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Isolation and identification of bacterial pathogens on the surfaces of gate passes from Umaru Musa Yar'adua University main gate

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Abstract
The bacterial contamination of shared objects in workplace settings could serve as a potential source for community acquired infections. The aim of this study was to isolate, identify and determine the bacterial load on the gate passes of Umar Musa Yar'adua University. A total of 60 samples of gate passes were swabbed and analysed for bacterial contamination. Samples collected were inoculated in nutrient broth and incubated at 37ºC for 24 hours after which they were plated out on nutrient agar, MacConkey and Mannitol salt agar (MSA) for bacterial isolation and for the estimation of the bacterial load. The samples were inoculated into nutrient broth after which they were serially diluted and plated out on nutrient agar and incubated at 37ºC for 24 hours. A Quebec colony counter was used for colony count. Out of the 60 samples collected, 30 samples were collected from car gate pass and 30 from motorcycle gate pass. The bacterial isolates and their percentage prevalence include *Enterobacter aerogenes* (34.7%), *Pseudomonas aeruginosa* (30.6%), *Staphylococcus aureus* (22%) and *Escherichia coli* (12.2%). The results also show that the motorcycle gate passes had higher contamination with four organisms isolated and three organisms were isolated from car gate passes. The contamination by microorganisms of gate passes and transmission of infection can be minimized by practising simple hand hygiene.

Keywords: bacteria, infections, handwashing, gate pass, formites, contamination

Introduction

The hands are the chief organs for physical manipulation of the environment. They serve as a medium for the propagation of microorganisms from place to place and from person to person. Although it is nearly impossible for the hand to be free of microorganisms, the presence of microbes may lead to chronic or acute illness. Human hands usually harbour microorganisms both as part of the body's normal flora as well as transient microbes contracted from the environment [1]. One common way by which organisms non-resident in the hand are picked up is by contact with surfaces such as gate pass, ATM machines, mobiles phones, currency notes, door handles etc. Microorganisms are ubiquitous in nature, therefore, exposure to pathogens on surfaces may take place either by direct contact with contaminated objects or indirectly through airborne particles. Some bacteria attach to surfaces as their predominant form of survival [2]. In some cases, they live as transient contaminants in hands or formites where they constitute a major health hazard as source of nosocomial and community-acquired infections [3]. Besides the daily interaction of people, the major source of transmission of community-acquired infection are the formites [4, 5]. Indeed, formites, when in contact with human or natural habitats of pathogenic organism constitute a major source of spread of infectious diseases [6]. These formites include door handles of conveniences, showers, toilet seats and faucets, sinks, lockers, and furnitures especially those found in public offices, hospitals, hotels, restaurants and restrooms [7]. The dominant resident microbes include *Staphylococcus epiderdimis* which is found on almost every hand [8]. and members of *Corynebacteria* and *Micrococcus spp* [9]. It has been estimated that the population of *Staphyloccocus epiderdimis* largely exceeds *Staphyloccocus aureus* on healthy hands. Certain members of *Enterobacteriaceae* may be present on the hands momentarily and include *Escherichia coli*, *Salmonella typhi*, *Shigella spp*, *Clostridium perfringes*, *Proteus mirabilis*, *Citrobacter freudii*, *Enterobacter spps*, *Streptococcus spp* and *Klebsiella spp* [10].

The human surface tissue (skin) is in persistent contact with environmental microorganisms and become colonized by certain microbial species [11]. Since the human hands harbour microorganisms both as residents and transients, [1] it is possible that the transfer of pathogens could occur between people who access the same area or surface. Appropriate handwashing can minimize microbes acquired by contact with contaminated surfaces. Handwashing is the simplest and most effective way of preventing the transmission of infection and thus reducing the incidents of healthcare associated infections [12, 13].

In the university, staff and students have access to gate pass every day. Given that the gate passes are not regularly disinfected, the likelihood for the transmission of microorganisms is increasing on a daily basis. Disinfection of surfaces is necessary to prevent infection from transient microbes especially surfaces that comes in frequent contact with the hand. Although these surfaces cannot be totally free from microorganisms, they can be minimized. The aim of this research study is to isolate and identify the microorganism(s) that contaminate Umaru Musa Yar'adua University gate passes.

Materials and Method

Study Area

The study was conducted at Umaru Musa Yar'adua University main gate, Katsina State, in northern Nigeria.

Study Duration

The study was conducted from July to September, 2017.

Sample Size

60 samples of the suspected infected gate passes were collected from UMYU main gate and transferred to Microbiology laboratory of the Department, UMYU, for analysis.

