Identification of Bacteria from under Finger Nails

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ABSTRACT

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This study was conducted to isolate and identify bacteria from human finger nails. A total of three nail samples were collected. The samples were collected from random people in which two of them were from females and one was from male. The isolated pathogens from finger nails include *Bacillus* species (2 isolates), coccus species (one isolate). Highest contamination of *Bacillus* species was isolated. After the colonies were being isolated, they were further characterized on the basis of biochemical characteristics including Indole test, Citrate test, Nitrate reduction test and Urease test. After that Antimicrobial susceptibility tests were performed to identify the resistance of a particular bacteria towards a given antibiotic i.e. Ampicillin, Chloromphenicol, Norfloxacin, Co-Trimoxazol and Ciprofloxacin. However the results obtained showed the resistance of isolates towards Ampicillin indicating the prevalence of potentially disease causing microbes under fingernails. This study showed the importance of nail hygiene.

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I. INTRODUCTION

Microorganisms are widely distributed all over the world be it air, water, soil and even human body. Human body is said to be the shelter of millions of bacteria, viruses, fungi, and other many other invisible organisms. These organisms are collectively called as microbes. The microbes belong to different communities and together called as micro biome. The human micro biome is a source of various genetic diversity and no two human micro biomes can be absolutely same. Different microbes reside on different places of human body and they are adapted

to the conditions in which they live. These microorganisms play an important role in maintaining the human health. (Kumar *et al.*, 2017).

The hands of the human body are in most contact with the outer world. The human hands are located at the end of each arm. Normally, a human has five fingers on each hand which includes: a thumb, index finger, middle finger and little finger (Baran *et al.*, 2002). People use their hands for multiple purposes every day. Therefore, it is very easy to come in contact with different microbes and to transfer them to objects and even to people. Surprisingly, fingernails

are the home for most of the bacteria found on human hands (Wachukwue *et al.*, 2017).

Fingertips are the areas of the human skin that contains the highest concentration of receptors. These areas of the nerve endings make the fingers very sensitive to heat, cold, moisture, vibration, pressure and various other stimuli (Wootton *et al.*, 2007). Fingernails are attached to the distal end of each finger. A nail is tough envelope like covering the terminal phalanges of fingers and toes in human. (Wang *et al.*, 2016).

The area under the fingernail is difficult to clean and therefore there resides the most pathogenic organisms. Activities which can increase the risk of bacterial nail infection include: having constantly wet hands, severe nail biting, eczema around the fingernails. It is seen that the fingernails are more sensitive to accumulate different kinds of bacterial pathogens due to constant change in the environment of the host and its surrounding. (Larson *et al.*, 2003).

The study under this research shows the importance of Nail hygiene as there is prevalence of pathogenic bacteria under the nails which may lead to numerous health issues.

II. MATERIAL AND METHODS

Collection of samples

This study was conducted between august to September, 2019, at Patna women's college.

Three nail samples were collected from people of different ages in profession randomly. Normal saline (0.9%) was prepared and sterilized in autoclave for 15 minutes at 15 psi, 121 °C. The swab sticks were dipped in normal saline and sterilized. The collected samples were rubbed all over the area under the nails from sterile swab sticks. The swab sticks were then

dissolved in 10 ml of normal saline mixed thoroughly in a sterilized test tube. The nutrient agar media (Aneja., 2017) was prepared in a flask and sterilized in autoclave. The prepared media was cooled (not solidified) after being sterilized. The second and third dilution was used for isolation of bacteria for all the 3 samples and plated on nutrient agar plates. The plates were incubated at 37 °C for 24 hours (Mengist *et al.*, 2018)

Isolation

After incubation for hours, the plates were checked for their growth of bacterial colonies. Then continuous or quadrant streaking was done with the loop near the flame. The Petri plates was sealed and incubated for 24 hours at 37 °C. After 24 hours the plates were observed for their growth and pure culture was obtained by streaking (Risan *et al.*, 2017).

Identification

Further to this Gram staining was done to identify the morphological characteristics whether it is gram positive or gram negative, the shape etc. The slide was observed in bright field microscope at 100x (oil immersion technique) and at 10x (Cohen *et al.*, 1992).

