isolates were obtained; the ratio of Gram-positive to Gram-negative organisms was (1.2 to 1.1). The bacteria isolated were *Staphylococcus. aureus* (21.7%),*Staphylococcus. epidermidis* (8.7%), *Streptococcus* spp. (8.7%), *Bacillus* spp. (13.0%), *Escherichia. coli* (26.1%), *Pseudomonas* spp. (8.7%), and *Klebsiella* spp. (13.0%).The isolation of pathogenic bacteria from fomites indicates that they can be vehicle for pathogens transfer.

Watutantrige *et al*. (2012) indicated that harmful microorganisms can be transferred to hands from contaminated surfaces people come into contact in daily life and can transfer disease to one self as well as to others. According to this hypothesis they conducted a study to determine the extent to which hand hygiene practices and toilet door handles contribute to the bacterial load of hands of toilet users in a medical school. They investigated 60 swabs taken from medical students for bacterial count from both hands before and after toilet use and from door handles of six toilets. They reported that bacterial load in the hands of both males and females showed an increase after toilet use. The increase was significant among male students. The mean bacterial load of male toilet door handles was 12 CFU/cm2.

An investigation of microbial contamination in 21 homes describes the environmental sites representing the various areas and activities in the home. The result revealed that several sites such as vegetable racks, bread bins, tin openers, door handles, walls and air samples contaminated with organisms such as Micrococci and Gram-positive bacilli and entero bacteria within those homes were from “wet sites” such as the toilet water, kitchen sink and others. The most frequently occurring species were *Escherichia* coli*, Citrobacter. freundii* , *Klebsiella. pneumoniae* and *Enterobacter. cloacae* (Scott *et al.*, 1982).

Bacteria that are often found in a healthcare environment include coagulase-negative *Staphylococcus, Bacillus* species, *Coryne bacterium* species, streptococci, *and clostridium. perfringens* , *Enterococcus* species, and *Staphylococcus. aureus.* Of significant importance in healthcare environments involve antibiotic resistant strains of microbes which include *Staphylococcus*. aureus, Vancomycin-resistant enterococci, and methicillin-resistant *Staphylococcus. aureus* (MRSA). The capability of these bacteria to survive for more than 24 hours further increase their chances of contamination in other places such as door handles (Rutala,et al. 2006).

Lynn et al. (2013) conducted a study about the prevalence of bacterial organisms on toilet door handles in secondary schools. They investigated a total 120 samples for bacterial isolates. 60(50%) yielded growth and 60(50%) showed no growth at all. The following organisms were isolated *Staphylococcus* species 26(43.3%), *Candida* species 6(10%), *Escherichia coli* (16.7%), *Citrobacter* species 1(1.7%), *Klebsiella* species 12(20%), *Proteus* species 4(6.7%) and *Salmonella* species 1(1.7%).

CHAPTER THREE

MATERIALS& METHODS

3.1. Study Design

3.1.1. Type of study

This is a descriptive cross-sectional study.

3.1.2. Study Area

The study was conducted in Sudan University of science and technology (SUST), Alneelain University and University of Khartoum from which sample were obtained. The experimental work was done in the Research Laboratory (SUST), collage of medical laboratory science.

3.1. 3. Study duration

The study was conducted during the period August-September,2014.

3.1.4. Sample size

A total of 200 office door handles were included in this study.

3.2. Experimental work

3. 2.1. Collection of samples

The specimens were collected from door handles by means of sterile cotton swabs moistened in sterile nutrient broth. The swab was wiped firmly on the entire surface of the door knob. Each swab was placed in small tube, labeled and immediately transported to the Research Laboratory.

3. 2.2. Culture

The swabs were used to inoculate nutrient agar plates. The plates were incubated aerobically at 37 ℃. Bacterial growth was checked after 24-48 hours.

3.3. Identification of Gram-positive bacteria

3. 3. 1. Colonial morphology

Different morphological features of the yielded colonies including color, size and elevation were recorded.