Media Preparation

All media used were weighed, prepared and sterilized according to the manufacturers' instructions, and these include Nutrient Broth, Nutrient Agar, Mannitol Salt Agar (MSA) and Eosin Methylene Blue (EMB).

Samples Preparation and Inoculation

Sterile swab sticks were made wet with 0.5 ml of physiological saline and rubbed throughout the entire surface of the plastic gate passes in order to ensure that the microorganisms on the surface of plastic gate pass adhere to the swab sticks appropriately. Each swab was inoculated into nutrient broth and incubated at 37°C for 24 hours after which they were plated out on nutrient agar, Eosin-methylene Blue, Mannitol salt agar and then incubated in an inverted position at 37°C for 24 hours. After the samples were inoculated into nutrient broth, 1 ml from each sample were serially diluted into test tubes containing 9 ml of distilled water. The tubes were labelled from 10^{-1} to 10^{-6} dilutions. Using aseptic technique, 0.5 ml from the last tubes (10^{-5} to 10^{-6}) were transferred into a petri plates after which nutrient agar was poured on each plates. The plates were incubated at 37°C for 24 hours. Plates containing 30-300 colonies were selected and counted using Quebec colony counter [14].

Identification of Bacterial Isolates

Routine laboratory techniques were carried out including Gram staining and biochemical identification tests.

Gram Staining

Gram staining reaction was carried out in order to differentiate the bacteria into Gram-positive and Gram-negative. A smear was made on a glass slide and fixed using Bunsen flame. The fixed smear was covered with crystal violet stain for 60 seconds, washed off with water and then covered with Lugol's iodine for another 60 seconds. It was then decolourised with acetone and washed off immediately with water. The smear was covered with safranin stain for two minutes and washed with water. Samples were examined under oil immersion objective [15].

Biochemical Identification

In order to identify the bacteria, the isolates were subjected to the following biochemical tests.

Catalase Test

Few drops of hydrogen peroxide solution were poured into a glass slide using a sterile wooden stick; several colonies of the test organism were removed and immersed in the hydrogen peroxide solution and observed for bubbles [15].

Coagulase Test

A drop of distilled water was placed on a slide. A colony of the test organism was emulsified on the drop to make a thick suspension. A loopful of plasma was added, gently mixed and observed for clumping within 10 seconds [15].

Motility Test

A sterile needle was used to pick the colony of bacteria. Motility media was stabbed, within 1 cm of the bottom of the tube, with organism under investigation. If the bacteria is motile, there will be growth going out away from stab or inoculation line indicative of positive test. If the test is negative, there will only be growth along the stab line [16].

Oxidase Test

Three drops of Kovac's oxidase reagent was added on a filter paper. Colony of the test organism was picked, smeared on the filter paper and observed for development of a blue purple colour within 10 seconds [15].

Indole Test Using Tryptone Water

The test organism was inoculated in a test tube of 4 mL of sterile tryptone water. It was incubated at 37°C for 72 hours. 15 drops of Kovac's indole reagent were added and gently shaken and examined within 10 minutes for a red colour in the surface layer [15].

Methyl Red and Vogas-Proskauer Test

MR-VP broth was prepared aseptically in sterile test tubes and inoculated with a pure culture of bacteria under investigation. The tubes were incubated for three days at 35^0C. Five drops of methyl red reagent (for methyl red test) and Barrit's reagent (for Voges-Proskeuer test) were added in each of the tube. Change of colour was observed. Red and yellow colour indicates positive and negative results respectively for both methyl red test and Voges-Proskauer tests [17].

Citrate Test Using Simmon's Citrate Agar

Slants of the medium were prepared in test tubes using a sterile wire loop. The slants were streaked with a suspension of the test organism and incubated at 35^0C for 48 hours and examined for a bright-blue colour in the medium [15].

Results

Of the 60 samples of gates passes collected from UMYU main gate, 30 each were from motorcycle and cars. Similarly, 4 different bacterial isolates were identified from the total number of samples investigated. The bacterial isolates comprise *Staphylococcus aureus* which is a Gram-positive bacterium, and three Gram-negative rod-shaped bacteria namely *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterobacter aerogenes*.

Table 1: Bacteriological load of car and motorcycle gate passes collected from UMYU main gate.