Biochemical tests

Biochemical tests are based on the ability of the given microbes to utilize a particular chemical and respond accordingly. Structural differences are not enough to identify bacteria, as many species of the same genus can have similar shapes too. Hence, biochemical tests are used for the identification based on different activities with different biochemical compounds. Following tests were performed to identify the samples:-

Indole test

This test is performed to check the ability of the bacterial samples to convert tryptophan into indole by an enzyme tryptophanase. The appearance of a cherry red colour indicates positive result. A negative result is indicated by no change in the broth after addition of Kovac's reagent (Michael *et al.*, 1986)

Citrate test

It is based on the ability of an organism to use citrate as a source of carbon and energy. Simmon's citrate agar media was used for this test. The bacterial colonies are inoculated and incubated at 37 °C for 24hrs. The positive result shows the appearance of blue colour and negative test shows no change in the colour of the media (Clarke *et al.* 1952).

Nitrate test

This test determines the production of an enzyme nitrate reductase which reduces nitrate into nitrite. For this test a heavy inoculum is inoculated into a nitrate broth, and is incubated for 24hrs at 37°C. Appearance of cherry red colour on addition of reagent A and B indicates positive result. No change in the colour indicates negative result to this test (Aneja *et al.*, 2018).

Urease test

This test is responded by a certain group of microorganisms which metabolize urea present in the media and convert them into ammonia and carbon dioxide in the presence of urease enzyme. During incubation the organism utilizing urea by urease enzyme which ultimately raise the pH of the media which is detected by the change in colour of the media from yellow to red or deep pink. Urea must not be sterilized in an autoclave as it is unstable and

breaks down at 15psi steam pressure. (+) ve result is indicated by change in color of the media from yellow to red whereas (-) ve test is indicated by no change in colour (Aneja *et al.*, 2018).

Antimicrobial susceptibility test

This test is performed by using simple disk diffusion method for both gram positive and gram negative bacteria. This method employs the phenotypic identification of susceptibility and therefore requires the following process.

The cultures were isolated carefully to prepare an inoculum. The inoculated test tubes were then incubated at 37°C for 24 hrs to obtain a moderate turbidity. A sterile cotton swab was dipped into the broth and rotated firmly on the upper inside walls of the test tube. The inoculums were swabbed uniformly on NA plated under sterile condition. The inoculum was allowed to dry for and the antibiotic discs were impregnated on the surface of the plates using sterile forceps. The plates were then incubated at 37 °C for 24 hrs. The zones of inhibition were measured in millimeters (Risan *et al.*, 2017; Aneja *et al.*, 2017).

The antibiotic disc used for checking the susceptibility of the samples were, chloramphenicol (30mcg), Ampicillin (10mcg), Norfloxacin (10mcg), Co-Trimoxazole (25mcg), Ciprofloxacin (5mcg).

III. RESULTS

Cultural characteristics

15 to 30 bacterial colonies were observed on second dilution plated media and 6 to 9 colonies on third dilution plated media. The bacterial samples were collected from nails of various age groups like sample 1 was from a female of 38 years suffering from fungal infection in her nails, sample 2 was of a 17 year old

boy and sample 3 was of a 12 year old girl. Isolates of Sample 1 showed the most abundant growth on nutrient agar media. Sample 2 and sample 3 isolates showed moderate growth. Sample 1 culture was purified by streaking twice to get a pure colony due to heavy growth.

Table 1- cultural characteristics of the selected isolates

Isolates	Colour	texture	Form	elevation	margin
Isolate 1	White	Dry	irregular	umbonate	wavy
Isolate 2	White	rough	irregular	umbonate	wavy
Isolate 3	Off white	Shiny	punctiform	Slightly raised	entire

Morphological characteristics

The following results were obtained after Gram's staining of the selected isolates as shown in the table 2.

Table 2- Gram's stain characterization of the selected isolates

Isolates	Gram's stain result
Isolate 1	Positive
Isolate 2	Positive
Isolate 3	Negative

Biochemical tests :

The isolate 1 and 2 showed orange colour in the top layer of the tube. This is a variable colour which is formed due to the presence of skatole, also called methylated indole which is another possible product of indole degradation. Isolate 3 showed positive red colour in the surface of alcohol layer of broth .

