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Isolation and Identification of Bacteria from under Fingernails

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A B S T R A C T

This study was conducted to isolate and identify bacteria contaminants of under nails (long nails). The samples were collected randomly from 20 samples under nails (belonging to volunteers for sampling). This study was conducted between October to December, 2016 at College of Biotechnology, Al-Nahrain University. The isolated colonies were then sub cultured in nutrient agar and slants in order to obtain pure culture of all colonies. All students (100%) found to harbour bacteria on their under nails. Bacterial pathogens isolated from the under nails of students include *Staphylococcus aureus* (5 isolates), *Bacillus cereus* (2 isolates), *Acinetobacter* spp (1 isolates), *Bacillus* spp (7 isolates), *Streptococcus* spp (1 isolates), *Pseudomonas aeruginosa* (6 isolates), *Klebsiella* spp (2 isolates) and Unknown (2 isolates). Highest contamination of *Bacillus* spp was isolated. Showed Percentage of bacterial isolates from the samples collected from under nails after calculating the total percentage of each isolate *Staphylococcus aureus* 19.23%, *Bacillus cereus* 7.69%, *Acinetobacter* spp 3.84%, *Bacillus* spp 26.92%, *Streptococcus* spp 3.84%, *Pseudomonas aeruginosa* 23.07%, *Klebsiella* spp 7.69% and Unknown 7.69%. The highest prevalence were in male were (16 isolates) and were percentage of bacteria isolated 61.53% while in female were (10 isolates) and percentage of bacteria isolated 38.46%. Also showed results Percentage of total bacteria isolated of female and male, were 38.46% and 61.53 % respectively.

Introduction

Foodborne diseases are known to contribute to both human morbidity and mortality as well as health care costs. Besides outbreaks of foodborne disease, health care costs associated with these outbreaks are enormous (Bean *et al.*, 1996; Campbell *et al.*, 1998). The human body surface is constantly in contact with environmental microorganisms and become readily colonized by certain microbial species, Gram-negative and Gram-positive pathogens in clinical specimens. It can cause a variety of community- or hospital-acquired infections, including those of the urinary tract, respiratory tract, wounds and burns, bacteriaemia, neonatal meningoencephalitis, empyema and osteomyelitis. The hand serves as a major vehicle of transmission of various microbes, including the enteric species (Prescott *et al.*, 2005). Various infections via hands and fingernails. Contaminated of hands play a major role in faecal-oral transmission of diseases (Ray *et al.*, 2011). The unhygienic habits of most of the people lead to the various infections via hands and fingernails. 80% of the diseases are associated with the poor domestic and personal hygiene. One of the ways of healthy living is hand hygiene (Patel *et al.*, 2010). Faecal contamination of hands is one of the important route by which

children are exposed to pathogenic organisms (Langford, 2009). Wachukwu *et al.*, (2007). Finding that artificial fingernails could serve as means for transmission of pathogens to foods and causing nosocomial infections in patients. Four genera of bacteria were isolated and identified, such as *Staphylococcus* sp., *Escherichia coli*, *Proteus* sp. and *Pseudomonas* sp. Among the organism identified, *Staphylococcus aureus* (41.7%) was predominant and frequently occurring, followed by *E. colI* (7.4%). Hedderwick *et al.*, (2000) Concluded in a study that artificial fingernails were more likely to harbor pathogens, especially gram-negative bacilli and yeasts, than native nails. Schools and Universities are an ideal environment for the spread of infection and infectious diseases. Transmission of bacterial enteric infections via hands has important consequences for students, as they are more likely to take meal and water without washing hands; therefore they are posed to risk of infection. (Lau *et al.*, 2012). This study was conducted to isolate and identify bacteria, contaminants on under the nails (long nails).

