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Microbiological assessment of the pedestrian hand rails of delta state polytechnic, Ozoro

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Abstract
The microbial contamination of handrails of the pedestrian walkway could serve as potential source for community acquired infections. This research work assessed the potential of bacteria and fungi pathogens in polytechnic campus environment. Twelve (12) samples were collected from the pedestrian handrails of Delta state polytechnic, Ozoro and the samples were coded as, Era, ELA, MRA, MLA, EXRA, EXLA, ERB, ELB, MRB, MLB, EXRB and EXRB. Contamination was higher in the morning sample for Bacteria but less for Fungi than in afternoon sample. A total of five(5) Bacteria species were isolated; *Proteus mirabilis, Streptococcus suis, Enterococcus species, Corynrbacterium species* and *Enterobacter aerogenes* and four (4) Fungi species; *Candida albican, Mold species, Aspergellus flavus* and *Penicillin species*. The total heterotrophic plate count for bacterial isolates ranges from 8.4 x 10^1 to 2.16 x 10^2 CFU/ML while that of fungal isolates ranges from 0.4 x 10^1 to 2 x 10^1 CFU/ML. Streptococcus suis (33. 33%) have the highest percentage of occurrence while *Enterococcus species* (8.33%) have the least percentage of occurrence amongst the bacterial isolates. *Aspergellus flavus* (52.38%) have the highest percentage occurrence while *Mold species* (4. 76%) and *Penicillin species* (9.52%) have the least percentage of occurrences amongst the fungal isolates. This study therefore, shows that the pedestrian handrails harbor highly pathogenic Bacteria and Fungi which have the potentials of causing epidemics in future.

Keywords: microbiological, assessment, pedestrian, hand, rails

Introduction
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) [1]. The major source of and spread of community acquired infections are fomites. Fomites when in constant contact with humans or natural habitats of pathogenic organisms constitute a major source and spread of infectious diseases (Osterhoim, *et al*., 1995) [2]. Therefore, fomite refers to as an inanimate object capable of carrying infectious agent such as: Bacteria, Viruses, and Parasites, thus passively enabling their transmission between hosts (http://wikitionary.org). Fomites such as; hand rails, door handles, etc are found in public places such as; public offices, hospitals, hotels, public pedestrian walkway, etc (Bright, *et al.*, 2010) [3].
Hand rail is referred to as a rail that is design to be grasped by the hand so as to provide stability, support, or guard (http://en.wikipedia.org/wiki/handrail). The presence of viable pathogenic Bacteria on fomite such as; hand rail, has been reported by researcher such as; Burke (2003) [4],therefore when the hands are in contact with the fomite, the hand serve as a medium for the propagation of micro-organism from place to place and from person to person. Although, it is nearly impossible for the hand to be free of micro-organism, therefore the presence of pathogenic Bacteria may lead to chronic or acute illness (Oranusi *et al*., 2013) [5]. The human hands also harbor micro-organisms both as part of body normal flora as well as transient microbes' contacted from the environment (Lindberg *et al.*, 2004) [6].
Eighty percent (80%) of infection are spread through hands contact with hands or other objects (Al-Ghamdi, *et al*., 2011) [7]. However, the risk of disease transmission through fomite is determined by the frequency of site contamination and exposure: level of pathogen excreted by the host; like hood of transfer of the infectious agent to a susceptible individual; virulence of the organism, immune-competence of the persons in contact; the presence of control measure such as; disinfectant use and personal hygiene (Reynolds, 2005) [8]. Hand washing is fundamental cautionary measure to protect against the spread of diseases and is one of the primary practice to reduce the transfer of Bacteria from person to person or from person to food contact surfaces (Chinakwe, *et al.*, 2012) [9]. Investigation of food borne illness showed

that poor personal hygiene, primarily ineffective hand washing is an important contributor to food borne illness (Lambrechts, *et al*., 2014) [10].

Micro-organism referred to tiny organisms which are invisible to the eye, which can only be seen with aid of microscope (Idodo-Urneh, 2004) [11]. Micro-organisms are found everywhere and constitute a major part of every ecosystem; they live either freely or as parasites (Sleigh and Timbury, 1998) [12]. Micro-organism live as transient contaminants in fomites or hands where they constitute a major health hazards, Bacteria and Fungi contaminate our body, houses, workplaces and whole environment. Fortunately among many billions of Bacteria, only 1,500 can be dangerous for health, causing different disease such as; Pneumonia or skin infection (Eltablawy and Elhinfnawi, 2009) [13].

