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Isolation and Molecular Characterization of Mycobiota and Other Microbiota from Fingernails

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Abstract: This study was carried out to ascertain the hygienic practices among tertiary institutions study and also to isolate and identify microbiota from their thereby conduct molecular screening of fingernails for potential pathogens. A total of 300 nail samples carefully aseptically collected from 30 consented individuals include artificial and natural fingernails from both male and female students of The Oke-Ogun Polytechnic, Saki. The students' consent were sought for and other ethical issues were complied with as stipulated by the Institutional Ethical Committee. The fingernails were swabbed with sterile swab sticks and thereafter inoculated on the surface of suitably prepared media plates and sub-cultured to obtain pure isolates**.** Morphological and biochemical tests were carried out on the isolates to confirm the isolates. All students were found to harbour diverse microbes on their undernails. The microorganisms isolated include: *E.coli* 16.6%, *Staphylococcus aureus* 22.2%, *Streptococcus spp* 13.88%, *Bacillus spp* 25%, members of the *Acinetobacter* 2.77%, *Salmonella spp* 13.88%, Fungi 5.55%. The suspected Fungi isolates were sent for sequencing for further identification and characterization. The highest prevalence was found to be more in females than in their male counterparts, which could be as a result of unhygienic practices especially nail-fixing related of artificial nails because those with artificial nails keep longer nails than keeping natural nails. This result further confirmed that fingernails are a possible reservoir of several microbes and could be implicated in the spread of more virulent microorganisms.

Keywords: Molecular Screening, Virulent, Artificial Nails, Microbiota, Fingernails, Unhygienic-practices

1. Introduction

Un-hygienic practices among all ages have variously contributed to food borne diseases and are known to contribute to both human morbidity and mortality in addition as health care costs. Besides, outbreaks of food borne disease, health care costs related to these outbreaks are enormous [1]. The body surface is continually in touch with environmental microorganisms and become ready colonized by certain microbial species, gram- negative and gram-positive pathogens in clinical specimens. It will be a spread of community or hospital acquired infections, including those of the tract, tract, wounds and burns, bacteremia neonatal meningoencephalitis, emphysema and osteomyelitis. Various infections via hands and fingernails. Contaminations of hands play a significant role in faecal-oral transmission of diseases [2]. The unhygienic habits of most of the people result in the varied infections through

hands and fingernails. 80% of the diseases related to the poor domestic and private hygiene. One in every of the ways of healthy living is hand hygiene [3, 4]. Wachukwu et al. [4] find that artificial fingernails could function a way for transmission of pathogens to foods and causing nosocomial infections in patients. Hedderwick et al., [5] concluded in a very study that artificial fingernails were more likely to harbour pathogens, especially gram- negative bacilli and yeasts, than native nails. The hand is a significant vehicle of transmission of assorted microbes, as well as the enteric species [6]. Feculent contamination of hands is one among the vital route by that youngsters square measure exposed to morbid organisms [7]. Colleges and Universities square measure a perfect atmosphere for the unfold of infection and infectious diseases. Transmission of microorganism enteric infections via hands has vital consequences for college kids, as they are a lot of doubtless to require meal and water while not laundry hands, therefore, they are expose to risk of infection [8]. Hand washing culture has long been identified as a crucial practices in prevention [9]. Research on food borne epidemics have shown that poor hygienic practices by food vendors have been a contributing factor in disease transmission. According to earlier study conducted researches for Disease Control and Prevention (CDC), lack of proper hygiene of food vendors was responsible for more than 30% of outbreaks in the late eighties and early nineties [10]. The present study aimed at identification thereby further preventing the spread of diseases and infection through fingernails which are reservoirs for diverse microorganisms. It is generally expected that the target age group under study are the most studious where hygiene practices is expected hence the need for the present study.

2. Materials and Methods

2.1. Collection of Samples

A total of 300 swab from both hands samples were collected randomly from both hands of 30 students' volunteers belonging to volunteers in whom their consent had being sought for. The study was conducted at The Oke-Ogun Polytechnic, Saki using sterile cotton swab sticks robbed all over the undernails and transported immediately to the laboratory to prevent dryness and for further deterioration on the samples.

Preparation of Culture Media and Identification of Isolates

All media used were prepared according to each manufacturer's specification.

(i) Inoculation of Media

The already accustomed swab of the fingernails were patterned on the surface of ready media plates. The plates were then incubated at 37°C for twenty-four hours. The plates were discovered for growth and a colonial description of the isolates. Designated colonies were sub cultured on agar, such as Sabroaud glucose agar, and EMB agar to isolate pure culture. When analytic pure cultures, the isolates were additional known and defined by size, shape, and their reaction to Gram's stain. (ii) Isolation and Identification Methods.