The second biochemical test done was citrate utilization test. Isolate 1 and 2 were slightly positive as it was not totally blue while sample was totally positive as it showed fully blue colour slant. The isolate 3 showed no change in colour of the slant and

remained green in colour, therefore it gave citrate negative test.

The third test done was nitrate reduction test which is based on the detection of nitrite and its ability to form red compound when it reacts with sulfanilic acid to form a complex nitrite sulfanilic acid which further reacts to give alpha naphthylamine to give prontosil, a red precipitate which is water soluble which was given by isolate 1, 2 and 3 all detecting positive tests for nitrate reduction.

Fourth test followed was urease test . All the three isolates gave a negative test for urease which shows

that bacteria is lacking in production of urease. The Gram's stain result however for confirmation 16s bacterial species were identified on the basis of rRNA sequencing is required.

Table 3- biochemical characterization of the selected isolates

Biochemical test/isolate	<i>Bacillus spp.</i>	<i>Bacillus spp.</i>	<i>Escherichia spp.</i>
Indole test	Negative	Negative	Positive
Citrate test	Slightly positive	Slightly positive	Negative
Nitrate reduction test	Positive	Positive	Positive
Urease test	Negative	Negative	Negative

Antimicrobial susceptibility tests

The antimicrobial susceptibility tests were performed by disk diffusion agar method to check the resistance to various antimicrobial compounds which are further useful in treatment of diseases. The presence or the absence of growth in the area surrounding the disk indirectly indicates the ability of the microorganism to inhibit the organism. Therefore 5 antimicrobial compound disks were used which are as follows: ampicillin, chloromphenicol, norfloxacin, Co-trimoxazol and ciproflaxin (Mengist *et al.*, 2018). The zone formation by all these compounds is depicted in the table in the next page. The zone measure was taken in millimeters. The results were

calculated based on standards .both the samples were sensitive to all the antimicrobial compounds except ampicillin. Since isolate 1 and 2 had 11.1 and 11 mm as diameter which was less than 13, therefore it indicated to be resistant. Isolate 3 had no zone formation so it proved to be sensitive. In case of chloromphenicol the diameters were far more than 18 mm which shows that the bacterium was susceptible (Hedderwick *et al.*, 2007). The samples were susceptible to Norfloxacin as their diameters were greater than 17 mm. The samples were susceptible to Co-trimoxazol and ciproflaxin as well as their diameters were far greater than standardized (Risan *et al.*, 2017).Also the results so obtained were compared with CLSI standard.

Table 4- Antimicrobial susceptibility test results for *Bacillus spp.*, *Bacillus spp.*, *Escherichia spp.* respectively

Antibiotic	Disc amount (microgram)	Zone of resistance (mm)	Zone of intermediate (mm)	Zone of susceptible (mm)	Zone diameter formed (mm)	Inference
Ampicillin (AM)	10	< or = 13	14-16	>or = 17	11.1	Resistant
	10	< or = 13	14-16	>or = 17	11	Resistant
	10	< or = 13	14-16	>or = 17	0	Resistant
Norfloxacin (NF)	10	< or = 12	13-16	>or =17	30	Susceptible
	10	< or = 12	13-16	>or = 17	30	Susceptible

	10	< or = 12	13-16	>or = 17	27	Susceptible
Chloramphenicol (C)	10	< or = 12	13-17	>or= 18	23	Susceptible
	10	< or = 12	13-17	>or = 18	22.8	Susceptible
	10	< or = 12	13-17	>or = 18	26	Susceptible
Ciprofloxacin (CIP-5)	5	< or = 15	16-20	>or= 21	39	Susceptible
	5	< or = 15	16-20	>or= 21	39	Susceptible
	5	< or = 15	16-20	>or= 21	29	Susceptible
Co-Trimoxazole (TM)	25	< or = 10	11-15	>or = 16	26.9	Susceptible
	25	< or = 10	11-15	>or = 16	27	Susceptible
	25	< or = 10	11-15	>or = 16	29	Susceptible

