# GENDER DIFFERENCE IN MICROBIAL LOAD AND FLORA OF HANDS AMONG SOME PHARMACY STUDENTS, UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

***Ahonkhai I. and Osazuwa E. O.**
Department of Pharmaceutical Microbiology
Faculty of Pharmacy, University of Benin, Benin City, Nigeria.
*Correspondence

**SUMMARY:**

The microbial load and flora on the hands of 114 healthy looking students (57 males and 57 females) of the Faculty of Pharmacy, University of Benin, Benin City were investigated.

The investigation involved washing of hands with sterile water, filtering the resultant "wash-water" by use of the membrane filter and thereafter resuspending the residual deposit on the membrane filter in appropriate medium for viable count, culturing, characterisation and identification.

The common bacterial isolates encountered were *Bacillus subtilis, Staphylococcus* species, *Escherichia coli, Klebiella pneumonia, Neisseria catarrhalis* while the fungal isolates were *Candida albicans, Aspergillus* species; *Trichophyton* species and *Penicillium* species. More microbial load was found on the male hands when compared to that on the female hands. The male hands harboured, on average, 12.50 x $10^5$ viable microbial cells while the female hands had 8.96 x $10^5$ viable cells. There were more *Eschericia coli* and fungi on the female hands than on the male hands. The type and species of micro-organisms on individual hands varied.

It is established that differences exist in the microbial load and flora on the hands of the male and female students. These findings call for greater hand hygiene by the students especially the males.

**KEY WORDS:** Hands, Gender difference, Microbes.

## INTRODUCTION

Normal human microbial flora refers to the population of micro-organisms that inhabit the internal and external surfaces of man. The micro-organisms live on the skin and mucous membranes of the upper respiratory tracts, intestine, orifices and vagina where they obtain nourishment from the secretions and food residues (1).

The human hand, an integral part of the body, is always in direct contact with the other parts of the body and its environment. The hands pick up micro-organisms prevalent in these parts. It is postulated that the different inherent roles and mannerisms exhibited by both sexes in terms of their appreciation of personal hygiene and general activities could be reflected by the resident and transient microbial load on the hands. Apart from revealing any gender variation, the characterization and identification of certain groups of organisms may suggest carrier status of such organisms. For example, it is known that *Staphylococcus aureus*, a common cause of acute pyogenic infections and even fatal septicaemia in men (2, 3, 4) may appear chronically or intermittently in healthy individuals who can be referred to as healthy carriers. The study of health of carriers have been undertaken by some investigators (5, 6). Carriage of micro-organisms on parts of the body has been the subject of study of several investigators (7, 8, 9). In this study, the microbial flora and load of the hands of some female and male pharmacy students who were adjudged to have similar occupational roles (studentship) will be investigated. Any distribution pattern as well as gender differences will be highlighted and possible reasons for such differences will be proffered.

## MATERIALS AND METHODS

**Subjects:** One hundred and fourteen (57 males and 57 females) students of Pharmacy of the University of Benin, Benin City were included in this study. The age range of the students was 18 - 25 years. They were healthy looking with no wounds

or bruises on the hands.

**Growth Media:** Nutrient Agar (Oxoid, Code CM3), Nutrient Broth (Oxoid, Code CM1), MacConkey Agar (Oxoid, Code CM7), MacConkey Broth (Oxoid, Code CM5), Sabouraud Dextrose Agar (Oxoid, Code CM41), Sabouraud Liquid Medium (Oxoid, Coke CM147) and Manitol Salt Agar (Oxoid, Coke CM85) were purchased and prepared according to the manufacturers instructions. The Sabouraud Dextrose Agar was modified before use by the addition of chloramphenicol 250ug $cm^3$ to prevent bacterial growth.