3. 3.2. Gram stain

Gram stain was essential step for the next experimental work to identify Gram-positive isolates. The procedure was carried out according to Cheesbrough (2006) as follows;

Smear was prepared from overnight culture on a clean and dry slide. The smear was left to air dry. Fixation was done by rapid pass the slide three times through the flame of a Bunsen burner then allowed to cool before staining. Crystal violet stain was added to smear for 30–60 seconds, and then washed by tap water. Lugol's iodine was added for 30-60minutes then washed by tap water and decolorized rapidly (few seconds) with acetone alcohol and washed immediately by tap water. Finally, the smear was covered with saffranin stain for 2 minutes and washed by tap water. The back of slide was wiped clean and placed in a draining rack for smear to air dry. Drop of oil was added to the dried smear and examined under the light microscope (Carl Zeiss, Germany) by oil lens 100X.

3.3.3. Spore stain

Smear was prepared and fixed with gentle heat. Malachite green stain was added. The smear was heated in Bunsen burner until steam can be seen rising from the surface.

The slide was left steaming for about three minutes, and then rinse with tap water.0.5 % Safranin was added as counter stain and left to act for 45 seconds, then washed off with water. The back of slide was wiped clean and placed in a draining rack for smear to air dry. Drop of oil was added to the dried smear and examined under the light microscope (Carl Zeiss, Germany) by oil lens 100X. Spore will appear green and cells red.

3. 3. 4. Biochemical tests

3.3.4.1. Catalase

A bout 2-3 ml of the hydrogen peroxide solution was transported into test tube, good growth of the test organism was removed with sterile wooden stick then immersed it in hydrogen peroxide solution. The positive result was showed as active bubbling that indicated staphylococcus species by Cheesbrough (2006).

3.3.4.2. Coagulase test

A drop of physiological saline was placed on each end of a slide, then the colony of the test organism was emulsified in each of the drops to make two suspensions, drop of the poold plasma was added to one of the suspension, mixed gently. The positive reaction was shows as clumping organisms within 10 seconds indicated *Staphylococcus aureus.*

3.3.4.3. Deoxyribo nuclease (DNAse) test

The test organism was cultured on medium which contains DNA after overnight incubation. The colonies were tested for DNAse production by flooding the plate with weak hydrochloric acid solution. The acid precipitates unhydrolyzed DNA. DNAse

producing colonies are therefore surrounded by clear areas indicating DNA hydrolysis (Cheesbrough, 2006).

3.3.4.4. Mannitol fermentation

Test organism was inoculated onto mannitol salt agar, incubated at 37^{o}C and examined after 24 hours for mannitol fermentation; it was indicated by formation of yellow color around the growth (Cheesbrough, 2006).

3.3.4.5. Novobiocin

To a filter disc of novobiocin was placed on the surface of inoculated plate with the test organism. The plate was incubated aerobically at 37^{o}C for 24 hours and examined at the end of incubation period, for the presence clear zone of inhibition (sensitive) or absence (resistant) (Cheesbrough, 2006).

3.3.4.6. Sugar fermentation test

Bacteria actively break down organic component to obtain energy as follows; (CH_2O) N $+6\ O_2 \rightarrow 6CO_2\uparrow + 6\ H_2O$ + energy o_2= electron acceptor.

Bromo thymol blue-carbohydrate broth complex was inoculated by the test organism and incubated up to 5 days. During aerobic respiration organism produced pink color due to break down of carbohydrate, while the organism not fermenting carbohydrate remain yellowish in color (Waghorn *et al.*, 2005).

3.3.4.7. Oxidase test

The oxidase test is used to identify all organisms that produce oxidase enzyme. A piece of filter paper was soaked with oxidase reagent, a colony of test organism is then smeared on filter paper .deep purple color indicate that phenylene diamine in the reagent oxidize by the oxidase in the test organism (Cheesbrough, 2006)

CHAPTER FOUR

RESULTS

A total of 200 doorknob swabs were collected from offices in different universities in Khartoum State. The universities include Khartoum University, Sudan University of Science and Technology (SUST) and Alneelain University. Frequency and percentages of these swabs are presented in table (1).

Cultivation of these swabs on nutrient agar plates yielded bacterial growth on 87 plates. The rest, 113 swabs showed no bacterial growth. Those yielded bacterial growth were obtained from Alneelain University 40(45.98%), SUST 24(27.58%) and University of Khartoum 23(26.44%) (Table2).

