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Research Article

Identification of Pathogenic Bacteria Isolated from Fingernails of Children in Shendi Locality

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Abstract

Background: Recently a lot of studies focused on the global occurrence of resistant bacteria carriages. Fingernails are a perfect area for harboring bacteria. Children are the most age categories who harbor pathogenic bacteria in their fingernails.

Objective: This was a prospective cross-sectional descriptive study aimed to identify the pathogenic bacteria isolated from the fingernails of children in the Shendi locality; it also studied their antimicrobial susceptibility patterns.

Methodology: The study included (70) participants, 37 (53%) males, and 33 (47%) females, within the age group (1- 10) years. Nails were collected and firstly cultured on nutrient broth then on blood agar and MacConkey incubated aerobically at 37c° for 24 hours. Isolated organisms were identified according to their colonial morphology, indirect gram staining reaction, biochemical tests, and antimicrobial susceptibility tests were done by the disc diffusion method.

Result: The study revealed that 61 (87%) of children showed bacterial growth from fingernails. The isolated bacteria were *Staphylococcus aureus18* (29.1%), *Bacillus spp* 11 (17.7%), *Escherichia coli* 2 (3.2%), *Klebsiella spp*12 (19.3%), *Enterobacter spp*8 (13%), *Citrobacter spp*6 (9.6%), *Proteus vulgaris*4(6.5%) and *Salmonella spp*1 (1.6%). Ciprofloxacin 58 (93.5%) and Gentamicin40 (64.5 %) were the most effective antibiotics against the isolated bacteria in contrast they were mostly resistant to Nitrofurantoin 53 (85.5%), Amoxiclav 45(72.6%) and Doxycycline 39 (63%).

Conclusion: Long nails and playing behaviors of children were the main factors for harboring bacteria in fingernails. *Staphylococcus aureus* was the most isolated bacteria while Ciprofloxacin was the most effective antimicrobial agent used.

Keywords: *Pathogenic Bacteria; Fingernails; Shendi; Children; Antimicrobial Susceptibility*

Introduction

The fingernail is a significant structure consisting of protein, keratin, and laminated layers. Nails have two key roles, despite their tiny number. They function as a protective lamina and by acting as a counter-force to improve the feeling of the fingertip. Every nail comprises several components including nail root, nail bed, nail plate, perionychium, and hyponychium [1]. The most in-touch human body components with the outside world are the hands. People are using their hands every day for a wide range of different activities. Contacting

distinct microbes and transferring them to other objects and perhaps even individuals is highly simple. Surprisingly, the larger number of bacteria discovered on human hands are under fingernails [2]. In many health-related problems, fingernails are progressively seen as a significant concern due to the ability to harbor many kinds of microorganisms [3]. Hand washing has long been recognized as an important hygienic procedure for preventing the transmission of infectious diseases [4,5]. Even when hands are washed, microbes can remain beneath fingernails [6]. Higher populations of microorganisms (2 to 3 log CFU per finger-nail) frequently occur beneath nails and are often more difficult to remove than in other locations on the hands [7]. Fingernail length and texture can also affect microbial removal from beneath nails. Studies revealed that long and polished nails generally harbor more microbes after hand washing than short and unpolished nails [8]. One of the ways of healthy living is hand hygiene [9]. Fecal contamination of hands is one of the important routes by which children are exposed to pathogenic organisms [10,11]. Effective hand washing techniques are crucial in stopping disease transmission via fingernails to remove microbes from artificial or natural [12]. The higher population of pathogenic microorganisms found under long nails were *Escherichia coli, Shigella*, *Salmonella*, *Enterobacter*, *Klebsiella*, *Serratia*, *Proteus*, *Bacillus*, and *Pseudomonas* [12,13]. Transmission of fingernail bacteria occurs through food, water, nails, and fingers contaminated with feces demonstrating the role of fecal-oral person-to-person transmission [14]. Various measures have been implemented to reduce the incidence of food-borne diseases both in developed and developing countries. However, there has been an increased occurrence of emerging and reemerging food-borne diseases. Among the factors responsible for this is the resistance of food-borne pathogens to antibiotics. Humans are exposed to resistant bacteria through sources such as food products, the environment, and food handlers [15].

Materials and Methods

Study design

This was a cross-sectional descriptive study that aimed for identifying the pathogenic bacteria in the fingernails of children in the Shendi locality in the period from August to November 2021.

Study population

Apparently healthy children who live at Shendi locality.

Sample size

Stratified random sampling, Included 70 participants.

Ethical considerations

Sample collection had been explained to participants undergoing tests. All children were informed about the research objectives and procedures during the interview period. Written valid consent was obtained from all participants. All results were with high privacy and confidentiality.