All the samples were primarily plated onto nutrient agar medium being general purpose medium. Demographic detailed information was collected based on sex, length and types. Sex (male and female), length (short and long), types (artificial and natural) from each respondent. The plated cultures were incubated at 37°C and checked for bacteria in 18-24 hours while suspected selected fungi plates were allowed growth until 48 hours. Separate colonies were sub cultured to obtain pure culture. Morphological and cultural characteristics of colonies were based on Gram staining techniques and biochemical tests [11].

(ii) Identification of Bacteria and Fungi

Further colonial morphological studies were allowed to be sub-cultured onto other selective and differential agar media such as Eosin Methylene Blue agar (EMB), Sabroaud Dextrose agar, Simon's Citrate Agar, Salmonella Shigella agar, MRS and incubated at 37°C for 24 hours while Sabroaud Dextrose agar waited for 48-72 hours. Morphological and biochemical properties of the isolates were identified, evaluated and compared according to [12]. Several biochemical identification methods such as Gram stain, indole test, oxidase test, catalase test were conducted for identification of the isolate [13].

2.2. Gram Staining Techniques

Prepare a clean smear from the pure culture of the organism which is less than 24 hours old and pass it through the flame in two times to heat fix the smear. To the already prepared thin smear, add few drops of crystal violet for 1 minutes before it is rinsed with water. The smear was flooded with Lugol's Iodine for 30 seconds and rinsed with water. A few drop of safranin stain was added after it was decolourized with 70% alcohol. The smears were placed respectively on a microscope and observed under oil immersion objective lens.

2.3. Oxidase Test

A piece of filter paper was soaked with few drops of oxidase reagent. Sterile inoculating loop was used to pick a colony of the test organism and smeared on the filter paper. If the organism is oxidase producing, the Phenylenediamine in the reagent will be oxidized to a deep purple colour.

2.4. Molecular Analysis Technique

2.4.1. Fungal/Bacteria DNA Extraction

Two milliters (2mls) of suspected plant life cells broth was more to a metallic element BashingTM lysis Tube. 750µl lysis resolution was more to the tube. It absolutely was secure in a very bead fitted with 2ml tube holder assembly and was processed at most speed for >5 minutes. The metallic element bashing BeadTM Lysis Tube was centrifuged in a very microcentrifuge at >10,000 x g for one minutes. It absolutely was transferred up to 400µl

supernatant to a Zymo-spin Tm IV Spin filter (orange top) in a very assortment tube and was centrifuged at 7, 000 x g for 1 minute. 1,200µl of fungal/bacterial deoxyribonucleic acid binding buffer was more to the filtrate within the assortment tube from step four. 800µl of the mixture from step five was transferred to a Zymo-spin Tm IIC column in a very assortment tube and was centrifuged at 10, 000 x g for one minute. The flow through from the gathering tube was discarded and the step was repeated. 200µl deoxyribonucleic acid pre- wash buffer was more to the Zymo-spin Tm IIC Column in new assortment tube and was centrifuged at 10, 000 x g for one minute. 500µl fungal/bacterial deoxyribonucleic acid wash buffer was more to the Zymo-spin Tm IIC column and centrifuged at 10, 000 x g for one minute. The Zymo-spin Tm IIC column was transferred to a clean 1.5ml microcentrifuge tube and 100µl (35µl minimum) deoxyribonucleic acid extraction buffer was more on to the column matrix. This was absolutely was centrifuged at 10,000 x g for thirty seconds to elute the DNA.

2.4.2. Electrophoresis for DNA and PCR

One gram (1g) of agarose (for DNA) and 2g of agarose for PCR was measured. Agarose powder with 100mL 1 TAE was mixed in a microwavable flask. It was microwaved for 1-3 minutes until the agarose is completely dissolved. Agarose solution is allowed to cool down to ab for 5 minutes. 10µl EZ vision DNA stain was added. EZ vision binds to the DNA and it allows to visualized the DNA under ultraviolet (UV) light. The agarose was poured into a gel tray with the well comb in place, newly poured gel was placed at 4°C for 10-15 minutes or allowed to sit at room temperature for 20-30 minutes until it has completely solidified.

2.4.3. Loading Samples and Running Agarose Gel

Loading buffer is added to each of the DNA samples or PCR products. When it was solidified, the agarose gel was placed into the gel box (electrophoresis unit). The gel box was filled with 1 TAE (or TBE) until the gel is covered. A molecular weight ladder is carefully load into the first lane of the gel. The samples was carefully load into the additional wells of the gel. The gel was run at 80-150V for about1-1.5 hours. The power was turned OFF, the electrode was disconnected from the power source, and the gel was carefully removed from the gel box. The DNA fragments or PCR product was visualize under UV trans illuminator.

