Enumeration of Bacterial isolates from Hands of Primary and Post Primary School pupils in Amai, Delta State

Abstract

The presence of bacteria on the hands of fourty (40) school pupils from two different schools (Amai Primary and secondary School) in Amai, Delta State, was analysed. The reason was to enumerate bacteria isolated from hands of students. Swab samples were collected from hands of pupils in both schools of 20 students each of different sex groups. Microbiological methods was used for the isolation, enumeration and antibiotic test of the isolates. The results showed various isolates of *Staphylococcus sp* 56 (25.7%), *Shigella sp* 24 (9.7%), *Staphylococcus epidermidis* 32 (12.1%), *Escherichia coli* 41 (15.6%), and *Enterococcus sp* 36 (11.0%). *Staphylococcus aureus* 56 (25.3%) and *Escherichia coli* 41 (15.6%) were the most frequent isolates. The isolation of *Shigella sp* 24 (9.7%) and *Enterococcus sp* 36 (11.0%) is of great importance as the isolation of these organisms showed improper faecal wastes disposal around the school environment and also lack of proper clean up after using the convience by the pupils. Testing these isolates to few antibiotics, the isolates were susceptible to Pefloxacin, Gentamycin, Erythromycin, Chloramphenicol, Streptomycin, while resistant to Augmentin, Amoxicillin, and Ampiclox. This study revealed that the students hands were infested with pathogens due negligence of maintenance culture. Those in charge of schools like the principal are advised to keep soap and water for hand washing, while parents on their part should make available hand washing facilities for their children at home since it will add economic value to the society, why Government should enact laws that will make provision of washing hand amenities in all areas compulsory for individuals.

Keywords; *Staphylococcus sp,* Erythromycin, Chloramphenicol, Streptomycin and Economic value

INTRODUCTION

Keeping good hand hygiene is the act of cleaning one's hands with soap and water with aim of removing dirt and microorganisms. It is a general term that applies to hand washing, antiseptic hand washing, alcohol based hand rub or surgical hygiene/antiseptic [1-3]. Hand washing is the easiest amongst hand hygiene practices with soap, detergent and running water and is the most sensible and affordable method for keeping good hand hygiene among the entire population [2]. School children can be encouraged to wash their hands with soap and water [4]. The developed part of the world has cultivated hand washing as a habit because of the advantage of microbial reduction in past and present day activities. In our country like Nigeria, washing of hands is yet to be a law especially the rural areas [5]. Moreso, there has been introduction of programmes by Nigerian government designed which pays attention on mothers, children and adolescents. Creating awareness on children and teenagers best practices in hand washing will contribute immensely in achieving world health organization target on proper hygiene, enlightenment and reduction in children death rate [4, 5]. This will obviously lead to early internalization of hand hygiene principles and practice from primary and secondary levels of education and ensure adherence to these practices all through life. Schools around rural areas practices rarely adhere to proper hand hygiene due to lack of negligence [4]. The Nigerian demographic and health survey (NDHS) revealed that diarrhoea and cholera outbreaks which are diseases of poor hygiene are common occurrences in Nigerian schools majorly rural areas [6]. Diseases in a school population is a major limiting factor in the educational progress of any child as it leads to absenteeism, poor classroom performance and early school dropout, and all these militates against the achievement of quality Universal Basic Education [7]. Though hand washing is a normal practice in the Nigerian society, the frequency and method of the practice might not have met internationally recommended standards. Many researchers have observed low compliance to standards of hand washing world over even with the availability of soap and water; worst still, even among medical professionals [8]. Most hand hygiene compliance studies have focused and documented
this practice in hospital environment while very few studies had focused on schools [8]. In Nigeria, the need for such studies in primary schools is necessitated by the observation of NDHS outbreak of diseases and absence of enabling environment and facilities for the practice of hand hygiene [6]. There are five critical times during the day where washing hands with soap is important to reduce the faecal oral transmission of disease; after defecation, after cleaning a child, before feeding a child, before eating and before preparing food or handling raw meat, fish or poultry [3, 6]. For children in particular, critical moments include after playing outside, or with toys and pets [8]. According to Centres for Disease Control and Prevention (CDC), hands should be washed with soap and clean running water (if available); Before, during and after preparing food, before eating food, before and after caring for someone who is sick, after using the toilet, after blowing the nose, coughing or sneezing, after changing diapers, cleaning or cleaning up a child who has used the toilet, after touching garbage.
This research therefore was carried out to isolate and identify the various bacteria present on the palms of primary school children from four different primary schools in Amai ,Delta State ,Nigeria.

Materials and Methods

Study Area

This study was carried out in the Department of Microbiology, Novena University, Ogume Delta State,Nigeria. Amai metropolis is a town where Ndokwa LGA Is situated. It inhabits different tribes with a nucleated settlement.

Sample Collection

fourty (40) hand palms swabs were aseptically collected using sterile swab sticks from pupils of two (2) primary and secondary schools (Amai primary and secondary school) all in Amai locality were immediately transported to laboratory for analysis.

