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Bacteria Load Assessment from Hands of Students in a Tertiary University in South-South Nigeria

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Abstract

Clean hands are the single most important factor in preventing the spread of pathogens and reducing the incidence of infections. The good hand hygiene practices promote health safety and prevent infections. This study was carried out to assess the presence of bacteria from hands of Ambrose Alli University Students. Of all fifty (50) samples analyzed, at least one or two bacteria were isolated. The colony counts of students from each site were properly recorded with the highest found amongst dwellers of male hostels i.e 4.85 x 105cfu/ml, compared to dwellers at female hostels. However, there was considerable increase amongst students at Microbiology laboratory, which may be attributed to lack of good hand hygiene procedures not been adhered to. The considerable increase in the frequency of *Escherichia coli* and as well as *Proteus* sp is indicative of poor hygiene practices. The low colony counts recorded amongst students from school gate could be as a result of the student have taken their bath before coming to school. The frequency of occurrence of the isolates includes: *Staphylococcus aureus* 50 (100%), *Escherichia coli* 35 (70%), *Klebsiella* sp 15 (30%), *Salmonella* sp 21 (42%), Streptococcus sp 10 (20%), *Bacillus* sp 4 (8%), *Pseudomonas* sp 2 (4%) and *Proteus* sp 2 (4%). From the study, it was shown that there was considerable increase in poor hygienic practices amongst students of Ambrose Alli University, Ekpoma Edo State, Nigeria.

Keywords: Hand; Handwash; Hygiene; Bacterial load; Health care.

1. Introduction

The concept of cleansing hands with any antiseptic agent to prevent infections probably merged in the early 19th century. In a paper published in 1825, a pharmacist declared that physicians and others attending patients with contagious diseases would benefit from moistening their hands with liquid chloride solution; however, as a result of the seminar studies by Ignaz Semmelweis and Holmes, hand washing gradually became accepted as one of the most important measures for preventing transmission of pathogens in health-care facilities.

Clean hands are the single most important factor in preventing the spread of pathogens and reducing the incidence of infections. The good hand hygiene practices promote health safety and prevent infections [1]. Every day, diarrhoea diseases from easily preventable causes claim the lives of approximately 5000 young children throughout the world. Personal hygiene and basic sanitation can cut this toll dramatically. A significant number of illnesses and deaths are reported annually as a result of unsanitary conditions. Diarrhoea-related illnesses alone are estimated to cause two to three million deaths per year; a majority of the mortality occurs in children. Infants and young children are the innocent victims of the worldwide failure to make basic sanitation services available to impoverished people.

Hygiene or health education helps the people to understand the causes of their ill health and their possible preventive measures. Infectious diseases commonly spread through faecal-oral route, which includes several gastrointestinal disorders, infectious diarrhea etc. In developing countries, 80% of the diseases are associated with the poor domestic and personal hygiene and about 2.2 million people; mostly children and school students die annually due to diarrhea. Poor domestic and personal hygiene, low health and lack of formal education predispose to

these diseases [2]. The unhygienic habits of most of the people lead to the various infections via hands and fingernails.

Transient bacterial flora which colonize the superficial layers of the skin, are more susceptible to removal by routine hand washing, and cause majority of healthcare-associated infections. Ideally, a hand hygiene preparation should at least have activity against bacteria, yeasts, and coated viruses. Health care workers have three opportunities for the post contamination treatment of hands: (i) the social hand wash, which is the cleaning of hands with plain, non-medicated bar or liquid soap and water for removal of dirt, soil, and different organic substances; (ii) the hygienic or antiseptic hand wash, which is the cleaning of hands with antimicrobial or medicated soap and water ("scrub"); most antimicrobial soaps contain a single active agent and are usually available as liquid preparations; and (iii) the hygienic hand disinfection, which normally consists of the application of an alcohol-based hand rub into dry hands without water. Several studies reveal a gap between knowledge about hand washing with soap and optimal hand washing behavior by staff and patients in healthcare settings, by students in schools, and by mothers whom are caregivers of children at the home and in the community.

Evidence from several studies suggests that the highest compliance by health professionals occurs after touching a patient and after body fluid exposure, and the lowest compliance occurs after touching the patient's surroundings and before performing aseptic procedure. Gaps also exist between attitudes toward compliance with safe behaviors, as well as awareness of the minimal amount of time needed for effective hand washing practice and the need to wash hands prior to putting on and removing surgical gloves [3].

Studies have found a discrepancy between knowledge and practice of hand washing. In one study, 85.6% of students had knowledge about the need to wash hands at critical times (before eating and after using the toilet), but only 24.9% practiced proper hand washing. In another, the extent of hand washing practice among secondary school students showed hand washing was seldom practiced, with hand washing occurring more frequently after touching genitals than before eating meals or after using toilets [4].

