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Bacteriological Qualities of Water, Hand and Vaginal Swab Samples of Female Adolescents in Aba, South-Eastern Nigeria

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ABSTRACT

Good personal hygiene among adolescent girls is essential for the prevention of bacterial infection. The objective of this study was to assess the bacteriological qualities of water, hand and vaginal swab samples of female adolescents in Aba, South-Eastern Nigeria. Water, hand and vaginal swab samples were collected from one hundred and thirty-four female adolescents residing in Aba metropolis in South-Eastern Nigeria. Sterile swab sticks were used to collect hand and vaginal swabs while a sterile container was used to collect water samples and taken to the laboratory for analysis. Results showed that out of the 134 water samples collected, *Staphylococcus aureus* was isolated in 47 (35.07%) samples; *Escherichia coli* 20 (14.93%); *Bacillus subtilis*; 12 (8.96%); *Streptococcus faecalis* 34 (25.37%); *Enterococcus faecalis* 11 (8.21%); *Micrococcus luteus*; 9 (6.72%). There was a significant amount (P< 0.05) of bacterial load in the water samples. Results also showed that out of the 134 hand swab samples collected, *Staphylococcus aureus* was isolated in 92 (68.66%) samples; *Escherichia coli* 24 (17.91%); *Bacillus subtilis* 17 (12.69%); *Streptococcus faecalis* 64 (47.76%); *Enterococcus faecalis* 23 (17.16%); *Micrococcus luteus* 16 (11.94%). There was a significant amount (P< 0.05) of bacterial load on the hand swab samples. Results also showed that out of the 134 vaginal swab samples collected, *Staphylococcus aureus* was isolated in 115 (85.82%); *Escherichia coli* 122 (91.04%); *Bacillus subtilis* 43 (32.09%); *Streptococcus faecalis* 102 (76.12%); *Enterococcus faecalis* 32 (23.88%); *Micrococcus luteus* 25 (18.66%). There was also a significant amount (P< 0.05) of bacterial load on the vaginal swab samples. In conclusion, there was a significant amount of bacteria isolates obtained from the water, hand and vaginal samples among the adolescent girls. Health education and enlightenment programmes for adolescent girls were recommended.

Keywords: Bacterium, Water, Hand, Vagina, Hygiene, Adolescent

INTRODUCTION

Adolescents are a large and growing segment of the world's population. More than half of the world's population is below the age of 25yrs, and one in every two young persons in the world is an adolescent. [1] A large variety of morbidities, such as nutritional deficiency disorders, menstrual disorders etc. prevail among adolescents. During adolescence, young people develop adult identity, move toward physical and psychological maturity. Physiologically, the hypothalamus produces growth hormone and gonadotrophins which initiates pubertal changes. [2] Menstruation is one of the most important physiological changes that occur among female adolescents. The menstruation cycle refers to the cycles in which a woman's uterus grows and sheds a lining (the endometrium) which could

support the development of a fertilized egg. It typically occurs in 28 day cycles. So a woman generally gets her period every 28 days. [3]

Menstruation is generally considered as unclean leading to isolation of menstruating girls and restrictions imposed on them in the family. These practices have reinforced negative attitude towards menstruation in girls. Dhingra et al. [4] opined that although adolescence is a healthy period of life, many adolescents are often less informed, less experienced and less comfortable accessing reproductive health information and services than adults. In many parts of the developing countries, a culture of silence surrounds the topic of menstruation and related issues. As a result, many young girls lack appropriate and sufficient information regarding menstrual hygiene. This may result in incorrect and unhealthy behaviour during their menstrual period. Arunmozhi and Antharam [5] reported in their study that schools did not have enough toilets for students and no separate toilets for girls and boys. When separate facilities are available, they are often unclean and lack water supply and cubicle doors. The majority of girls in the rural areas use cloth to manage their flow. These girls need adequate water for changing their commodities in schools, and a private space to wash. Consequently, since girls cannot do this at school, they are forced to go home half way through the day, missing at least one hour at school in the process Many girls avoid going to school altogether when menstruating and fall behind on school work. According to Shivaleela et al. [6], adolescent girls constitute a vulnerable group not only with respect to their social status but also in relation to their health. The issue of menstrual hygiene is inadequately acknowledged and has not received proper attention.