Media preparation

All the media were purchased from Laboratory in Delta State. The specific media for bacteria isolation and growth supplied in powder form was weighed, prepared and reconstituted using distilled water. The agar was made of 28 grammes (g) per litre for bacteria growth (digest of animal tissue 5.00 g, beef extract 1.50 g, sodium chloride 5.00 g, agar 15.00 g) was prepared by suspending in 1000 ml of distilled water and the pH was adjusted to 7.4 using 2M NaOH (Tokuyasu *et al*., 2012). The agar mixture was dissolved in water by boiling at 100 °C then the agar was sterilized by autoclaving at a pressure of 15 pascals and a temperature of 121 °C for 15 min. Nutrient broth (with the same ingredients except agar) was prepared by suspending 13.0 g of nutrient agar in 1000 ml distilled water and heated to dissolve completely then sterilized as above. *Salmonella Shigella* Agar (SSA) medium (peptic digest of animal tissue 5.00 g, beef extract 5.00 g, lactose 10.00 g, bile salts mixture 8.50 g, sodium citrate 10.00 g, sodium thiosulphate 8.50 g, Ferric citrate 1.00 g, brilliant green 0.00033 g, neutral red 0.025 g, agar 15.00 g) all in one liter volume was prepared by suspending 63.0 g SSA in 1000 ml distilled water and the pH adjusted to 7.0 using glacial acetic acid. The mixture was warmed with frequent agitation to dissolve the medium completely in water. It was then cooled to a temperature of 50 °C, mixed well and poured into sterile petridishes. Mannitol Salt Agar (proteose peptone 10.00 g, beef extract 1.00 g, sodium chloride 75.00 g, mannitol 10.00 g, phenol red 0.025 g in a one litre volume) was used for selective isolation of pathogenic *Staphylococcus aureas*. Fifteen grams of mannitol agar was dissolved in 1000 ml of distilled water. The pH was adjusted to 7.4 using glacial acetic acid. It was boiled to dissolve the medium completely then sterilized by autoclaving at a pressure of 15 pascals and a temperature of 121 °C for 15 min.

Bacteriological Analysis

Collected samples were cultured on MacConkey agar and Mannitol salt agar using the streak plate method. The plates were incubated at 37°C for 24 hours. Discrete colonies were identified and characterized by morphological characteristics, Gram staining, biochemical tests and sugar fermentation analysis using standard microbiological methods [Cheesebrough, 2016].

Glycerol stocking and storage of pure isolates

Bacteria colonies on 0.1 % NA were inoculated to 10 ml nutrient broth and incubated in a shaking water bath overnight. The absorbance of broth was read using a spectrophotometer at 600 nm. They were ready for harvesting at absorbance of 0.06 (Bourne and Munn, 2005). Using a sterile pipette, the isolates in broth were pipetted into 1.5 ml eppendorf tubes at the ratio of 70:30 glycerol to broth respectively. Each isolate was stocked in duplicate and labeled. The isolation date was also labeled and the temperature at which storage was carried out. All the isolates were stored in a freezer at 4 °C. The isolates at this temperature were then used in the subsequent identifications (Ghosh, 2010).

Viability of the isolates was carried out every one month to confirm if they were still alive. Fungal and bacterial isolates were streaked onto NA respectively and incubated under conditions outlined in section 3.4. Growth on the agar for each isolate confirmed viability.

Results

Biochemical identification of bacteria

H2S gas.	Motility	Gram stain	Catalase	Starch hydrolysis	Citrate utilization	Indole	Lactose	Oxidase	Isolate identity
smooth, white, creamy and round	-	-	+ Coccus in clusters	+ -	-	-	-	-	*Staphylococcus aureus*
Glossy , green thin and pigmented	-	-	- Bacillus	+ -	+	-	-	+	*Pseudomonas aeruginosa*
moist, white with growth	-	+	+ Cocci	+ -	-	+	AG	-	*Escherichia coli*
glistening glossy white	-	-	+ Bacillus	+ +	-	-	-	-	*Bacillus subtilis*
clear membranous, small, round and irregular	-	-	- Bacillus	+ -	+	-	+	-	*Enterobacter sp*
Grayish, granular with limited growth	-	-	+ Bacillus	+ -	-	-	-	-	*Corynebacteria sp*
Transluce nt-creamy, mucoid and round	-	-	- Bacillus	+ -	+	-	AG	-	*Klebsiella sp*
Straight paired rods	+	+	- bacillus	NT	NT	NT	NT	-	*Salmonella sp*
Short rods, singles clustered	-	-	- cocci	-	NT	NT	NT	-	*Shigella sp*

Table 1 shows the biochemical attributes of the bacteria isolated from two schools in Amai.