There are two types of microbes colonizing hands: the resident flora, which consists of microorganisms residing under the superficial cells of the stratum corneum and the transient flora, which colonizes the superficial layers of the skin, and is more amenable to removal by routine hand hygiene. Transient microorganisms survive, but do not usually multiply on the skin. They are often acquired by health care workers (HCWs) during direct contact with patients or their nearby contaminated environmental surfaces and the organisms are most frequently associated with HCAIs [5].

Microorganisms (germs) responsible for Health Care Acquired Infections (HCAI) can be viruses, fungi, parasites and, more frequently, bacteria. HCAI can be caused either by micro-organisms already present on the patient's skin and mucosa (endogenous) or by micro-organisms transmitted from another patient or health-care worker or from the surrounding environment (exogenous). In most cases, health-care workers' hands are the vehicle for transmission of microorganisms from the source to the patient but patients themselves may also be the source. Generally, microorganisms are transmitted from one patient to another, from one body site to another and from the environment to the patient or vice versa. Health-care workers' hands can become progressively colonized by germs and potential pathogens during patient care. In the absence of hand hygiene, the longer the duration of care, the higher the degree of hand contamination and potential risks to patient safety [2].

2. Material and Method

2.1. Geographical Description of the Study Area

This study was carried out in Ekpoma, Esan West Local Government Area of Edo State, Nigeria. Edo state lies between longitude 06^{o} 04^{l}E and 06^{o} 43^{l}E and latitude 05^{o} 44^{l}N and $07^{o}34^{l}$N with a land mass of 17,450 sq.km located in the South-South geopolitical zone of Nigeria with a population of 3.1 million people. Ekpoma is a semi-urban town with the major occupation of farming, trading, civil servants and students [6].

2.2. Collection of Samples

A total of 100 hand swabs of 50 students were collected from school hostels. Hand swab was collected from the subjects using sterile cotton swab-stick, moistened with autoclaved normal (0.85%) saline. Palmar creases and interdigital spaces were also swabbed, and sample was collected by gently rolling the swab stick over the areas for 6-7 seconds. The volunteers were also asked when they last washed their hands before this procedure. These swabs were directly added into saline solutions of various dilutions under aseptic conditions and 0.2mL from each dilution was inoculated on sterilized MacConkey agar plate and uniformly spread and incubated at 37^{0}C for 24h. After incubation, numbers of CFU were counted and different types of colonies were isolated. The distinct colonies were screened and selected on the basis of morphology, cultural characteristics and identified using standard biochemical tests.

Media used include; MacConkey agar, Deoxycholate agar (XLD agar), *Salmonella-Shigella* agar (S-S agar), Mannitol salt agar, were prepared according to the manufacturer's instruction and were sterilized using autoclave at 121^{0}C for 15 min while glassware was sterilized using hot air oven at 160 ^{0}C for 90 min.

2.2.1. Isolation of Bacteria

The bacteria were isolated using pour plate method. 0.1 ml and 1.0 ml of each sample was aseptically inoculated in a Nutrient Agar and incubated for 24 h at 37^{0}C for the isolation of the bacteria. The microorganisms were sub-cultured into freshly prepared media (CLED) for pure culture isolation.

2.2.2. Identification of Isolates

Tentative identification of isolates was made by Gram staining, motility, oxidase test and cultural characteristic by subculturing on CLED such as yellow-colored colonies of lactose fermenting *E. coli*, greenish colour colonies of *Proteus* spp. greenish blue or blue colonies of *Ps. aeruginosa*, mucoid yellow to whitish blue colonies of *Klebsiella* spp. and deep yellow opaque colonies of *S. aureus* [7]. Confirmation of various bacterial pathogens were made by subculturing on Xylose Lysine Deoxycholate agar (XLD agar), Salmonella-Shigella agar (S-S agar) for *Salmonella* spp, Mannitol salt agar for *Staphylococcus aureus*. MacConkey agar for other enteric pathogens and various special biochemical tests. Catalase test**,** Oxidase test, Urease test, Sugar Fermentation test, Indole test, Motility test and Coagulase test was carried out in identification of the organisms according to procedures by Chessbrough [7].

2.2.3. Gram Staining

Gram staining also called Gram's Method is always the first step in the identification of a bacterial organism. It differentiates bacterial species into two large groups (Gram positive and Gram-negatives) by the chemical and physical properties of their cell walls by detecting peptidoglycan which is present in a thick layer in gram-positive bacteria.