Study on colonial morphology of bacterial isolates, showed different patterns of feature. Most isolates were white-cream in color. The size of the colonies ranged from small to large size. The majority of the colonies were entire circular and few were rhizoid.

Bacterial load was recorded semi-quantitatively. The average of bacterial load estimated at different universities were as follows; Alneelain University high (++++), SUST and Al Khartoum University moderate (+++) (Table 2).

Gram-positive bacteria were recovered from 32 (36.78%) samples investigated that we concern on them and G-ve 55(63.22%) sample. Seven were recovered from University of Khartoum, ten from SUST and fifteen from Alneelain University

(Table 3) . Biochemical tests adopted for identification of Gram-positive isolates are tabulated in table (4). The most prevalent bacteria identified was S. *aureus*, followed by *Bacillus* spp., but considerable number of other staphylococci was also recovered (Table 5).

Table1. Number of office door handles sampled according to university

University	Offices	
	No	**%**
University of Khartoum	60	30
SUST	70	35
Alneelain University	70	35
Total	200	100

Key: SUST= Sudan University of Science & Technology

Table2. Bacterial growth after primary cultivation of swabs according to university

University	Swabs yielded bacterial growth	
	Frequency	**%**
University of Khartoum	23	26.44
SUST	24	27.58
Alneelain University	40	45.98
Total	87	100

Key: SUST= Sudan University of Science & Technology

Table 3. Distribution of the isolated Gram –positive bacteria according to university

University	Gram-positive bacteria isolated	
	Frequency	%
University of Khartoum	7	21.9
SUST	10	31.3
Alneelain University	15	46.8
Total	32	100

Key: SUST= Sudan University of Science & Technology

Table 4 . Biochemical tests adopted for identification of Gram-positive bacteria

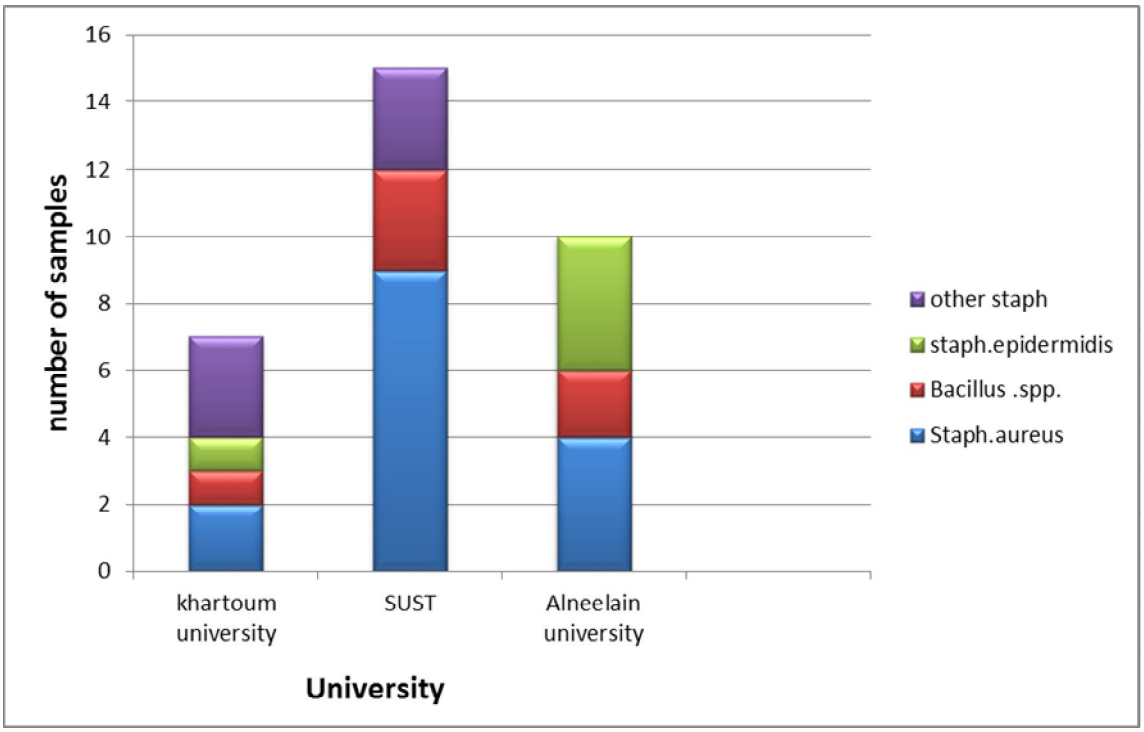
Isolate code	Biochemical Tests										Suggested organism
	Cat	Co	DNAse	Novo	Glu	Mal	Suc	Man	Mano	Tre	
1D	+	+	+	S	+	+	+	+	+	+	**S. *aureus***
2D	+	-	-	S	+	+	+	-	$+^{SI}$	-	**S. *epidermidis***
3D	+	-	-	R	+	+	+	V	-	+	**Staphylococci Sabrophyticus**
4D	+	-	NT	NT	+	+	+	-	+	+	***Bacillus* spp.**