Data collection

Individual interviewing method.

Study area

Shendi locality- River Nile State- Sudan. Shendi is a town in northern Sudan on the east bank of the River Nile 150 km northeast of Khartoum (16°41'N 33°25'E).

Specimen collection

Nails were clipped by sterilized nail clippers and cultured in tubes containing nutrient broth and incubated at 37 ° C for 24 hours for cultivating the bacteria.

Specimen culture

To achieve pure colonies; inoculum from the nutrient broth was sub cultured on MacConkey agar and blood agar for isolation of both gram-negative and gram-positive bacteria and incubated at 37 ° C for 24 hours.

Antimicrobial susceptibility test

Muller Hinton agar was prepared then suspension of micro-organism in sterile N.S was also prepared and compared with McFarland stander, then organism was cultured on Muller Hinton agar, then antimicrobial disc was added and incubated for 24 hours at 37° C. The zone of sensitivity was measured after incubation time. Five disks had been used: Ciprofloxacin 5 mcg, Nitrofurantoin 300 mcg, Amoclav 30 mcg, Doxycycline 30 mcg, and Gentamicin 10 mcg.

Data analysis

Data was analyzed using SPSS19 and manually using odd ratio [16]

$$\text{Odd ratio} = \frac{\text{(n) exposed cases / (n) unexposed cases}}{\text{(n)exposed non-cases/ (n) unexposed non-cases}}$$

Result

In the current study; bacteria that existed in the fingernails of children in the Shendi locality were determined. The study included 70 samples collected from fingernails of males and females- children- with numbers 37 (53%), and 33 (47%) respectively. Participated children were within three educational levels; the baby daycare, KG students, and primary school students, bacterial growth was mostly in KG students 14 out of 15 (93%).34 (89%) of children who had long nails in the collection process showed bacterial growth in culture, while 27 (84%) had short nails but showed bacterial growth too, Odd ratio for bacterial growth in fingernails and nail length was 1.6.60 of the children had contacted different objects; 53 (88%) showed bacterial growth while 8 (80%) of children showed bacterial growth were uncontacted, Odd ratio for bacterial growth in fingernails and children different contacts was 1.89, such objects they contacted were; animals, mud, and unclean toys. 56 (86%) of children were using their right hands and showed bacterial growth on culture, while 5(100%) using their left-hand showed bacterial growth, Odd ratio for bacterial growth in fingernails and the most used hand was 0**.**Despite 57 children washing their hands after using the toilet, 50(88%) showed bacterial growth on culture, Odd ratio for bacterial growth in fingernails and hand washing after using the toilet was 0.77 (Table 1). 61of children with bacterial growth; 25 (41%) of them had a recent history of GIT infection, Odd ratio for bacterial growth in fingernails and children with recent GIT infection was 0.86 (Table 2). Of 70 children there were 61(87%) children found that they were harboring bacteria on their fingernails; while 9 (13%) showed no growth of bacteria on fingernails. The Microorganisms that were isolated and diagnosed from fingernails were *Staphylococcus aureus*18 (29.1%), *Bacillus spp* **11 (17.7%),** *Escherichia coli* **2 (3.2%),** *Klebsiella spp*12 (19.3%), *Enterobacter spp*8 (13%), *Citrobacter spp*6 (9.6%), *Proteus vulgaris*4 (6.5%) and *Salmonella spp*1 (1.6%)are found in (Table 3). The isolated bacteria tested for antimicrobial susceptibility, five antibiotics were used as mentioned in the material and methods, and antibiotic resistance patterns were shown in (Table 4). The most sensitive antimicrobial agent used was Ciprofloxacin 58 (93.5%) while the most resisted agent was Nitrofurantoin 53 (85.5%).

Children	Bacterial growth	No bacterial growth	% of bacterial growth according to washing	Odd ratio
No Washing after toilet	11	2	85%	**0.77**
Washing after toilet	50	7	88%	
Total	61	9	70	

Table 1: *Bacterial growth among children who wash their hands after using the toilet.*

Children	Bacterial growth	No bacterial growth	% of bacterial growth according to GIT infection	Odd ratio
GIT infection	25	4	86%	**0.86**
No GIT infection	36	5	88%	
Total	29	41	70	

Table 2: *Bacterial growth among children who had recent GIT infection.*

Isolated organism	NO	%
Staphylococcus aureus	18	29.1%
Bacillus sp.	11	17.7%
Escherichia coli	2	3.2%
Klebsiella spp.	12	19.3%
Enterobacter spp.	8	13%
Citrobacter spp.	6	9.6%
Proteus vulgaris	4	6.5%
Salmonella spp.	1	1.6%
Total	**62**	**100%**