**Recovery of Micro-organisms from Hands:** After, due appraisal of the modus operandi and consent, each student volunteer washed his or her hands. The washing was effected by rubbing the hands together for 30sec under a stream of sterile distilled water (250$cm^3$) poured gently onto the hands. The resultant "wash-water" was collected in a sterile bowl and filtered immediately after the wash, through a membrane filter (Watchman, 0.22um pore size and 47mm diameter) in a filteration unit fitted to a vacuum pump (Gallenkamp, U. K). The membrane filter was aseptically removed and the micro-organisms screened off by the filter were resuspended in 10$cm^3$ sterile water. The resultant suspension was utilised in further investigations.

**Viable Count, Culture and Characterisation:** The microbial suspension obtained as described above, was serially diluted using sterile distilled water and viable counts of an appropriate dilution was performed in accordance with the surface spread technique. The experiments were done in quadruplicate, 2 plats for bacterial count using Nutrient Agar and 2 plats for fungal count using Sabouraud Dextrose Agar. Inoculated bacterial count plates were incubated at 37°C for 24 - 48h while the fungal count plates were incubated at room temperature (28 - 30°C) for 2 - 5 days. The aggregate number of colonies counted on the plates was used to calculate the microbial load on the hands of each subject.

After appropriate incubation of the viable count plates, representative colonies were further screened for characterisation and identification using standard protocols (10, 11).

## RESULTS

The microbial load on the hands of a representative sample of the female and male students are compared in Table 1. The total viable microbial load on the male hands was $5.07 \times 10^7$ cells. The minimum and maximum viable microbial loads on the males hand were $3.01 \times 10^5$ cells and $75.51 \times 10^5$ cells respectively, with a mean value of $12.15 \times 10^5$ cells. Thus, the maximum viable microbial load on the male hands was 25.09 times the minimum load. Similarly, the total viable microbial load on the female hands was $3.53 \times 10^7$ cells. The minimum and maximum viable microbial load on the female hands were $3.04 \times 10^5$ cells and $71.03 \times 10^5$ cells respectively with a mean value of $8.92 \times 10^5$ cells. Hence the maximum viable microbial load on the female hands was 23.37 times the minimum load. From the foregoing, there was greater variation in microbial load on the male hands than on the female hands. In addition, between the male and female hands, there was a difference in microbial load of 43.63% the males having the greater load.

Table 2 presents the frequency of microbial load among the students. It can be deduced from Table 2 that more than half the female population, approximately 64.91%, harboured less than $5.0 \times 10^5$ viable cells on their hands whereas 43.86% of the male population had microbial loads greater than or equal to $6 \times 10^5$ viable cells.

The bacterial isolates recovered from the hands of the students were *Bacillus subtilis*, *Staphylococcus* species, *Escherichia coli*, *Klebsiella aerogenous* and *Neisseria catarrhalis*. The fungal isolates were *Rhizopus* species, Yeasts (*Candida albicans* and *Trichosporon beigelii*), *Penicillium* species, *Aspergillus* species and *Trichophyton* species. This list of isolates includes known pathogens. The Gram positive organisms *Bacillus subtilis* and *Staphylococcus aureus* were the predominant isolates. These two isolates accounted for 68.10% of

all isolates recovered from the female hands. In contrast, the two isolates accounted for 70.4% of the isolates from the male hands. Thus more of these two isolates were on the male hands than on the female hands.

Table 3 presents a comparative frequency distribution of the micro-organisms found on the student hands. Generally, there were more bacteria than fungi on the students' hands. There were more bacteria on the male hands than on the female hands. There were more fungi on the female hands than on the male hands. With regard to individual isolates, it is important to note that the frequency of *Staphylococcus aureus* was lower on the female hands (28.2%) than on the male hands (34.4%). In addition, the frequency of *Neisseria catarrhalis* on the female hands was higher at 2.5% when compared to the male hands with a frequency of 0.2% for this organism. Furthermore, *Escherichia coli* was more frequent on the female hands (5.1%) than on the male hands (2.7%). However, the coliforms, *Escherichia coli* and *Klebiella aerogenous* put together were equally frequent on the male and female hands.