2.4.4. PCR Mix Components

The PCR cocktail contains12.5ul of Taq 2X as has been conditioned from England Biolabs (m0270); Also, the kit contain 1µl each of 10µlM forward and reverse primers; 2µl of DNA template and then made up with 8.5ul nuclease free water.

Primer Sequences for Bacterial identification

Forward primer sequences: AGAGTTTGATCMTGGCTCAG

Reversed primer sequences: AAGGAGGTGWTCCARCCGCA

2.4.5. Cycling Conditions

Initial denaturation at 94°C for 5 minutes, followed by 36 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds and elongation at 72°C for 45 second. Followed by a final elongation step at 72°C for 7 minutes and hold temperature at 10°C forever. The lids are replaced and after solidification. The agar plates are incubated 30°C for 24-48 hours in bacterial, and fungal colony forms counter and expressed as cfu × 106/g. Data represented in the table.

2.5. Statistical Analysis

The statistical analysis was performed by using SPSS version for the analysis of data collected.

2.6. Ethical Considerations

The confidentiality of data collected was kept very intact and other ethical issues were complied with as stipulated by the Institutional Ethical Committee.

3. Results

Microbial contamination of undernails has become a global health problem thus a total of 30 under nails swabs from the undernails in left and right hands of 30 students of The Oke-Ogun Polytechnic, Saki were collected. All students were found to harbour bacteria on their undernails. Bacterial pathogens isolated from the under nails of students include both gram positive and gram negative bacteria. Gram-positive isolates were *Staphylococcus aureus, Bacillus spp, Streptococcus* while the gram-negative bacteria include *E.coli, Acinetobacter spp, Salmonella spp.*

Table 1 shows the percentage of probable organisms isolated from the samples collected from undernails after calculating the percentage of each isolate. It was observed that the prevalence of *E.coli* 16.6%, *Staphylococcus aureus* 22.22%, *Streptococcus spp* 13.88%, *Bacillus spp* 25%, *Acinetobacter spp* 2.77%, *Salmonella spp* 13.88%, Fungi 5.55% are a greater concern for health. These colonies isolates were identified using biochemical tests, colony morphology and staining techniques with their reaction to Gram stain which categorized them into gram positive and gram negative respectively. Table 2 shows the morphological and biochemical characteristics of probable isolates under the finger nails in which *E.coli* was gram negative, having a rod like shape and biochemical test testing indole positive (+), oxidase (-) and catalase positive (+). *Staphylococcus aureus* was gram positive having a cocci shape and its biochemical tests testing indole negative, oxidase positive, catalase positive. *Staphylococcus* was gram positive having a cocci shape and its biochemical test having indole negative, oxidase negative and catalase negative. *Bacillus spp* was gram negative having a bacilli shape and its biochemical test testing indole negative, oxidase negative and catalase positive. Opportunistic pathogens such as bacteria, viruses and fungi can survive on inanimate surfaces for long periods and items such as watches, pens and mobile phones are

permanent surfaces of transmission of this type of infections [13]. Table 3 showed the isolates result from under nails of female and male and it was found out that the highest prevalence was found in female. Table 4 also shows the percentage of the isolates from under the fingernails. The results showed that high contaminations were found in females than in males, which could be as a result of artificial nails being fixed by female and keeping of long nails because artificial and long nails harbour more microorganisms than short nails. Table 5 shows the growth of fingernails samples on media. Long nails were found to have much more growth on media because long nails are prone to harboring pathogens. Short nails was found to have medium growth which could be as a result of unhygienic practices such as improper washing of hands after visiting the toilet, taking care of household pets which could lead to the contamination of the fingernails.

Table 1. *Morphological and Biochemical profiles of isolates from under nails samples.*

Gram stain	Shape	Indole test	Oxidase test	Catalase test	Probable organism
-	Rod	+	-	+	*E.coli*
+	Cocci	-	+	+	*S. aureus*
+	Cocci	-	-	-	*Streptococcus*
+	Bacilli	-	-	+	*Bacillus spp*
-	Bacilli	-	-	+	*Acinetobacter*
-	Rod	-	-	+	*Salmonella*

Keywords: + Reaction, - No reaction.