2.2.4. Procedure

This technique was carried out by making a smear on a grease-free glass slide, using a sterile loop heated after which, the smear was heat fixed to affix the bacteria properly to the slide by passing it through flame 4-5 times before air drying. The primary stain (crystal violet) was applied for 1min then, washing with water, followed by the application of Lugol's iodine acting as a mordant and was washed off after 1min. A decolorizer acetone was applied and rigorously washed off after 5secs after which, counter staining was carried out for 1min using a secondary dye neutral red. The stained slide was then allowed to air dry after which, microscopy was carried out by adding a drop of immersion oil on the stained slide which was then viewed with the aid of a microscope using x 100 objective lens.

3. Results

The aim of the study is to isolate and evaluate the bacteria load of palm of hands of students of Ambrose Alli University, Ekpoma Edo State, Nigeria. This study is limited to the isolation and evaluation of bacteria from the palms of hands of the students.

In this study, a total of fifty (50) student palm samples were examined out of which 5 were collected from school gate, 10 from microbiology laboratory, 10 from female hostel in the main campus (Female Hostel 1 and Female Hostel 2) with 5 palm samples collected from each of the hostels while 25 palm samples were collected from male hostels which are Male hostel 1 (10), Male hostel 2 (10) and Male hostel 3 (5) (Table 1). The colony counts of the bacteria isolated are presented in Table 2.

Table 3 shows the Mean±SD of Colony Counts of Bacteria isolated. It was observed that Male hostel 2 has the highest prevalence ($0.9 \times 10^5 \pm 1.7$ cfu/ml) in the study and among the male hostels sampled, followed by Male hostel 3 ($2.2 \times 10^4 \pm 2.0$ cfu/ml), Microbiology Laboratory ($0.9 \times 10^4 \pm 0.7$ cfu/ml), School Gate and Male hostel 1 has similar counts of $0.5 \times 10^4 \pm 0.6$ cfu/ml, Female Hostel 1 ($0.4 \times 10^4 \pm 0.4$ cfu/ml) and Female Hostel 2 ($0.3 \times 10^4 \pm 0.4$ cfu/ml).

Table 4 presents the frequency of occurrence of the bacteria isolates. It was observed that al the samples had *Staphylococcus aureus* (100%), *Escherichia coli* was isolated from 35 samples (70%), *Klebsiella* sp 30%, *Salmonella* sp 42%, *Streptococcus* sp 20%, *Bacillus* sp 8% while *Proteus* spp and *Pseudomonas* sp were 4%

The result of the cultural, morphological and biochemical characteristics of the bacteria isolates is shown in Table 5. Among the organisms isolated, some were Gram positive and others were Gram negative. The organisms on the agar plate showed circular to irregular shape, they were bright yellow and pale colour with dark center with surface appearances (consistency) which were moist, mucoid and dry.

Table-1. Sampling Location of Students

Locations	**No. of Student Samples Examined**
School Gate	5
Microbiology Laboratory	10
Female Hostel	
Female Hostel 1	5
Female Hostel 2	5
Male hostel	
Male hostel 1	10
Male hostel 2	10
Male hostel 3	5
TOTAL	**50**

Table-2. Colony Counts of Bacteria (cfu/ml)

School Gate	Microbiology Laboratory	Female hostel 1	Female hostel 2	Male hostel 1	Male hostel 2	Male hostel 3
1.1×10^2	1.2×10^4	1.5×10^3	1.0×10^2	1.0×10^4	4.8×10^5	4.5×10^4
1.3×10^4	3.2×10^2	1.1×10^4	1.0×10^3	1.0×10^2	1.6×10^3	3.2×10^2
1.3×10^1	1.3×10^2	2.0×10^4	1.1×10^2	1.3×10^4	3.3×10^4	3.2×10^4
1.2×10^3	2.4×10^1	1.2×10^4	1.4×10^3	1.3×10^1	3.0×10^4	3.1×10^4
1.0×10^4	3.3×10^3	1.0×10^4	1.0×10^4	1.0×10^1	3.0×10^2	1.1×10^2
	1.6×10^4			1.2×10^3	1.2×10^4	
	1.4×10^4			1.0×10^4	1.1×10^5	
	1.1×10^4			1.1×10^4	1.5×10^3	
	2.0×10^4			1.2×10^2	1.2×10^2	
	1.0×10^4			1.3×10^3	3.2×10^5	

Table-3. Mean±SD of Colony Counts of Bacteria (cfu/ml)

Location	Mean±SD (cfu/ml)
School Gate	$0.5 \times 10^4 \pm 0.6$
Microbiology Laboratory	$0.9 \times 10^4 \pm 0.7$
Female Hostel 1	$0.4 \times 10^4 \pm 0.4$
Female Hostel 2	$0.3 \times 10^4 \pm 0.4$
Male hostel 1	$0.5 \times 10^4 \pm 0.6$
Male Hostel 2	$0.9 \times 10^5 \pm 1.7$
Male hostel 3	$2.2 \times 10^4 \pm 2.0$