(+): positive reaction; (-): negative reaction; ($+^{SI}$): slow positive reaction; (V):variable; (S): sensitive; (Cat)= Catalase; (Co)= Coagulase; (Novo)= Novobiocin; (Glu)= glucose; (Mal)= Maltose; (Suc)= Sucrose; (Manni)= Mannitol; (Manno)= Mannose; (Ttre)= Trehalose; NT= not tested

Table 5. Frequency and percentage of Gram-positive bacteria isolated during this study in different university

Organism identified	Frequency (%)			
	Khartoum University	SUST	Alneelain University	Total
Staphylococcus. aureus	4 (26.7%)	2 (13.3%)	9 (60%)	15 (100%)
Bacillus spp.	2 (33.3%)	1 (16.7%)	3 (50%)	6 (100%)
Staphylococcus. epidermidis	4 (80%)	1(20%)	0	5(100%)
Other staphylococci	0	3 (50%)	3(50%)	6(100%)
Total	10	7	15	32

Bacterial growths in door handle swabs



CHAPTER FIVE

DISCUSSION

5.1. DISCUSSION

Bacterial contamination of door handles is well documented. These formites in turn serve as vehicles for cross-infections (Monarca *et al*., 2000). Some of the contaminants can be highly pathogenic and can be transferred from one person to another or may result in auto-inoculation (Kennedy *et. al*., 2005)

In this study a total of 200 door handles swabs from different Universities in Khartoum State were investigated. 87 (43.5%) of them yielded bacterial growth. This result is lower than that obtained by Nworie *et al*., (2012) in Abuja Metropolis, Nigeria, who reported that out of 180 door handle swabs assessed, 156 (86.7%) showed bacterial growth. This may be due to difference in the source of swabs ; the present study sampled a door handles of service office, while the study of Nworie and his colleagues (2012) investigated swabs taken from door handle of toilet and bathroom that contain moist environment help in the growth of bacteria as well as the common knowledge that large population usage of toilets than office.

The present study showed that *Staphylococcus. aureus* (*S. aureus*) was the most prevalent organism (46.87%), followed by *Bacillus* spp. (18.75%). This result is in consistent with that reported by Kennedy *et.al.* (2005) who found that the most common bacterial contaminant are *S.aureus* (30.1%), followed by *Klebsiella. pneumoniae* (25.7%). In fact these contaminants were of a great concern. It must be borne in mind that even small numbers of organisms such as *S.aureus* may proliferate

and become hazardous if transferred to food (Elizabeth *et.al.*, 1982). *S . aureus* and *Bacillus.* spp. recovered during this study constitute a major part of normal skin flora; they may be passed from person to person by direct contact or via surfaces, including door handles. The two organisms are potentially pathogenic and may cause disease due to their high resistance such as food poisoning, abscesses, if enter the body can lead to bacteremia and sepsis, pneumonia, meningitis osteomyelitis.

Several studies have indicated that bacteria such as *S. aureus* survive on hands and surfaces like door handles for hours or even days (Jiang & Doyle, 1999; Scott et al., 1990). The present study confirmed their findings.