Sample	CGP (CFU/ML)	MCGP (CFU/ML)
1	2.2×10^5	4.1×10^5
2	1.62×10^5	3.0×10^5
3	1.7×10^5	TNTC
4	1.13×10^5	1.6×10^5
5	1.14×10^5	1.3×10^4
6	NG	4.1×10^4
7	1.59×10^5	NG
8	2.52×10^5	1.38×10^4
9	NG	5.8×10^2
10	4.4×10^5	NG
11	TNTC	3.2×10^4
12	NG	2.6×10^5
13	TNTC	1.16×10^5
14	2.4×10^5	3.6×10^4
15	2.5×10^5	TNTC
16	NG	TNTC
17	2.17×10^5	TNTC
18	TNTC	NG
19	NG	4.2×10^5
20	3.4×10^4	2.62×10^5
21	2.7×10^5	TNTC
22	NG	2.49×10^5
23	4.6×10^4	TNTC
24	TNTC	TNTC
25	NG	1.43×10^6
26	2.1×10^5	2.7×10^6
27	3.2×10^5	TNTC
28	NG	TNTC
29	2.2×10^5	TNTC
30	2.5×10^5	TNTC

Key: NG -no growth**,** TNTC - too numerous to count

Biochemical tests, Gram staining from the samples collected show the presence of *E.coli, S. aureus, P. aeruginosa* and *E. aerogenes* as shown in table 2.0

Table 2: Morphology, Gram reaction and biochemical characterization of the bacterial isolates

Media	Colony morphology	Gram reaction & microscopic appearance	Biochemical tests								Inference
			MR	**VP**	**IND**	**CIT**	**OXI**	**CAT**	**CO**	**MO**	
MSA	Yellowish colonies	G(+), Cocci in Chain	-	-	-	-	-	+	+	-	*S. aureus*
EMB	Greenish Metallic sheen	G(-), rod-shaped	+	-	+	-	-	-	-	+	*E. coli*
	Pinkish colonies	G(-), rod- shaped	-	+	-	+	-	+	-	+	*E. aerogenes*
NA	Bluish-green colonies	G(-), rod -shaped	-	+	-	+	+	+	-	+	*P.aeruginosa*

Key:

MR - Methyl red test
VP - Voges-Prokeur test
Indn- Indole test
CIT - Citrate utilization
OXI - Oxidase test
Cat - Catalase
Co - Coagulate
Mo - Motility
MSA - Mannitol salt agar
EMB - Eosin-methylene blue
NA - Nutrient agar
G (+) - Gram-positive
G (-) - Gram-negative

Table 3: Distribution of bacterial isolates from motorcycle and car gate passes.

S/N	Isolates	MCGP	CGP
1.	*S. aureus*	5	6
2.	*E. coli*	6	0
3.	*P. aeruginosa*	8	7
4.	*E. aerogenes*	8	9
	Total	27	22

Key: MCGP - Motorcycle gate pass, CGP - Car gate pass

Table 4: Percentage of the bacterial isolates from car and motorcycle gate passes

S/N	Bacterial Isolates	No. of Isolates	% of Isolates
1.	*S. aureus*	11	22.4%
2.	*E. coli*	6	12.2%
3.	*P. aeruginosa*	15	30.6%
4.	*E. aerogenes*	17	34.7%
	Total	49	99.9%

Discussion

The results obtained from the study showed that gate passes

are important reservoir of microorganisms. These formites in turn serve as vehicles for cross infections [18]. Some of the contaminants can be highly pathogenic and transferable from one person to another. The isolation of pathogenic bacteria contaminants in workplace setting as seen in the present study is not unexpected. Bright *et al.* [7] reported that frequently used formites were most likely contaminated with microorganisms. Different kinds of organism can be picked up from the environment especially from easy contact surfaces and the hands can be the most important means by which enteric pathogens are transmitted.

The results of table 1.0 showed the bacteriological load of the car and motorcycle gate passes used for the study. In some of the samples for both vehicle, no growth was observed which suggests absence of contamination on those passes. This is more obvious in car gate passes seen which had eight instances whereas motorcycle gate passes had only three. On the other hand, some samples recorded high number of bacterial colonies that are too numerous to count. Comparatively, there were four and eleven instances of the too-numerous-to-count colonies for car and motorcycle gate passes respectively. Table 2.0 shows the biochemical characteristics of the four isolates identified in the study namely *S. aureus*, *E. coli*, *P. aeruginosa,* and *E. aerogenes*. *S. aureus* tested positive to only catalase and coagulase tests. With the exception of *S. aureus*, all other three organisms tested negative to coagulase test. Coagulase is a protein enzyme produced by several microorganisms that enables the conversion of fibrinogen to fibrin. Coagulase test is used to distinguish between different types of *Staphylococcus* isolates. [19] Only *P. aeruginosa* was positive to oxidase test among the four isolates. Oxidase is part of the electron transfer system used by some organisms that utilize molecular oxygen as a terminal electron acceptor. The oxidase test is a useful test for distinguishing between the Gram negative rod *Pseudomonas* and the Enterobacteriaceae. [19] Similarly, for indole test, all isolates showed negative results other than *E. coli.* Indole production test is significant in the identification of Enterobacteriaceae as most strains of *E. coli, P. vulgaris, P. rettgeri, M. morganii,* and *Providencia spp*. break down the amino acid tryptophan with the release of indole. [19,17] Table 3.0 showed the distribution of bacterial isolates from the vehicle gate passes where motorcycle had the higher number of the bacterial isolates indicating possibly more contamination. Table 4.0 showed the percentage prevalence of the four organisms. *E. aerogenes* were the most prevalent organism (34.7%), followed by *P. aeruginosa* (30.6%), *S. aureus* (22.4%) and *E. coli* (12.2%) has the least prevalence as observed. The findings of this study highlight the fact that bacterial contamination in a workplace could serve as a source of infections.