Table-4. Frequency of Occurrence of the Isolates

Isolates	Frequency of Occurrence n = 50	Percentage Prevalence (%)
Staphylococcus aureus	50	100
Escherichia coli	35	70
Salmonella sp	21	42
Klebsiella sp	15	30
Pseudomonas sp	2	4
Bacillus sp	4	8
Proteus sp	2	4
Streptococcus sp	10	20

Table-5. Cultural, Morphological and Biochemical Characteristics of Bacterial Isolates

Organisms Isolated	Cultural characteristics			Morphological characteristics		Biochemical characteristics							Organism
	Shape of colony	Consistency	Colou	Gram	Shape	Motility	Citrate	Coagulase	Catalase	Oxidase	Urease	Indole	
B_1	Circular	Moist	Golden yellow	+	Cocci in cluster	-	-	-	+	-	-	-	*Staphylococcus aureus*
B_2	Irregular	Moist	Bright yellow	-	Rod	+	-	+	+	-	-	+	*Escherichia coli*
B_3	non-capsulate	Non-sporing	Pale colour with dark center	-	Rod	+	-	-	+	-	-	-	*Salmonella* sp
B_4	Circular	Mucoid	Transluce nt cream	-	Rod	-	+	-	+	-	+	-	*Klebsiella* sp
B_5	Irregular	Moist	Light green	-	Rod	+	+	-	+	+	-	-	*Pseudomonas* sp
B_6	Convex	Moist	White	+	Rod	+	-	-	+	-	-	-	*Bacillus* sp
B_7	Flat and Swammy	Buterious	Light Pink	-	Rod	+	+	-	+	-	+	-	*Proteus* sp
B_8	Spherical	Moist	Shinning greyish white	+	Cocci in chains	-	+	-	-	-	-	-	*Streptococcus* sp

KEY: B = Bacteria; + = Positive; - = Negative

4. Discussion

The colony count from the students living in the male hostel was found to be very high being 4.8 $x10^5$±1.7 cfu/ml in Male hostel 2 compared to dwellers of female hostels (Table 2). However, there was considerable increase amongst students sampled in the Microbiology laboratory, which may be attributed to lack of hand hygiene procedures not been adhered to. This study is in accordance with the work of which suggested that females tend to be more hygienic than their male counterparts [8]. The considerable increase in the frequency of *Escherichia coli* and as well as *Proteus* sp is indicative of poor hygiene practices. The low colony counts recorded amongst student from school gate could be as a result of the students having taken their bath before coming to school.

Of all fifty samples analysed, at least one or two bacteria were isolated, it could be a reflection of the level of exposure and thus cross contamination, as the hand is the main organ used for manipulating the environment and for picking microbes in diverse environments. The frequency of occurrence of the isolates as shown in Table 4 includes; *Staphylococcus aureus* 50 (100%), *Escherichia coli* 35 (70%), *Klebsiella* sp 15 (30%), *Salmonella* sp 21 (42%), *Streptococcus* sp 50 (100%), *Bacillus* sp 4 (8%), *Pseudomonas* sp 2 (4%), *Proteus* sp 2(4%).

The high prevalence of *Staphylococcus aureus* and *Streptococcus* sp in all hand swabs could be attributed to the fact that they are normal flora of the human skin. The presence of predominantly *Staphylococcus aureus* on the hands suggests poor hygiene among the students. This does not imply that a disease hazard is probable, but it points to the possibility. These organisms are indigenous to man and are easily and readily recoverable in large numbers from the body surfaces, although they may tend to be pathogenic. Several theories may be advanced concerning the origin of these bacteria. Organisms of this genus are found readily everywhere in the hand as a normal flora. *Staph. aureus* is a relatively common contaminant in and on many items in the environment, but these organisms are related to human contamination to some degree. This bacterium may survive heat, cold and drying as they are usually present in the hand and skin. Again, the person servicing the dispenser may be guilty of handling the laundered towel improperly. These organisms denote environmental contamination, such as dust, and their presence alone on towels does not suggest the existence of a public health hazard but can be pathogenic. However, Williams reports that several investigators, who have searched extensively and carefully, failed to find these organisms in appreciable numbers on the skin of normal adults.

5. Conclusion

Our findings may have implications for the university authority, health professionals and medical educators aiming to design effective programs to promote hand hygiene amongst students and the general populace.

From the analysis, it is therefore concluded that the bacterial loads in the palms of students studied are relatively high with normal flora been more. This indicates a poor hygienic level among the students. Hand hygiene has the potential to prevent diseases and reduce health care–associated infections. The proper drying of hands after washing should be an essential component of effective hand hygiene procedures. From the study, it was shown that there was considerable increase in poor hygienic practices amongst students of Ambrose Alli University, Ekpoma.

Conflict of Interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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Authors' Contributions

The entire study procedure was conducted with the involvement of all writers.

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