Table 3: *Types and Numbers of bacteria isolated from fingernails.*

Bacterial isolate	***N***	***Sensitivity pattern***					
			CIP	**NIT**	**AMC**	**DO**	**CN**
S. aureus	18	*S*	16 (89%)	0 (0%)	4 (22%)	3 (17%)	3 (17%)
		R	2 (11%)	18(100%)	14 (78%)	15 (83%)	15 (83%)
Bacillus spp.	11	*S*	10 (91%)	1 (9%)	2 (18%)	1 (9%)	9 (82%)
		R	1 (9%)	10 (91%)	9 (82%)	10 (91%)	2 (18%)
E. coli	2	*S*	2 (100%)	1 (50%)	2 (100%)	2 (100%)	2 (100%)
		R	0 (0%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)
Klebsiella spp.	12	*S*	12(100%)	2 (17%)	2 (17%)	5 (42%)	8 (67%)
		R	0 (0%)	10 (83%)	10 (83%)	7 (58%)	4 (33%)

Enterobacter sp.	8	*S*	7 (87.5%)	2 (25%)	2 (25%)	6 (75%)	8 (100%)
		R	1 (12.5%)	6 (75%)	6 (75%)	2 (25%)	0 (0%)
Citrobacter spp.	6	*S*	6 (100%)	2 (33%)	3 (50%)	4 (67%)	6 (100%)
		R	0 (0%)	4 (67%)	3 (50%)	2 (33%)	0 (0%)
P. vulgaris	4	*S*	4 (100%)	1 (25%)	1 (25%)	1 (25%)	3 (75%)
		R	0 (0%)	3 (75%)	3 (75%)	3 (75%)	1 (25%)
S. paratyphi A	1	*S*	1 (100%)	0 (0%)	1 (100%)	1 (100%)	1 (100%)
		R	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)
Total	62	*S*	58 (93.5%)	9 (14.5%)	17 (27.4)	23 (37%)	40 (64.5)
		R	4 (6.5%)	53 (85.5%)	45 (72.6)	39 (63%)	22 (35.5)

Table 4: *Antimicrobial susceptibility patterns of isolated bacterial species from children fingernails (compared with NCCLS guidelines).*

Key: *CIP- Ciprofloxacin, NIT – Nitrofuranton, AMC – Amoxiclav (Ampicillin + Clavulinic acid), DO – Doxycycline, CN – Gentamicin.*

In addition, the mean for the inhibition zones of the antimicrobial susceptibility pattern of the isolated pathogen was calculated and shown in (Table 5).

Bacterial isolate	***Sensitivity zones***					
	Mean	**CIP**	**NIT**	**AMC**	**DO**	**CN**
S. aureus	*S*	24.3	0	18	17.3	20
	R	14	8.8	8.1	7.4	13.86
Bacillus spp.	*S*	21.8	18	18	24	18.75
	R	12	9.1	9.2	8.9	12
E. coli	*S*	29	20	22	17	16
	R	0	10	0	0	0
Klebsiella spp.	*S*	23	9.1	16	16.8	15.5
	R	0	17	8.3	8.4	10.75
Enterobacter sp.	*S*	22.8	16	17	16.6	16
	R	12	10	7	12	0
Citrobacter spp.	*S*	24.66	16	19.33	16	15.3
	R	0	8.75	9.3	8.5	0
P. vulgaris	*S*	22.6	16	20	14	16
	R	0	11	9.3	7	12
S. paratyphi A	*S*	26	0	20	14	18
	R	0	12	0	0	0

Table 5: *Mean of inhibition zones of anti-microbial susceptibility pattern of isolated bacterial species from finger nail.*