Good hygienic practices such as use of sanitary pads and adequate washing of the genital areas are essential during menstruation period. In Nigeria, most secondary schools do not have available pads or sanitary towels within the school to maintain good hygiene practices. The practices of good menstrual hygiene reduce the incidence of Urinary Tract Infection (RTI) thus, the consequences of RTIs are severe and may result in significant negative impact to a woman's health including chronic pelvic pain, dysmenorrhoea (painful periods) and in severe cases infertility. [6]

Every year approximately 10% of women worldwide are exposed to urinary tracts infections and bacterial vaginosis and 75% of women have a history of a genital infection. [7] Specifically, the common risk factors for vaginal infections include pregnancy and poor hygiene both perineal and menstrual hygiene. [8] Good personal hygiene practices among adolescent girls are very essential toward the prevention of infections. Contaminated water used for cleansing can be a source of bacterial infection. These bacteria can also be found on their hands and vagina. This study aims at isolating the bacteria that can be found in water used by adolescent girls and also on their hands and vagina.

MATERIALS AND METHODS

Collection of samples

Water, hand and vaginal swab samples were collected from one hundred and thirty-four female adolescents residing in Aba metropolis in South-Eastern Nigeria. Sterile swab sticks were used to collect hand and vaginal swabs while a sterile container was used to collect water samples. The samples were transported to the laboratory for analysis.

Preparation of Media and Diluents

All bacteriological media were prepared according to specific requirements of the manufacturers. Nutrient agar was used to isolate heterotrophic bacteria, MacConkey Agar for faecal coliform bacteria, Eosin Methylene Blue Agar for *Escherichia coli*, Blood Agar for *Staphylococcus aureus* and Salmonella Shigella Agar for the isolation of *Salmonella* and *Shigella* species.

Characterization and Identification of Microbial Isolates

Microbial isolates were characterized based on cultural (colonial), microscopic and biochemical methods with reference to standard manuals. [9] Biochemical tests carried out include catalase test, coagulase test, oxidase test, sugar fermentation, hydrogen sulfide production test, urease test, IMViC test, indole test, and citrate utilization test.

RESULTS

Table 1 showed that *Staphylococcus* species (sp) were circular moist and shiny golden yellow colonies on Nutrient Agar and light yellow on Mannitol Salt Agar; *Enterococcus* sp were small circular moist and shiny low convex cream colonies on Nutrient Agar; Shigella sp were light pink mucoid moist and shiny colonies on Salmonella Shigella Agar; *Escherichia coli* were greenish metallic sheen on Eosin Methylene Blue Agar. *Bacillus* sp were Serrated dull and dry flat cream colonies on Nutrient Agar; *Micrococcus* sp were small smooth moist and shiny low convex yellow colonies; *Streptococcus* sp were complete (beta) haemolysis on blood agar with zone of clearance around colonies. Table 2 showed that *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Micrococcus luteus* showed a positive result for catalase test while *Enterococcus faecalis* and *Streptococcus faecalis* gave a negative result. The table also explained the positive and negative results for the bacterial isolates in each of the biochemical tests. Table 3 showed that out of the 134 water samples collected, *Staphylococcus aureus* was isolated in 47 (35.07%) samples; *Escherichia coli* 20 (14.93%); *Bacillus subtilis*; 12 (8.96%); *Streptococcus faecalis* 34 (25.37%); *Enterococcus faecalis* 11 (8.21%); *Micrococcus luteus*; 9 (6.72%). There was a significant amount [P (0.015) < 0.05] of bacterial load in the water samples. Table 4 showed that out of the 134 hand swab samples collected from the adolescent girls in Aba, *Staphylococcus aureus* was isolated in 92 (68.66%) samples; *Escherichia coli* 24 (17.91%); *Bacillus subtilis* 17 (12.69%); *Streptococcus faecalis* 64 (47.76%); *Enterococcus faecalis* 23 (17.16%); *Micrococcus luteus* 16 (11.94%). There was a significant amount [P (0.036) < 0.05) of bacterial load on the hand swab samples. Table 5 showed that out of the 134 vaginal swab samples collected, *Staphylococcus aureus* was isolated in 115 (85.82%); *Escherichia coli* 122 (91.04%); *Bacillus subtilis* 43 (32.09%); *Streptococcus faecalis* 102 (76.12%); *Enterococcus faecalis* 32 (23.88%); *Micrococcus luteus* 25 (18.66%). There was a significant amount [P (0.011) < 0.05] of bacterial load on the vaginal swab samples.