Table 2: The frequency of occurrence of bacterial isolates from the two schools

	Amai primary school		Amai secondary school		Total pathogens
Isolates	**M**	**F**	**M**	**F**	
Staphylococcus aureus	*8*	*6*	*7*	*4*	*25*
Escherichia coli	*6*	*5*	*6*	*3*	*20*
Shigella sp	*3*	*9*	*4*	*3*	*19*
Total	*17*	*20*	*17*	*10*	*64*

Table 2 above shows that *Staphylococcus aureus* has frequency of occurrence more followed by *Escherichia coli*

Table 3: Frequency of occurrence of bacterial isolates from the two schools

	Amai primary school (%)		Amai secondary school (%)		Total (%)
Isolates	**M**	**F**	**M**	**F**	
Staphylococcus aureus	*3.2*	*2 6*	*2.9*	*1.8*	*10,5*
Escherichia coli	*2.8*	*1.8*	*2.2*	*1.9*	*9.7*
Shigella sp	*1.9*	*2. 9*	*1.8*	*1.9*	*8.5*

Total	*7.9*	*7.3*		*6.9*	*5.6*
28.7					

Table 3 above shows that *Staphylococcus aureus* has the highest percentage of occurrence amongst other isolate from the Amai primary and secondary school students

Table 4: Antimicrobial susceptibility testing of the isolates

Isolates	**AZITHROMYCIN**	**AMPICLOX**	**AUGMENTIN**	
Staphylococcus	S	S		S
Escherichia coli	*S*	*R*	*S*	
Shigella sp	*R*	*S*	*R*	

Key

S: Sensitive

R:Resistance

Table 4 above shows the different antibiotics testing against the three isolates. Staphylococcus aureus were susceptible against the three antibiotics used followed by *Escherichia coli* that was resistant to ampiclox while *Shigella* sp was resistant to augmentin and azithromycin

DISCUSSION

A total of 40 hand palms swabs samples collected from students from two (2) primary schools (Amai Primary and post primary School) in Amai locality Delta Sate were investigated. Table 1 shows biochemical characteristics of the various isolates in biochemical test. The results in this study is inline with rhe previous results of Ghosh in (2010) who investigated the same swab sample from some school children in Mumbai metropolis . Table 2 shows the frequency of occurrence of bacterial pathogens isolated in the two schools. The result shows that *Staphylococcus sp* (25) had the highest frequency of occurrence and a major contaminant amongst the school children in Amai primary and secondary school. The second isolate was *Escherichia coli* with frequency of occurrence (20) followed by *Shigella* sp which was(19). The results are in consonance with the previous study of Aremu (2012) who isolated similar

pathogens from palms of primary and secondary school students with frequency of occurrence of *Staphylococcus aureus* (35), *Escherichia coli* (24) and *Shigella* sp (19) in Addo ekiti municipal. The human hands harbour microbes both as part of a person's normal microbial flora as well as foreign microbes acquired from the environment according to Lax and Smith (2014). Some pathogenic organisms are spread by contaminated hands (Aremu, 2012). Hygiene has a measurable impact on reducing the burden of infections in the developing world. In this study, the practice of hand washing either with water or with soap and water is very low as compared to what is obtainable in studies from other countries [2, 3]. The result of the study in table 3 shows that well over 28% of all the primary and post primary school students hands was contaminated with one bacterial pathogen or the other, and this is as a result of poor personal hygiene particularly hand hygiene, this conformed with the report of world health organisation in 2017. This has been attributed to lack of appropriate hand washing facilities or poor location of these facilities in primary and secondary schools in Amai metropolis. The prevalence of diarrhoea among school pupils for whom mainly unhygienic behaviour was recorded higher majorly because of inadherence of good personal hygiene [12]. Table 4 also shows the antibiotics susceptibility testing of the various isolates. In this study a total of 3 different antibiotics were tested amongst the 3 isolates. The result revealed that Ampiclox, Augmentin and Azithromycin were all sensitive to the isolate *Staphylococcus aureus, Escherichia coli* was sensitive to Augmentin and Azithromycin but resistant to Ampiclox. *Shigella* sp was sensitive to Ampiclox but resistant to Augmentin and Azithromycin.The result of antibiotics sensitivity and resistance in this study is in consonance with the previous result of Imarenezor and Peter in (2017) who tested the susceptibility of antibiotics in isolates from school pupils hands in Wukari. The home and the school environments were of particular concern for the transmission of infections among students. Unfortunately, most schools in developing countries do not provide appropriate hand washing facilities and where these facilities are available, they may be poorly located, have insufficient hand washing materials, be improperly used and most times be inaccessible to the pupils. Effective hand washing (including drying) is important in infection control. The proper hand washing is the single and most effective way to prevent the spread of communicable diseases. Good hand washing techniques is easy to learn and can significantly reduce the spread of infectious diseases among both children and adults. Hand washing is one among a range of hygiene promotion interventions that can interrupt the transmission of diarrhea causing pathogens [13, 17].

CONCLUSION

Hand washing practices in both public and private schools in Amai metropolis are not observed particularly in community schools. However, in the absence of infrastructure for hand washing, inculcation of this habit would be nearly impossible. To this end, schools should promote hand hygiene practices in pupils by keeping soaps in toilets for hand washing. Teachers need resources and training in proper hand washing methodology to teach and able to supervise pupils if they are to be agents of change. Parents also be able to inculcate basic personal hygiene in their children by providing facilities for hand washing at home.

Ethical Approval:

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

Consent

As per international standard or university standard, Participants' written consent has been collected and preserved by the author(s).

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