Materials and Methods

Collection of samples

A total of 20 swab samples were collected randomly from 20 samples under nails (belonging to volunteers for sampling). This study was conducted between October to December, 2016 at College of Biotechnology, Al-Nahrain University, with sterile cotton swab sticks. Sampling was done using sterile cotton swab sticks. The swab sticks were rubbed all over the surface of under nails. The cotton swabs were transferred immediately to the laboratory with one hour of collection to prevent dryness. The nutrient agar was prepared in 500ml flask and was sterilized by autoclaving at 121^{o}C at 15 psi for 20 minutes. 20 ml of the media was poured in the petri plates before getting solidified. The swab was immediately streaked on three plates of Nutrient agar. The plates were incubated at 37°C for 24 hours. The plates were then observed for growth and a colonial description of the isolates. Selected colonies were again sub- cultured on nutrient agar in petri- plates to isolate pure culture. After isolating pure cultures, bacterial isolates were further identified and characterized by size and shape and gram staining of colonies.

Isolation and Identification Methods

Cultural Methods

The nutrient agar medium was used in the study for isolation of the bacteria. The detail information was collected based on age and sex during collection under the nails (long nails) samples. The cultures were incubated at 37C° and checked for bacterial growth at 24 hours. Separate colonies were subcultured onto nutrient agar to obtain pure culture. Morphological Characteristics of colonies and gram stain tests and biochemical tests were, according (Ramos, 2004; Ekrakene and Igeleke 2009) that used for bacterial identification.

Identification of bacteria

Subculture was done from each plate on Nutrient Agar and incubated at 37°C for 24 hours. Morphological and biochemical properties of the isolate were identified, evaluated, and compared, according (Ramos, 2004; Ekrakene and Igeleke, 2009).

Several biochemical identification methods such as shape, Gram stain, Indole Test, Oxidase test, Catalase test, Citrate Utilization Test, Urease Test, Coagulase Test and Nitrate reduction test were conducted to identify the isolated bacteria (Holding and Collee, 1971; Abdulkadir and Waliyu, 2012).

Results and Discussion

Microbial contamination of the under nails has become a global health problem. Thus a total of 20 under nails swabs from under the nails, in left and right hands of 20 students were collected.

All students (100%) found to harbour bacteria on their under nails. Bacterial pathogens isolated from the under nails of students. Gram-positive bacteria isolates were *Staphylococcus aureus* (5 isolates), *Bacillus cereus* (2 isolates), *Bacillus* spp (7 isolates) and *Streptococcus* spp (1 isolates) while Gram negative bacteria were isolated from under nails, *Acinetobacter* spp (1 isolates), *Pseudomonas aeruginosa* (6 isolates), *Klebsiella* spp (2 isolates) and Unknow (2 isolates). Highest contamination of *Bacillus* spp was isolated. (Table 1).

Showed Percentage of bacterial isolates from the samples collected from under nails after calculating the total percentage of each isolate *Staphylococcus aureus* 19.23%, *Bacillus cereus* 7.69%, *Acinetobacter* spp 3.84%, *Bacillus* spp 26.92%, *Streptococcus* spp 3.84%, *Pseudomonas aeruginosa* 23.07%, *Klebsiella* spp 7.69% and Unknow 7.69% (Figure 1) as the number and types of bacteria associated with the hands are of greater concern for health.

Seven colonies isolated and identified using biochemical tests, colony morphology, and staining properties and sugar fermentation were in (Table 2). Gram negative bacilli, *Acinetobacter* spp, *Pseudomonas aeruginosa*, and *Klebsiella* spp and Gram positive cocci and some bacilli, *Streptococcus* and *Staphylococcus* spp., *Bacillus* and *Bacillus cereus*. Staining helps in the identification of the organism's morphology and cell arrangement.

Opportunistic pathogens such as bacteria, viruses and fungi can survive on inanimate surfaces for long periods of time and items such as watches, pens, and mobile phones are permanent surfaces for transmission of these types of infections (Akinyemi *et al.,* 2009). Ryan *et al.,* (2004) Explain *Pseudomonas* spp. are Rugged and opportunistic.