Hediet et al. (2012) found a significant correlation between the frequency of movement through a door and the degree to which it was contaminated in our study further more concentration needed on the time of peak and the door location that affect the frequency of movement further more need to use other sources such as Blood agar and Mackoncey .

5.2. Conclusion

The load of bacterial contamination in the door handles of service offices in universities is considerable. The isolated bacterial are gram positive bacteria mainly Staphylococcus species. Some of these isolates are potentially pathogenic organisms.

5.3. Recommendations

1. The door handles are instrumental in the spread of many infections thus the use of self-disinfecting door handles (e.g. copper) is highly recommended particularly important in service offices.

2. Regular surface cleaning and disinfection is also highly recommended to reduce chances of transmission of these potentially pathogens.
3. Further studies are recommended to validate the results of the present study by using more different culture media, concentrate on the peak time of movement that will increase the load of bacterial contamination also, the material of the door handles perhaps it's the reason of the negative bacterial growth.

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APPENDICES

Appendix 1: Ingredient of media

1. Nutrient agar

Formula and preparation	gram /liter
Lab-lemco……	1.0
Yeast extract……..	2.0
Peptone……..…….	5.0
Sodium chloride ……..…..	5.0
Agar ……..	15.0

2. MacConky agar

Peptone ……..…….	20.0
Lactose ……..	10.0
Bile salt ……..	5.0
Sodium chloride ……	5.0
Neutral red ……..……..	0.075
Agar ……..	12.0

3. Mannitol salt agar

Lab –lemco powder ……..	1.0
Peptone …….	10.0
Mannitol…….	75.0
Phenol red …….	0.025
Agar ……..……	15.0

4. **DNAse agar**

Tryptose …….. 20

Deoxyribonucleic acid ……... 2

Sodium chloride ……. 5

Agar …… 12

5. **Blood agar**

Nutrient agar …… 500 ml

Sterile defibrinated blood ……. 25ml

Appendix 2: Reagents and stain

1. Sodium chloride, 8.5g/l (0.85%w/v)

Sodium chloride …… 8.5g

Distilled water…… 1 liter

2. Acetone –alcohol decolorize

Acetone …… 500ml

Ethanol or methanol, absolute …… 475ml

Distilled water …… 25ml

3. Crystal violet Gram stain

Crystal violet …… 20g

Ammonium oxalate …… 9 g

Ethanol or methanol, absolute ……..…… 95ml

Distilled water …… 1liter

4. Lugols iodine solution

Potassium iodide …… 20g

Iodine …… 10g

Distilled water …… 1liter

5. Safranine

Safranine O …… 2.5 g

Ethanol …… 100ml

Distilled water …… 90ml

Appendix 3: Biochemical table:

Table 4 . Gram stain and biochemical test adopted to identify Gram +ve bacteria

Number Of the Swab Label	Gram stain From culture	Gram stain From nutrient agar culture	Cat	Coag	DNase	Spore Stain	Oxi	Manit	Suspected organism
2-15	G+ve cocci	G+ve cocci	+	+	+			Moderate growth manitol ferment	S.aureus
2-17	G+ve bacilli	G+ve bacilli	+		-	+	+	Moderate growth	Bacillus
2-20	G+ve cocci	G+ve cocci	+	+	+			Light growth yellow colonies	S.aureus
2-23	G+ve cocci	G+ve cocci	+	-	-			Light growth (NMF)	S.epidermidis

Number Of the Swab Label	Gram stain From culture	Gram stain From nutrient agar culture	Cat	Coag	DNase	Spore Stain	Oxi	Manit	Suspected organism
2-25	G+ve cocci	G+ve cocci	+	+	+			Heavy growth, small manitol ferment	S.aureus
2-21 Big Coloni	G+ve cocci	G+ve cocci	+	-	-			Light growth (NMF)	S.epidermidis
2-21 Small coloni	G+ve cocci	G+ve cocci	+	-	+			moderat egrowth	S.epidermidis
2-29	G+ve bacilli	G+ve bacilli	+		-	+	+	Heavy growth	Bacillus
2-37	G+ve cocci	G+ve cocci	+	-	-			moderat egrowth (NMF)	S.epidermidis