Table 2. *Severity of Growth of fingernails samples on Culture Media.*

Types of nails	Growth on media
Long nails	+++
Short nails	++
Artificial nails	++++

Table 3. *Isolates from undernails of participants per gender.*

Microbiota isolated	Females n (%)	Males n (%)
E.coli	10 (16.16)	6 (15.00)
S. aureus	12 (19.12)	8 (20.00)
Streptococcus	10 (16.12)	4 (10.00)
Bacillus spp	14 (22.58)	10 (25.00)
Salmonella spp	8 (12.90)	6 (15.00)
Acinetobacter	4 (6.45)	2 (10.00)
Fungi	4 (6.45)	4 (5.00)
Total	62 (60.8)	40 (39.2)

Table 4. *Percentage of isolates from undernails samples.*

Isolates	Number	Percentage (%)
E.coli	12	16.66
S. aureus	16	22.22
Streptococcus spp	10	13.88
Bacillus spp	18	25.00
Acinetobacter spp	2	2.77
Salmonella spp	10	13.88
Fungi	4	5.55
Total	72	100

Table 5. *Prevalence of isolates from female and male respondents.*

Isolates	Female %	Male %
E.coli	16.12	15.00
S. aureus	19.35	20.00
Streptococcus	16.12	10.00
Bacillus spp	22.58	25.00
Salmonella spp	12.90	15.00
Fungi	6.45	10.00
Acinetobacter	6.45	5.00

Accession Number: EU645733

Aspergillus aculeatus Strain JO6

Isolate Molecular Sequences

GTTCAAAGCTTCATCCTCGATTTATGGATGGCCATC
TGGTGACGAGTTTTGCCCCGGCCGGGCCTTAGAGCG
GGTGACAGAGCCCCATACGCTCGAGGACCGGACGG
T

GCCGCCGTTTCTCTCGAGGCCCGCCCCCGGGAGG
CGCGGCGAGCAACCAGCGG

GCCTGGAGGGGAGAAATGACGCTCGGACAGGCAT
GCCCCCCGGAATACCAGGG

GGCGCAATGTGCGTTCAAAGACTAAATGATTCACT
GAATTCTGCAATTCACATTA

ATTATCGCATTTCGCTGCGTTCTTCATCGATGCCGG
AACCAAGAGATCCATTGTG

GAAAGTTTTGACTGATTGATATCAATCGACTCAGA
CTGCACGCTTTCAGACAGTG

TTCCATTGGGGTCTCAGGCGGGCGCGGTCCCGGG
GGCAGGCCCCGGGCCGCCCG

CCCCCGAAAGGAACCAGCACTCGGTAATGATCCTT
CCGCAGGTTCACCTTACGGA

AACCTTTTTACACTTTTATTTCAAT

4. Discussion

Artificial nails were determined to harbour extra microbes, having numerous increase on media due to the fact fingernail duration and texture the performance of microbial elimination from underneath the nails. Long and polished nails usually harbour extra microbe even after hand washing hence, carrying artificial nails may be an issue influencing the efficacy of hand washing because synthetic nails commonly polished are commonly longer than natural nails. Thus, synthetic nails serves as a reservoir of microorganisms and are extra at risk of get contaminated than natural nails. Lau et al., opined that lengthy nails tend to harbor greater microorganisms than brief nails. Visibly smooth nails were located merely by using look of fingernails of students, showed presence of 62% bacterial infection on their palms. Ray et al., confirmed a lower in colony depend following hand washing with cleaning soap in 60% of the samples. Tambekar et al., Also discovered highest bacterial infection (70%) was discovered in the palms of the kingdergaten students followed via 67% at the fingers of number one students, 66% on secondary college students, and 64% on Post-graduate students and at the least 57% at the hands of undergraduate students. Ray et al., observed that hand swab samples of 61% children harbour capacity pathogens before

taking food, also mentioned presence of pathogenic microbes on the fingers of the scholars which consist of *S. Aureus, E. coli, Pseudomonas spp, Klebsiella spp*. This work was similar to the work by Akinwumi and others isolated pathogenic bacteria from their study on cell phones [14] and [15, 16], showed the presence of *E. coli, Pseudomonas spp, Salmonella spp, Enterobacter spp and S. aureus* from the hand swab samples of students. The presence of gram negative bacteria in fingernails may also explain contamination of faecal material. The frequency of organisms isolated was extra in artificial nails than herbal nails and will harbour diverse microorganisms. Four fungi isolates were isolated from undernails of the scholars and morphological and biochemical tests were executed on the isolates wherein one was tough to confirm. The isolate was dispatched for sequencing for further identity and characterization. The presence of *E. coli* may explain contamination of faecal material, which may be because of unhygienic practices, or even fallacious washing of palms after going to the toilet, managing domestic animals together with goats, dogs, ducks, fowls that are vulnerable to motive sickness and infection. The detection of *S. aureus* from fingernails of college students may also pose considerable health threat on college students as those organisms are able to generating enterotoxins that causes food poisoning, which can cause the malfunctioning of the body gadget and may even lead to death. The detection of *Salmonella* from fingernails could be an outright possible indication of food-borne infections among the Polytechnic students as result of their unkempt habit of purchasing and meal sharing without proper hand washing hygiene.

5. Conclusion

Fingernails remain one of the channel of food contamination and it serves as probable reservoir for pathogenic organisms in this study especially the females who prepare meals for both domestic and commercial purpose

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