The highest prevalence were in male were (16 isolates) and were percentage of bacteria isolated 61.53 % while in female were (10 isolates) and percentage of bacteria isolated 38.46%.

The highest infection was in male by two bacteria *Bacillus* spp (5 isolates) and *Staphylococcus aureus* (4 isolates) (Tables 3 and 4). Showed results Percentage of total bacteria isolated of female and male, were 38.46% and 61.53 % respectively (Figure 2).

Table.1 Percentage of bacteria isolated from samples under nails

Bacterial isolates (Species)	Number	Percentage %
Bacillus cereus	2	7.69
Acinetobacter spp	1	3.84
Bacillus spp	7	26.92
Streptococcus spp	1	3.84
Pseudomonas aeruginosa	6	23.07
Staphylococcus aureus	5	19.23
Klebsiella spp	2	7.69
Unknow	2	7.69
Total	26	100%

Table.2 Morphological and biochemical characteristics of Bacterial isolates from under nails samples

Bacterial isolates (Species)	**Gram stain**	**Shape**	* Ind.	Oxid.	Cata.	Cit.	Ure.	Coag.	Nit.	Sugar fermentation	
										Glucose	Lactose
Bacillus cereus	+	Bacilli	-ve	-ve	+ve	+ve	-ve	-ve	**v.	+ve	-ve
Acinetobacter spp	_	Bacilli	-ve	-ve	+ve		v.	-ve	-ve	+ve	+ve
Bacillus spp	+	Bacilli	-ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	v
Streptococcus spp	+	Cocci	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve
P. aeruginosa	_	Bacilli	-ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	-ve
Staphy. aureus	+	Cocci	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Klebsiella spp	-	Bacilli	-ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve

* Ind: Indole Test, Oxid: Oxidase test, Cata: Catalase test, Cit: Citrate Utilization Test, Ure: Urease Test, Coag: Coagulase Test and Nit: Nitrate reduction test, **V: Variable

Table.3 Bacteria isolated from under nails of female and male

Bacterial isolates (Species)	**female**	**male**
Bacillus cereus	1	1
Acinetobacter spp	0	1
Bacillus spp	2	5
Streptococcus spp	1	0
Pseudomonas aeruginosa	3	3
Staphylococcus aureus	1	4
Klebsiella spp	2	0
Unknow	0	2
Total	10	16

Table.4 Percentage of bacteria isolated from under nails of female and male

Bacterial isolates (Species)	**Percentage %**	
	female	**Male**
Bacillus cereus	50	50
Acinetobacter spp	0	100
Bacillus spp	28.57	71.42
Streptococcus spp	100	0
Pseudomonas aeruginosa	50	50
Staphylococcus aureus	20	80
Klebsiella spp	100	0
Unknow	0	100