Table 1: Colonial and microscopic characteristics of bacteria isolated from water samples

Colonial Characteristics	Motility Test	Spore Formation	Capsule Formation	Gram morphology/ Reaction	Probable Identity
Circular moist and shiny golden yellow colonies on Nutrient Agar	-	-	-	Gram positive cocci predominantly in clusters, few in tetrads and pairs	*Staphylococcus* species
Small circular moist and shiny low convex cream colonies on Nutrient Agar	-	-	-	Gram positive cocci predominantly in chains and pairs	*Enterococcus* species
Serrated dull and dry flat cream colonies on Nutrient Agar	+	+	-	Large Gram-positive rods with central spores in chains	*Bacillus* species
Greenish metallic sheen on Eosin Methylene Blue Agar	+	-	-	Gram negative rods predominantly in single and pairs	*Escherichia coli*
Small smooth moist and shiny low convex yellow colonies on nutrient agar	-	-	-	Cocci predominantly in tetrads and few in pairs and irregular	*Micrococcus* species
Complete (beta) haemolysis on blood agar with zone of clearance around colonies	-	-	-	Gram positive cocci predominantly in chains	*Streptococcus* species
Light pink mucoid moist and shiny colonies on Salmonella Shigella Agar	+	+	-	Gram negative single and short rods	*Shigella* species

Table 2: Biochemical characteristics and carbohydrate fermentation of bacteria isolated from water samples

Cat	Oxi	Coag	IN	VP	Cit	NO_3	Ure	G	S	L	M	Mn	Xyl	Ara	MR	Identity of Isolates
+	-	+	-	+	-	+	+	+	+	+	+	+	-	-	-	*Staphylococcus aureus*
-	-	-	-	-	+	+	-	+	+	+	=	+	-	+	-	*Enterococcus faecalis*
+	-	-	+	-	-	+	-	+	+	+	+	+	+	+	-	*Escherichia coli*
+	-	-	-	+	+	+	-	+	-	-	-	+	+	+	-	*Bacillus subtilis*
+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	*Micrococcus luteus*
-	-	-	-	+	+	+	-	+	-	-	-	+	-	-	-	*Streptococcus faecalis*

Cat, Catalase; Oxi, Oxidase; Coag, Coagulase; IN, Indole; VP, Voges Proskaeur; Cit, Citrate; NO_3, Nitrate reduction; Ure, Urease; G, Glucose; S, Sucrose; L, Lactose; M, Maltose; Mn, Mannitol; Xyl, Xylose; Ara, Arabinose; MR, Methyl Red;

Table 3: Distribution of bacterial isolates from water samples

Bacteria	n	%
Staphylococcus aureus	47	35.07
Escherichia coli	20	14.93
Bacillus subtilis	12	8.96
Streptococcus faecalis	34	25.37
Enterococcus faecalis	11	8.21
Micrococcus luteus	9	6.72
P value	0.015	

Table 4: Distribution of bacterial isolates from hand swabs

Bacteria	n	%
Staphylococcus aureus	92	68.66
Escherichia coli	24	17.91
Bacillus subtilis	17	12.69
Streptococcus faecalis	64	47.76
Enterococcus faecalis	23	17.16
Micrococcus luteus	16	11.94
P value	0.036	

Table 5: Distribution of bacterial isolates from vaginal swabs

Bacteria	N	%
Staphylococcus aureus	115	85.82
Escherichia coli	122	91.04
Bacillus subtilis	43	32.09
Streptococcus faecalis	102	76.12
Enterococcus faecalis	32	23.88
Micrococcus luteus	25	18.66
P value	0.011	

DISCUSSION

The bacterial isolates were *Staphylococcus aureus* 101 (35.31%), *Escherichia coli* 43(15.03%), *Bacillus subtilis* 26(9.09%), *Streptococcus faecalis* 74(25.87%), *Enterococcus faecalis* 23(8.04%) and *Micrococcus luteus* 20(6.99%). These results corroborated the findings of Balaban and Rasooly [10] who identified bacteria isolated from different water samples as including faecal coliforms, faecal streptococci *Staphylococcus aureus*, Pseudomonas species, *Escherichia coli*, *Klebsiella* species, *Enterococcus* species, *Salmonella* species and *Enterobacter* species. Onyango et al. [11] conducted a study on the microbiological quality and contamination level of water sources and also reported *Staphylococcus aureus*, *Clostridium perfringens*, *Coliforms* and *Escherichia coli* as the major bacterial isolates. Microorganisms tend to flourish in an unhygienic environment. Water samples could become contaminated due to poor hygiene practices. The bacterial isolates from hand swab samples collected indicated *Staphylococcus aureus* 197 (68.88%), *E. coli* 52 (18.18%), *Bacillus subtilis* 34 (11.89%), *Streptococcus faecalis* 137 (47.90%), *Enterococcus faecalis* 48 (16.78%) and *Micrococcus luteus* 34 (11.89%). These results confirmed the findings of Otsuka et al. [12] who isolated *E. coli* in 98.7% of the study population (children) and *Staphylococcus* (76.9%), *Micrococcus* sp. (19.2%), *Bacillus* sp. (8.2%), *Pseudomonas* sp. (6.0%), *Proteus* sp. (1.6%) and *Escherichia coli* (0.5%). The results also corroborated the findings of Parvez et al. [13] who reported similar findings. From the vaginal swab samples, the bacteria isolated were *Staphylococcus aureus* 245 (85.66%), *E. coli* 260 (90.91%), *Bacillus subtilis* 92 (32.17%), *Streptococcus faecalis* 217 (75.87%), *Enterococcus faecalis* 68 (23.78%) and *Micrococcus luteus* 54 (18.88%). Several studies [14-16] have also reported similar findings.