Discussion

Microbial contamination of the fingernails of hands with resistant bacteria had become a global health problem. Thus nail samples from the most used hand of children of the Shendi locality were collected to determine the pathogenic bacteria in their fingernails. Isolated bacteria were more in males than in females 34 (56%), 27 (44%) respectively, as in the study of Mohsen Hashim Risan [13] that showed the percentage of bacteria isolated from males was (61.53 %) and females were (38.46%); this could be due to types of games such as football and other activities of the boys. The study found that; the isolated bacterial pathogen was most in KG students 14 out of 15 (93%) and in baby daycare 10 out of 11 (90%) were more than children in primary school 37 out of 44 (84%), this finding is in agreement with Tambekar., *et al*. that also found 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 undergraduate students [17]. This could be due to their less application of hand and nail hygiene in comparison to older children. Lin indicated that more microorganisms tend to harbor lengthy fingernails than brief nails [18] in addition; Rayan and Flournoy clarify the presence of large bacterial growth under fingernails over 1 mm in length, showing that volunteers with long nails and short fingernails were (67%, 64%) respectively [6]. Both studies agreed with the current study which 34 (89%) out of 38 children with long nails were harboring bacteria in comparison to those with short nails 27(84%). In the current study, 53 (88%) of 60 children had bacterial growth in their fingernails, and have contact with different contaminated objects and games. Children playing with mud were the highest group among them 26 (49%); this agreed with the study conducted by G Duffy; who revealed the affection for handling contaminated mud in the transmission of bacteria [19]. 56(86%) of children using their right hand showed bacterial growth, while all of the left-hand users gave bacterial growth 5 (100%). The odds ratio for the most hand used was (0) that disapproved the affection of left nor right hand in increasing the bacterial growth on fingernails of children in agreement with the study done by WatutantrigeRanjit De Alwis., *et al*. who studied the CFU count of hands and showed no significant difference between both hands (right-hand $P = 0.097$, left-hand $P = 0.096$) [20]. Abeba Mengist., *et al*. [21] agreed with the current study that found the insignificant association between handwashing after using the toilet and bacterial growth on fingernails with a P value (0.837) while it disagreed with Watutantrige Ranjit De Alwis., *et al.* who studied The mean CFU count of both hands of the students and it was significantly higher after toilet use (right-hand $P = 0.001$, left-hand $P = 0.003$) [20]. However, the other expected risk factors (i.e., recent GIT infection) were not associated with bacterial fingernail rate.61 (87%) out of 70 children found they were harboring bacteria on their finger nails. Bacterial pathogens isolated from finger nails of children were both Gram-positive and Gram-negative bacteria. Gram-positive was *Staphylococcus aureus* 18 isolates (29.1%), *Bacillus spp* 11 isolates (17.7%) while Gram-negative bacteria were *Escherichia coli* 2 isolates (3.2%), *Klebsiella spp* 12 isolates (19.3%), *Enterobacter spp* 8 isolates (13%), *Citrobacter spp* 6 isolates (9.6%), *Proteus Vulgaris* 4 isolates(6.5%) and *Salmonella spp* 1 isolate(1.6%). A lot of studies isolated the same bacteria from fingernails such as; Ukaegbu-obi *et. al.* and Mengist., *et al.* [12,21] 58 (93.5%) of the isolated bacteria were sensitive to Ciprofloxacin as in the study conducted by AbebaMengist *et. al.* who found also; all of the isolated bacteria from fingernails were more sensitive to ciprofloxacin [21] 40 (64.5%) of isolated bacteria were sensitive to Gentamicin, except for *Staphylococcus aureus* as Kelechi M. Ukaegbu-Obi., *et al.* also reported the sensitivity for Gentamicin by all of the isolated bacteria except for *Staphylococcus* spp. and *Streptococcus* spp [12]. Zahraa AL-Taee reported that most of the isolated bacterial groups from fingernails were resistant to Nitrofurantoin [22] who agreed to this study which revealed high resistance to Nitrofurantoin 53 (85.5%).39 (63%) of isolated bacteria were resistant to Doxycycline. The isolated *Staphylococcus aureus*, *Klebsiella spp,* and *Proteus spp* groups were more resistant to Doxycycline, this agreed with the study of Kelechi M., *et al* [12]. In contrast to *E. coli*, *Enterobacter spp* and *Citrobacter spp* were more sensitive to it than Abeba., *et. al*. [21] who also revealed the same results for these groups. Waleed Al Momani *et. al.* found that all of the isolated bacteria from students' hands were sensitive to (Amoxicillin - clavulanic acid) in contrast to the current study that showed 45 (72.6%) of the isolated bacteria from fingernails were resistant to it [23].

Conclusion

The study concluded that 61 (87%) of children under study were harboring bacteria in their fingernails. KG students were the group with bacterial growth 14 (93%). It approved the relation of risk factors of having long nails, dealing with animals, playing with mud and unclean toys in increasing the bacterial growth in fingernails of children; while there was no statistically significant association between neither left nor right-handed children, hand washing after using the toilet, recent GIT infection, and bacterial growth on fingernails of children. Bacterial isolates were *Staphylococcus aureus*, *Bacillus spp*, *Escherichia coli*, *Klebsiella spp*, *Enterobacter spp*, *Citrobacter spp*, *Proteus Vulgaris*, and *Salmonella spp*. All of the isolated bacteria were sensitive to Ciprofloxacin and the majority of them were sensitive to gentamicin. Most of the isolated bacteria were highly resistant to Nitrofurantoin, Amoxiclav, and Doxycycline.

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