Fig.1 The percentage of Bacterial isolates from samples under nails

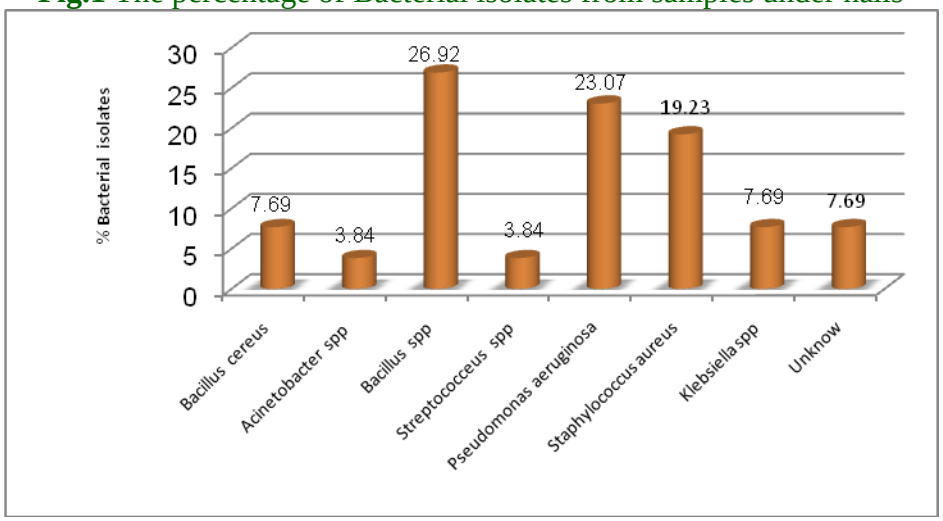
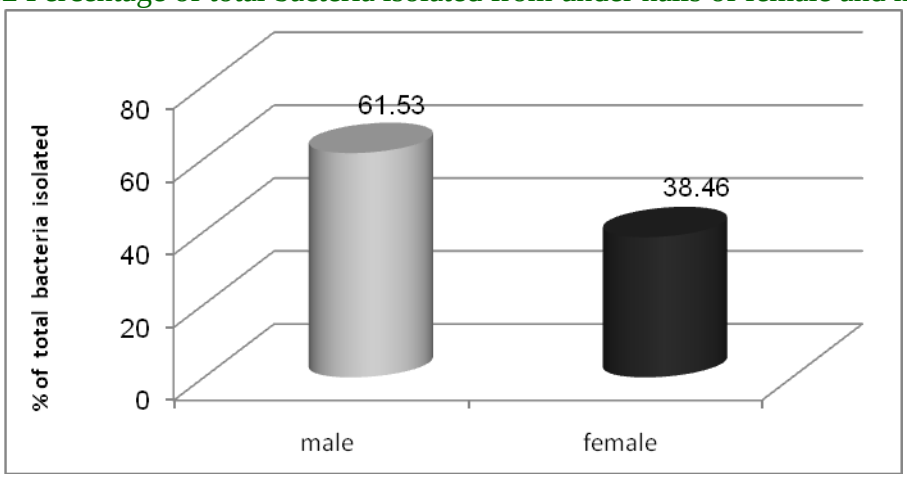


Fig.2 Percentage of total bacteria isolated from under nails of female and male



Rayan and Flournoy (1987) had reported heavy bacterial growth under fingernails that were more than 1mm in length and showed that students with short finger nails (properly cut) had 64% bacterial contamination (bacterial count) and students with long finger nails showed more (67%) contamination of bacterial count on their hands.

Lin *et al.*, (2002) reported that long fingernail tends to harbors more microorganisms than short nails. Visibly clean nails were observed merely by appearance of finger nails of students, showed presence of 62% bacterial contamination on their hands. Ray *et al.*, (2009) observed a decrease in colony count following hand washing with soap in 60% of the samples.

Tambekar *et al.*, (2009) also observed highest bacterial contamination (70%) was observed on the hands of the KG students followed by 67% on hands of primary students, 66% on secondary students, 64% on PG students and least (57%) on the hands of under graduate students.

Ray *et al.*, (2011) found that hand swab samples of 61% children harbours potential pathogens before taking food, also reported presence of pathogenic microbes on the hands of the students which included *S. aureus, E.*

coli, *Enterococcus faecalis*, *Klebsiella* spp. Tambekar and Shirsat, (2012) reported the presence of *E. coli*, *Pseudomonas* spp., *Proteus* spp., *Citrobacter* spp., *Klebsiella* spp., *Salmonella* spp., *Enterobacter* spp. and *S. aureus* from the hand swabs of students. Chinakwe *et al.*, (2012) also isolated *E. coli*, *S. aureus*, *Enterobacter* spp., *Klebsiella* spp., *Enterococcus* spp., *Pseudomonas* spp., *Shigella* spp. and *Corynebacterium* spp. from the hand-wash water samples. Oniya *et al.*, (2006) isolated microorganisms transmissible through hand-shake and also reported prevalence of microorganisms was higher in primary and secondary school students than in the under graduate students. The reduction in the number of pathogens after hand washing was also reported by Tambekar *et al.*, (2009).

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