Staphylococcus aureus was one of the most isolated bacteria in all the samples collected. It is a commensal and opportunistic pathogen that can cause wide spectrum of infections, from superficial skin infections to severe, and potentially fatal, invasive diseases. [17] Girls who do not wash their hands regularly especially after toilet use can contaminate their hands with *Staphylococcus aureus*. Cross contamination of the bacteria when they get in contact with other girls can occur. The water kept in their rooms can also get contaminated. Staphylococcal water-borne

disease is one of the most common water-borne diseases and is of major concern in public health programs worldwide. [18] A typical water-borne disease caused by *S. aureus* has a rapid onset following ingestion of contaminated food. This is due to the production of one or more toxins by the bacteria during growth at permissive temperatures. [19] However, the incubation period of Staphylococcal water-borne disease depends on amount of toxin ingested. The onset is abrupt. Symptoms include hyper salivation, nausea, vomiting, and abdominal cramping with or without diarrhea. If significant fluid is lost, physical examination may reveal signs of dehydration and hypotension. [10]

Escherichia coli was also another major bacterium that was isolated from the samples. It is commonly found in the lower intestine of warm-blooded organisms. Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination. [20] *E. coli* is expelled into the environment within fecal matter. The bacterium grows massively in fresh fecal matter under aerobic conditions for 3 days, but its numbers decline slowly afterwards. [14] *E. coli* and other facultative anaerobes constitute about 0.9% of gut micro biota, and fecal–oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Hence, cross contamination may occur from the hand of the girls who do not wash their hands after toilet use. They can transfer the bacterium to anything they touch which can then be transferred to another girl that touches the same item. Flies can also transport the bacteria after perching on faeces that was disposed in the open in bushes or drainages and then find their way into the girls' rooms. Most *E. coli* strains do not cause disease, but virulent strains can cause gastroenteritis, urinary tract infections, neonatal meningitis, hemorrhagic colitis, and Crohn's disease. Common signs and symptoms include severe abdominal cramps, diarrhea, hemorrhagic *coli*tis, vomiting, and sometimes fever. [21]

Enterococcus faecalis was isolated in the water samples as well as hand and vaginal swab samples of the girls. These bacteria live in the mouth and vagina. They are very resilient, so they can survive in hot, salty, or acidic environments. [22] *E. faecalis* bacteria do not usually cause problems in healthy people but people with underlying health conditions or a weakened immune system are more likely to get sick. *E. faecalis* infections spread from person to person through poor hygiene. Being that these bacteria are found in faeces, these girls can transmit the infection if they do not wash their hands properly after using the toilet. The bacteria can get into food or onto surfaces such as doorknobs, telephones, and computer keyboards, then they can pass to others who touch same. These are very common conditions found in the hostels of institutions where young students reside. Due of the overcrowded nature of the rooms, it is inevitable that roommates would at one time or another make contact with one another's belongings. Thus, anyone whose hands were contaminated with *E. faecalis* would have transferred the bacterium to other household items and invariably to other roommates. [21] Symptoms include fever, chills, fatigue, headache, abdominal pains, nausea and vomiting.

CONCLUSION

There was a significant number of bacteria isolates obtained from the water, hand and vaginal samples among the adolescent girls that participated in this study. The findings of this indicated that hygiene practices among adolescent girls were very poor. Health education and enlightenment programmes for adolescent girls in both and urban and rural areas were recommended.

Ethical Approval: Ethical approval for this study was obtained from the Ethics Committee of the College of Medicine and

Health Sciences, Abia State University, Nigeria.

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