This present study was designed to access and determine the level of microbial (Bacteria and Fungi) contamination on the pedestrian hand rails.

Materials And Methodology

Study Area

Ozoro is a town in Isoko-North Local Government Area populated with students. This research work was carried out on the pedestrian hand rails of Delta state polytechnic, Ozoro.

Sample Size

A total of twelve (12) samples were used in this research work.

Sample Collection

Sterile swab stick was used to swab the hand rails of pedestrian walkway of Delta state Polytechnic, Ozoro. Six (6) samples were collected in the morning and labeled as follows;

1. Entrance, Right — ERA
2. Entrance, Left — ELA
3. Middle, Right —MRA
4. Middle, Left — MLA
5. Exit, Right —Exra
6. Exit, Left — EXLA

Note: A means Morning sample collection

Then, in the afternoon, six (6) samples were collected and labeled as follows;

1. Entrance, Right —ERB
2. Entrance, Left — ELB
3. Middle, Right — MRB
4. Middle, Left — MLB
5. Exit, Right — ExRB
6. Exit, Left — EXLB

NOTE: B means Afternoon sample collection

The above samples were collected using sterile swab stick and normal saline was added to it and properly covered at the place of collection. Both were transported to the laboratory where analysis was carried out immediately.

Method

Sterilization of Glass Wares

The glass wares that were used for this project were washed with detergent, rinsed thoroughly and sterilized using autoclave at 12 1°c for 15 minutes.

Analysis Isolation of Test Organisms

The swab samples were inoculated onto twelve(12) plates of prepared nutrient agar (for Bacteria growth) and twelve(12) plates of saboroud dextrose agar (SDA) (for Fungi growth) and subculture was carried out on the growth after 24hours (for Bacteria) of inoculation in the incubator. Media prepared was according, to the manufacturer instruction and then used for isolation of Bacteria and Fungi. The plates were incubated in the incubator at 37°c for 24hours for Bacteria and 28°c for 48hours for Fungi. Pure isolates were identified according to their morphological characteristics and reactions to biochemical test for Bacteria and Fungi were identified according to morphological characteristics and microscopic characteristics.

Morphological characteristics

Gram staining

Smear of each Bacterial isolate was made on a grease free clean glass slide with a drop of normal saline, air dried, and heat fixed by quickly passing the slide flame. The smear was flooded with crystal violet for one minute (1 mm.) then wash, Lugol's iodine solution was added for one minute and then washed with water which it was decolourized with 95% alcohol for 15 seconds and rinsed off with water again. The slide was then flooded with safranine red for one minute to counter stain and washed off with water, dried and examined under the microscope using oil immersion and x l00 objective.

Biochemical Test

The biochemical analysis carried out was in accordance with procedures reported by Cheesbrough (2002) [14].

Citrate Test

The bacterial isolates were tested for their ability to utilize citrate as the sole carbon source. Simmons citrate medium was used. Bacterial isolates were inoculated into simmons citrate medium in test tubes and incubated at 37°c for 24 - 48hours. The culture media was observed for a colour change from green to blue. Positive showed no growth with intense blue colour, while negative test showed no growth and the colour of the medium remained green (Bello, 2002) [15].

Triple Sugar Iron Agar Test (TSI)

Bacterial isolates were stabbed into TSI slant media and also streaked on the surface of the slant after which the medium was incubated at optimal temperature of 37°c for 24hours. The TSI slant medium was used to check for the present of the following;

GAS: If bubble is present in the media (gas positive)

H_2S: If black is present in the media (H2S positive)

LACTOSE: If the top of the media turn from pink to yellow (lactose positive)

GLUCOSE: If the bottom of the media turn from pink to yellow (glucose positive).

Catalase Test

This test detects the presence of catalase enzyme when present in a bacterium, it catalyse the breaking down of hydrogen peroxide (H_20_2) with the release of oxygen as bubble.

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

With a wire loop, a colony was packed from the pure culture and was transferred to the centre of a glass slide. 1-2 drops of 8% hydrogen peroxide was added to the Bacterial isolates.

Immediate production of bubble indicates positive result and if no bubble, indicates negative result.