E. aerogenes is an opportunistic pathogen which mainly cause disease in healthy individuals. It causes various community-acquired infections among which are urinary tract infections, skin and wound infections. *E. coli* is a major cause of infection by Enterobacteriaceae. It causes 90% of urinary tract infection. It is also an indicator of faecal contamination and when taken in food, it can result to gastroenteritis and diarrhoea. This might be due to the fact that most people use the toilet and contaminate their hands with faecal matter due to failure to wash their hands. Many microbes have been isolated from other formites such as door handles/knobs in public conveniences including multidrug resistant *S. aureus*, *K. pneumoniae*, *P. aeruginosa* and *E. faecalis* which pose a threat to the wellbeing of the population [20, 21]. Similarly, Maori *et al.* [22] reported the high prevalence of bacteria (*Staphylococcus* spp., *Candida* spp., *E. coli*, *Citrobacter* spp., *Klebsiella* spp., *Proteus* spp., *Salmonella* spp.) on the toilet door handles in secondary schools in Bokkos L. G. A., Jos, Plateau Sate, Nigeria.

P. aeroginosa can be spread by contaminated surfaces e.g. gate pass that is not properly cleaned or on the hands. It is an opportunistic, nosocomial pathogens of immunocompromised individuals. *S. aureus* may colonize the human body as a part of the normal flora. Approximately 30% of healthy people are inhabited by *S. aureus*, mostly in the anterior nares.[23] *S. aureus* is also a leading cause of nosocomial and community-acquired bacterial infections in humans, associating with numerous mild skin and soft tissue infections, as well as life-threatening pneumonia, bacteraemia, osteomyelitis, endocarditis, sepsis and toxic shock syndrome [24]. Studies have indicated that bacteria including *E. coli, S. aureus* and *Salmonella spp* can survive on hands and surfaces for hours or even days after an initial contact with the organisms [25-27].

Microorganisms may be picked by the hands from contact surfaces and spread from one person to another. Thus, this could be the reason why S. *aureus, E. coli, P. aeroginosa* and *E. aerogenes* were isolated from this study because these organisms would have been picked up from one place and then spread to another. The knowledge of the bacterial contamination in workplace setting is imperative as this would help in identifying the source of an infection with the view of taking preventive measures [21]. Adequate preventive measures is necessary to avoid contamination and cross-contamination of contact surfaces used in daily routine at workplaces and homes. Hand hygiene is a simple, effective and prophylactic measure to curtail the transmission of pathogens. Microbial contamination of gate passes' surfaces may account for the transmission of potentially pathogenic bacteria among users of the object. *E. aerogenes*, *P. aeroginosa*, *E. coli* and *S. aureus* are the microbes isolated from this study. The list of bacterial isolates identified from this study may not be exhaustive of the bioburden on the gate passes of UMYU considering the small sample size. Hence, a larger sample size and longer duration of the investigation should be considered in further studies.

Conclusion

This study demonstrates that public contact surfaces such as gate pass are often colonized by several bacteria and could serve as potential source of infection. Transmission of this infection can easily occur through contact with the hands. Adequate hand hygiene and public enlightenment on the role of easy contact surface in the spread of infectious agent should be advocated. The isolation of pathogenic bacteria from formites indicates that they can be vehicles for disease transmission. Hand hygiene such as simple handwashing should be practised by everyone especially after using the restroom, clinic visit, contact with sink and other potential pathogenic surfaces. Furthermore, gate passes and other

frequently used contact surfaces should regularly be disinfected in order to minimize and prevent the spread of infection.

Conflict of interest
The authors declare no conflict of interest.

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