## DISCUSSION

The microbial load and flora of human hands have been investigated with particular reference to female and male students of the Faculty of Pharmacy, University of Benin. Moderate to relatively high number of viable micro-organism were recovered from the students' hands. The microbial load varied considerably within the population and gender investigated. Revealing as these may be, it should be noted that the number of microorganisms recovered were only part of the total number of microorganisms actually on the hands. The method of microbial recovery adopted in this study could not have completely removed all the organisms on the hands. Nonetheless, this method of recovery is considered adequate for its purpose as previous workers (12, 13, 14) have used the method and reported microbial recoveries of between 90% and 100%.

Occupation, environment and age are important determinants of an individuals microbial load and flora (1). In this study, the occupation (studentship), environment (University campus) and age (18 - 25 yrs) cut across both sexes investigated. The only other major factor left is the sex. Interestingly, the greater microbial load was found on the male hands than on the female hands. This difference is probably due to the difference between the sexes in attitude to hand hygiene, the females being generally more concerned in this regard.

The micro-organisms identified in this study are similar, in terms of species, to those variously reported in the literature as members of the microbial flora of human skin and the environment i.e. the soil and air from where they could easily be picked up by the hands (2, 15). The number and frequency of occurrence of the major isolates e. g. Staphylococcus species, in this study is not surprising. Osuide et al (9) studied the nasal carriage of *Staphylococcus* aureus among students of Edo State University, Ekpoma and found that the males carried this organism more than the females. Of the other micro-organisms encountered in this study, it is important to note the frequency of *Neisseria catarrhalis*. This organism is a frequent commensal of the throat and nose. It can easily be picked directly with the fingers or through the use of an handkerchief.

No explanation can be found from this study for the greater presence of micro-organisms on the male hands nor is there any for the greater presence of *Escherichia coli* and fungi on the female hands. This not withstanding, knowledge of the presence of these organisms on the hands dictate the need for constant and repeated hand washing among relevant persons. Such persons include medical and health care delivery personnel as well as students during microbiology practical classes. The suggested hand washing practice is expected to reduce considerably from the hands, micro-organisms that could cause cross or autogenous infections.

**TABLE 1 Comparative Microbial Load on the Hands of Representative Pharmacy Students of University of Benin.**

| SER. NO. | VIABLE COUNT ($X10^5$) ON HAND | |
|---|---|---|
| | MALE | FEMALE |
| 1 | 5.0 | 71.0 |
| 2 | 3.5 | 23.5 |
| 3. | 75.5 | 4.0 |
| 4. | 41.5 | 9.0 |
| 5. | 3.0 | 16.0 |
| 6. | 65.6 | 6.0 |
| 7. | 6.0 | 3.5 |
| 8. | 4.0 | 7.0 |
| 9. | 5.5 | 3.0 |
| 10. | 12.0 | 4.5 |
| 11. | 8.0 | 4.0 |
| 12. | 15.5 | 6.0 |
| 13. | 4.0 | 5.5 |
| 14. | 5.5 | 3.5 |
| 15. | 4.0 | 3.5 |
| 16. | 5.0 | 5.0 |
| 17. | 4.0 | 3.0 |
| 18. | 8.0 | 3.5 |
| 19. | 7.5 | 5.5 |
| 20. | 4.0 | 4.5 |
| 21. | 3.5 | 4.0 |
| 22. | 4.5 | 7.0 |
| 23. | 5.0 | 7.5 |
| 24. | 6.0 | 8.0 |
| 25. | 6.5 | 6.5 |
| Range | 3.0 - 75.5 | 3.0 - 71.0 |
| Mean | 12.50 | 8.96 |
| Std. Deviation | 19.10 | 13.65 |

**TABLE 2 Frequency of Microbial load encountered among male and female students**

| MICROBIAL LOAD (Viable Count x $10^5$) | FREQUENCY | |
|---|---|---|
| | Male (57) | Female (57) |
| 3.00 | 1 | 12 |
| 3.50 | 7 | 11 |
| 4.00 | 7 | 9 |
| 4.50* | 8 | 5 |
| 5.0 | 7 | 1 |
| 5.50 | 2 | 3 |
| $\geq 6.0^+$ | 25 | 16 |

KEY:

* = More than half the female population, approximately 64.91% harboured $< 5.0 \times 10^5$ viable organisms on their hands.