Indole Test

This test demonstrates the ability of certain Bacteria to decompose the amino acid tryptophan to indole, which then accumulates in the medium for indole production. Bacterial isolates were inoculated into peptone water medium contained in sterile test tubes then incubated at 37°c for 48hours. After the incubation period, about 3 drops of kovac's indole reagent was added to the peptone water culture. The test tubes were shaken thoroughly and allowed to stand and observed for colour development. A red colour ring at the interface of the medium denotes a positive result. And if the isolate is negative, the reagent layer will remain yellow or slightly cloudy (Bello, 2002) [15].

Results and Discussion

Results

The following organisms were isolated from the pedestrian handrails of Delta state polytechnic, Ozoro; *Corynebacterium species, Proteus mirabilis, Streptococcus Suis, Enterobacter aerogenes, Enterococcus species, Candida albican, Aspergellus flavus, Mold species, and Penicillin species*. The Bacterial isolates have the ability to utilize sugar as their substrate as shown in Table 1. Table 1. Shows the morphological and biochemical characteristics of isolated Bacteria of the pedestrian hand rail. Table 2 shows Bacterial isolates identified in different pedestrian hand rail samples and heterotrophic plate count. Table 3 shows the mean colony forming unit (cfu/ml) count of Bacterial isolates from the various pedestrian hand rail samples. Table 4 Shows Bacterial isolates, number of occurrence and percentage of occurrence. Table 5 shows the morphological and microscopic characteristics of isolated Fungi of the pedestrian hand rails. Table 6 Shows Fungal isolates identified in different pedestrian hand rail samples and heterotrophic plate count. Table 7 shows the mean of colony forming unit (cfu/ml) count of Fungal isolates from various pedestrian hand rail samples. Table 8 Shows Fungal isolates, number of occurrence and percentage of occurrence.

Table 1: Cultural, morphological and biochemical characteristics of Bacterial isolates.

Gram stain	Morphol ogical	Cit	Ox	Cat	In	G1	Lat	H_2S	Gas	Organism
GPC	Cocci	+	-	+	-	+	+	-	-	*Streptococcus suis*
GPB	Rods	+	-	+	-	+	+	-	-	*Enterobacter aerogenes*
GPB	Rods	-	-	+	-	+	-	-	+	*Corynebacter species*
GPC	Cocci	+	-	+	-	+	+	-	-	*Enterococcus species*
GNB	Rods	+	-	+	+	+	-	-	+	*Proteus mirabilis*

Key

+ = positive
- = negative
GPB = Gram positive bacillus
GNB Gram negative bacillus
GPC = Gram positive bacillus
Cit Citrate test
Ox = Oxidase test
Cat = Catalase test
In = Indole test
Gl Glucose test
Lat = Lactose test
H_2S Hydrogen Sulphate

Table 2: The various pedestrian hand rail samples heterotrophic plate count

Sample	Colony forming unit(cfu/ml)
ERA	$2.16x10^2$
ELA	$1.2x\ 10^2$
MRA	$1.48\ x\ 0^2$
MLA	$2.12x\ 0^2$
ExRA	$1.44x0^2$
EXLA	$1.2x\ 10^2$
ERB	$1.88x10^2$
ELB	$L52x10^2$
MRB	$1.20x10^2$
MLB	$8.4x10^2$
ExRB	$1.2x10^2$
EXLB	$1.0x10^2$

Table 3: Mean of colony forming unit (cfu/ml) count of Bacteria isolates from various pedestrian hand rail samples

Sample	Mean of cfu/ml
A	160
B	127

Key: A = Morning samples
B = Afternoon samples

Table 4: Bacterial isolates, number of occurrence and percentage of occurrence

Bacterial isolates	Number of occurrence	Percentage of occurrence
Enterobacter aerogenes	2	16.67
Streptocuccussuis	4	33.33
Cotynebacterium species	3	25.00
Enterococcus species	1	8.33
Proteus mirabilis	2	16.67
	12	100

Table 5: Morphological and microscopic characteristics of fungal isolates

Morphological	Microscopic	Organism
The colony is circular about 4.0 -4.5cm in diameter, Colour is yellowish-green with age. Reverse is creamish-yellow.	Stipe is long, vesicle is dome-shaped. Metulae is small.Conidia is globose, rough and yellowish-green.	*Mold species*
Blue-green fluffy growth on plate.	Blue-green conidiospores borne in multi-link chains.	*Penicillin species*
The colony is circular about 4.0 -4.5cm in diameter, Colour is yellowish-green with age. Reverse is creamish-yellow.	Stipe is long, vesicle is dome-shaped. Metulae is small. Conidia is globose, rough and yellowish-green.	*Aspergellusfiavus*
The colony are creamy without profuse growth.	Hyphae and conidiospores are non-septate.	*Candida albican*