IV.DISCUSSION

The bacterial pathogen found under the human nails are found to be resistant to many antibiotics. Nails are considered to be the home of many bacteria and these pathogens can be transmitted to healthy individuals and can even worsen a patient existing medical condition. The pathogenicity and virulence of these bacteria is associated with its capacity to produce several virulence factors. Antibiotic susceptibility test was performed via the disk diffusion agar method tests in order to determine which antimicrobials will inhibit the growth of the bacteria causing a specific function. The gram-negative disc and gram-positive disc were used against the bacterial isolates according to the gram reaction. Plates are incubated for 24 hours at 37° C. The antibiotics used in this study includes ampicillin, chloramphenicol, norfloxacin, co-trimoxazole, ciprofloxacin. The result of the antibiotic susceptibility test of the isolates showed that the gram-negative species is found to be 80% susceptible to all the antibiotics used whereas no zone of inhibition is seen where the ampicillin disc is used, this showed that the species is resistant to ampicillin. Trimoxazole as well as ciprofloxacin showed the high inhibitory activity and is effective. The gram-positive species are 80% susceptible to the antibiotics used whereas ampicillin was resistant. Ciprofloxacin showed the highest inhibitory action on the gram-positive isolates and is very effective. These

information are used to select an appropriate antibiotics to fight a specific infection. The result obtained was in accordance with Risan et al., 2017 however the result differed with Mengist et al., 2018. This study conducted showed that the fingernails contains a lot of pathogenic bacteria and the unhygienic nails can serve as a medium for transmitting many harmful bacteria to healthy individuals (Prescott et al., 2005). Nail hygiene is becoming an important aspect in our day to day lives. Nail hygiene etiquettes are a major factor which are taken into consideration by the professional medical practitioner as untidy nails can worsen a patient's existing medical conditions. It is extremely important to keep the nails and the area under the nails clean because surprisingly, fingernails are the home of the many bacterial pathogen. However, it is difficult to clean to area under the fingernails as compared to the other body parts. Fingernails are more susceptible to the constant changing environment of the host and its environment (Brannon et al., 2008).

This study conducted showed the importance of nail hygiene as many bacteria resides on our nails. Chewing on nails directly puts those harmful bacteria in our mouth. These bacteria are also responsible for many of the disease caused in humans (Wootton et al., 2019). Transmission of these bacteria occurs through food, water and even fingers. The food handlers accumulate many such bacterial pathogen and

contaminate food by their fingers and then to food preparation and serving. Thus, infecting healthy persons. *Bacillus* spp. bacterium is a common cause of most of the bacterial infection of the nail (Hunter et al., 2002).

V. CONCLUSION

This study was conducted to isolate and identify bacteria from human finger nails. The isolated pathogens from finger nails included *Bacillus* species (2 isolates), coccus species (one isolate). Highest contamination of *Bacillus* species was isolated. After isolation further characterization on the basis of biochemical characteristics including Indole test, Citrate test, Nitrate reduction test and Urease test; based on these tests and ABIS results possible microorganisms indicating isolate one and two can be *Bacillus licheniformis* (similarity 94.1%), *Bacillus cereus* (similarity 94.0%), *Bacillus thuringiensis* (similarity 94.1%) or *Bacillus mycoides* (similarity 92.3%) where as isolate three can be *Escherichia coli* (similarity 99%), *Escherichia fergusonii* (similarity 98%). After that Antimicrobial susceptibility tests were performed to identify the resistance of a particular bacteria towards a given antibiotic based on CLSI standards i.e. Ampicillin, Chloromphenicol, Norfloxacin, Co-Trimoxazol and Ciprofloxacin which showed that isolate one and two were susceptible to 4 antibiotics and resistant to one. The resistant group as indicated by ABIS online was beta-lactams where as susceptible groups indicated were quinolones and phenicols for isolate one and two. Hence the results obtained showed the resistance of isolates towards Ampicillin indicating the prevalence of potentially disease causing microbes under fingernails. This study showed the importance of nail hygiene as pathogenic microorganisms prevail under our nails which is needs our attention for healthy life.

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