\+ = 43.86% of the male population harboured $\geq 6.0 \times 10^5$ viable organism on their hands

**TABLE 3 Percentage frequency distribution of isolated organisms.**

| ORGANISM | FREQUENCY DISTRIBUTION (%) | |
|---|---|---|
| | Male | Female |
| BACTERIA: | | |
| Bacillus subtilis | | |
| Staphylococcus species | 32.40 | 28.20 |
| Escherichia coli | 2.72 | 5.14 |
| Klebiella aerogenes | 2.73 | 0.32 |
| Neisseria catarrhalis | 0.21 | 2.51 |
| FUNGI: | | |
| Trichophyton species | 1.35 | 1.30 |
| Penicillium species | 6.70 | 5.13 |
| Aspergillus species | 2.74 | 3.80 |
| Yeasts - Candida albicans and Trichosporon beigelii | 1.35 | 2.51 |
| Rhizopus species | 17.50 | 19.22 |

## REFERENCES


1. Jawetz E, Melnick JL and Adeberg E A (1974). "Normal Microbial flora of the human body". Review of Medical Microbiology, Los Altos; California, pp 255 - 257.
2. Burnette GW, Henry WS and Schuster SW (1976) "Staphylococci and staphylococcal infections". Oral Microbiology and Infectious Disease, 1st Ed. Williams & Wilkins, pp 405 - 419.
3. Kosimidis J, Polychronopoulou C, Milona - Petropoulou D, Mavrogonis N et al (1988) "Staphylococcus Infections. The Greek experience". J. Hosp Infection, Vol 11, pp 104 - 115.
4. Ike E. I, Adebayo E O, Okunoghae HO and Ighogboja is (1993) "Bacteriology of Chronic discharging ears in children in Jos, Nigeria". J. Med. Lab. Sci, Vol 3, pp 27 - 30.
5. Doig CM (1971) "Nasal carriage of Staphylococcus aureus in a General Surgical Unit", Brit. J. Surg; Vol 58, p113.
6. Page M1, Hawn CVZ and Cannon JH (1963) "The nasal carriage of staphylococci among children in a rural community". J. Am. Med. Assoc. 183, 1063.
7. Noble WC, Valkenbury HA and Walters CHL (1967) "Carriage of Staphylococcus aureus in random samples of normal population". J. Hyg. (camb), 65, 657.
8. Aly R, Maibach HL, Shinefield HR and Mandel AD (1974) "Staphylococcus aureus carriage in Twins". Am. J. Dis. Child. Vol. 127, p.486.
9. Osuide MI, Agbonlahor DE, Imarenezor of & Odenema E M (1996) "Carriage of Staphylococcus aureus among studens of Edo State University, Ekpoma, Nigeria" J. Med. Lab. Sci. Vol. 5, pp 65 - 68.
10. Cowan ST and Steel J. (1974) "Manual for the identification of Medical Bacteria". Cambridge Univeristy Press, pp42 - 112.
11. Cooper BH. Manual of Clinical Microbiology 2nd Edition Edwin HL et al (1974). Am. Soc. Microbiology. Washington DC pp463 - 7.
12. Lowbury EJL, Lilly HA and Bull JP. (1964) "Disinfection of Hands: Removal of transient organisms" Brit. Med. J. 2, pp 230 - 3.
13. Sprunt K, Redman W and Leidy G (1973) "Antibacterial effectiveness of routine hand washing. Paediatrics Vol 52, pp 264 - 271.
14. Casewell M and Phillips 1 (1977) "Hands as a route of transmission of Klebsiella species". Brit. Med. J. 2 1315 - 1317.
15. Ivler D (1974) Staphylococcus. Am Soc. Microbiology, Washington DC, pp 91 - 95.