Table 6: Different pedestrian hand rail samples and its heterotrophic plate count

Sample	Colony forming unit(cfu/ml)
ERA	$1.2x10^1$
ELA	$2.4x10^1$
MRA	$2.4x10^1$
MLA	$1.7x10^1$
ExRA	2.0xl0[1]
EXLA	1.0xl0[1]
ERB	$3.2x10^1$
ELB	$3.4x10^1$
MRB	$1.8x10^1$
MLII	3.0xl0[1]
ExRB	2.lxl0[1]
EXLB	$1.2x10^1$

Table 7: Mean of cfu/ml count of fungal isolates from various pedestrian hand rail samples.

Sample	Mean of cfu/ml
A	17.83
B	24.5

Table 8: Fungal isolates, number of occurrence and percentage of occurrence

Fungal isolates	Number of occurrence	Percentage of occurrence
Candida albican	7	33.33
Mold species	1	4.76
Aspergellus flavus	11	52.38
Penicillin species	2	9.52
	21	100

Discussion

Hand rails are mostly found in public place and are commonly touched by hands. Hand rails are contaminated with microbes from human secretions as saliva, urine and skin origin and in turn these hand rails serve as vehicle for cross-infections and recontamination of washed hands (Monarca, *et al*., 2000) [16]. Moreover, majority of isolated Bacteria and Fungi in this research work are potentially pathogens and can be transferred from one person to another (Kennedy, *et al*., 2005) [17].

This research work shows that the level of contamination of pedestrian hand rails from the samples collected in the morning (A) is higher than from samples collected in the afternoon (B) with morning mean count of l60cfu/ml while afternoon is l27cfu/ml (Table 3). This study also shows that the level of Fungi contamination is less in the morning samples while the afternoon samples are high. Morning samples count is 17.83cfu/ml and afternoon samples count is 24.5cfu/ml (Table 7).

Hand rails contamination assessed in this study resulted in the isolation of mostly Gram-positive Bacteria and the Bacterial isolates from morning samples were three (*Corynebacterium species*, *Enterobacter aerogenes*, and *Streptococcus suis*) while from afternoon samples were five (*Proteus mirabilis*, *Enterococcus species*, *Corynebacterium species, Enterobacter aerogenes*, and *Streptococcus suis*) (Table 1). But for Fungal isolates from morning samples were four (*Candida albican*, *Mold species*, *Aspergellus flavus* and Penicillin species) (Table 5.)

The result of this research work is in line with Nworie, *et al*., (2012) [18]. that most of the Bacteria contaminants are coliforms. Also the result is in line with the research carried out by Sabra, (2013) [19] with similar organisms such as; *Proteus mirabilis* and *Enterococcus species* were isolated.

From Table 4; *Streptococcus suis* (33.33%) have the highest percentage of occurrence in morning samples while *Proteus mirabilis* (16.67%) have the highest percentage of occurrence in the afternoon samples. But *Streptococcus Suis* have the most number of occurrence in both morning and afternoon samples when sum together.

Also from Table 7; *Aspergellus flavus* have the highest percentage of occurrence (52.3 8%) in both morning and afternoon samples and also have the most number of occurrence in both morning and afternoon samples when sum together.

The presence of these pathogenic organisms re-occurring in this study has attributed to the fact that these organisms cause disease and infection to students and staff on campus. To better protect public health on campus, it is vital to highlight the need for, effective disinfection to minimize the hazard caused or to reduce Bacterial and Fungal contamination that may come in contact with the pedestrian hand rails on campus.

Conclusion and Recommendation

Conclusion

This study has revealed the presence of Bacterial isolates (*Corynebacterium species, Enterobacter aerogenes, Streptococcus suis, Proteus mirabilis and Enterococcus species*) and Fungal isolates (*Candida albican, Mold species, Aspergellus flavus and Penicillin species*) The presence of these pathogenic organisms re-occurring in this study has attributed to the fact that these organisms cause disease and infection to students and staff on campus, thus individual should maintain their oral health at a high level to avoid any of these somatic problems.

Recommendations

1. The hand rails just be cleaned with disinfectant at regular intervals
2. Students should avoid holding the hand rails when walking through the walk way
3. The use of hand rails made of a heavy metal such as; silver or copper reduce microbial load.
4. The use of self-disinfecting technology on the hand rails minimize the attachment of microbes or delay the development of bio flim.

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