Number Of the Swab Label	Gram stain From culture	Gram stain From nutrient agar culture	Catalase Test	Coagulase Test	DNASE test	Spore Stain	Oxidase test	Culture in Manitol salt agar (MSA)	Suspected organism
2-56	G+ve cocci	G+ve cocci	+	+	+			yellow colonies manitol ferment	S.aureus
1-21	G+ve cocci	G+ve cocci	+	-	+			Heavy growth (NMF)	Other staph/ S. epidermidis
1-24	G+ve cocci	G+ve cocci	+	-	-			Light growth (NMF)	Other staph/ S. epidermidis
1-27	G+ve bacilli	G+ve bacilli	+	-		+	+	Light growth (NMF)	Bacillus

Number Of the Swab Label	Gram stain From culture	Gram stain From nutrient agar culture	Cat	Coag	DNase	Spore Stain	Oxi	Manit	Suspected organism
1-28L	G+ve cocci	G+ve cocci	+	+	+	-		Heavy yellow colonies manitol ferment	in purity culture clear S.aureus (odour& colonies)
1-50NL	G+ve cocci	G+ve cocci	+	+	+			Yellow colonies manitol ferment	S.aureus
1-53	G+ve cocci	G+ve cocci	+	-	+			Heavy growth (NMF)	S. epidermidis
1-57	G+ve cocci	G+ve cocci	+	-	+			Light growth (NMF)	Other staph/ S. epidermidis

Number Of the Swab Label	Gram stain From culture	Gram stain From nutrient agar culture	Cat	Coag	DNase	Spore Stain	Oxi	Manit	Suspected organism
3-9L Big Coloni	G+ve cocci	G+ve cocci	+	+	+			Light growth (NMF)	S.aureus
3-13	G+ve bacilli	G+ve bacilli	+	-		+	+	Light growth (NMF)	Bacillus
3-20NL	G+ve cocci	G+ve cocci	+	-	-			Light growth (NMF)	Other staph/ S. epidermidis
3-20L	G+ve cocci	G+ve cocci	+	+	+			Heavy yellow colonies manitol ferment	S.aureus
3-25	G+ve cocci	G+ve cocci	+	+	+			yellow growth (NMF)	S.aureus

Number Of the Swab Label	Gram stain From culture	Gram stain From nutrient agar culture	Cat	Coag	DNase	Spore Stain	Oxi	Manit	Suspected organism
3-39	G+ve cocci	G+ve cocci	+	-	-			Light growth yellow colonies (NMF)	Other staph/ S. epidermidis
3-40	G+ve cocci	G+ve cocci	+	+	+			Heavy yellow colonies manitol ferment	S.aureus
3-42	G+ve bacilli	G+ve bacilli	+	-		+	+	moderat e growth (NMF)	Bacillus

Number Of the Swab Label	Gram stain From culture	Gram stain From nutrient agar culture	Cat	Coag	DNase	Spore Stain	Oxi	Manit	Suspected organism
3-43 small	G+ve cocci	G+ve cocci	+	+	+			Heavy yellow coloni (MF)	S.aureus
3-43 Big	G+ve bacilli	G+ve bacilli	+	-		+	+	(NMF)	Bacillus
3-46	G+ve cocci	G+ve cocci	+	+	+			Moderat e yellow growth (NMF)	S.aureus
3-57	G+ve cocci	G+ve cocci	+	+	+			Heavy yellow colonies manitol ferment	S.aureus

Number Of the Swab Label	Gram stain From culture	Gram stain From nutrient agar culture	Catalase Test	Coagulase Test	DNASE test	Spore Stain	Oxidase test	Culture in Manitol salt agar (MSA)	Suspected organism
3-63	G+ve cocci	G+ve cocci	+	-	-			Light growth (NMF)	Other staph/ S. epidermidis
3-68L	G+ve cocci	G+ve cocci	+	+	+			Heavy yellow colonies manitol ferment	in purity culture clear S.aureus (odour& colonies)
3-70	G+ve cocci	G+ve cocci	+	+	+			Heavy yellow colonies manitol ferment	S.aureus