Possible Pathogenic Bacteria Present on Stumps of Amputees applying Prostheses: A Case of Mulago National Referral Hospital, Uganda.

Sulaiman Mahmood Kakooza[a,1,2], Kizito Muwonge[b,3]

[a] Faculty of Health Sciences, Uganda Martyrs University, Kampala, Uganda
[b] Faculty of Health Sciences, University of Kisubi, Kisubi, Uganda

Abstract

Background: [a]
The number of amputees using prostheses is decreasing, and the victims complain that prostheses make stumps itchy and cause sores and they thus opt to go without artificial limbs. This has led to increased joblessness among persons with orthopedic disabilities. This research, therefore, sought to determine whether the prostheses increase the number of species of bacteria found on the stumps of amputees since bacteria are known to be the primary cause of the discomfort in form of itches and sores.

Methodology:
Thirty participants were selected for this study, and were divided into two groups; 22 candidates were in the experimental group and four were in the control group, while six withdrew from the research. Members in both groups had their stump surfaces examined for the species of bacteria present at the time of prosthesis fitting. Members in the experimental group used the prostheses while those in the control group did not use the prostheses and both groups were re-examined three weeks later.

Results:
The use of prostheses generally increased the mean number of species of bacteria found on the skin surface of stumps of amputees three weeks after starting to use the artificial limbs although the increase was not found to be statistically significant. No significant change was observed in the mean number of species of bacteria in the control group at the time of prosthetic fitting and after the three weeks of disuse. This implied that the observed increase in bacterial load was truly due to the use of prostheses in the experimental group.

Conclusions:
Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Clostridium tetani, and non-hemolytic *streptococci* were identified on the skin surfaces of the stumps of amputees.

[a]**Email: kakoozasulaiman@gmail.com**
Received: 12th/12/2020 Accepted: 15th/12/2020 Journal of Microbiology

1 Background:

Amputations occur for various reasons and afflict people of all ages with diverse comorbidities. Stump problems are very common, disrupt the day-to-day use of artificial limbs, and hence interfere with the independence and lifestyle of amputees (Salawu *et al.,*2006). In medicine, a missing body part due to trauma, disease, or congenital conditions can be replaced with an artificial device called a prosthesis at the amputee stump. The use of prostheses in lower limb amputees has been observed to lead to skin problems of the amputation stump. Stump skin is an altered cutaneous landscape. The trauma of amputation leaves scars, invaginations of the skin, and bony protrusions, making the skin more fragile and prone to skin problems. This altered skin is not physiologically adapted to support prostheses. However, it is placed in the enclosed environment of the prosthesis socket and made to withstand high compressive and shear forces, increased temperature, and high humidity(Meulenbelt *et al.*, 2011; Kathryn *et al*., 2014). The healing of the stump after amputation has also been reported to bear important local complications associated with this exercise. The affected individuals abandon the prostheses. However, there are also cases of those who receive proper healing after amputation and begin to use prosthetic devices but later develop infections at the stump site. The fitting of prostheses generally presents difficulties at amputated sites resulting in characteristic symptoms of local pain, skin ulceration, and discomfort. Also, symptomatic heat and sweating of the stump, development of sores, or skin irritation at the prosthetic socket may be observed in some amputees. Consequently, these challenges lead to reduced prosthetic use and hence reduced quality of life.

Bacterial sampling from the infected sites usually shows *Staphylococcus aureus, Staphylococcus epidermidis, diphteroids,* and alpha-hemolytic *streptococci*. These are natural inhabitants of healthy skin (Gillespie *et al.,* 2014), however, some of them are also opportunistic pathogens of the skin, that can also be resistant to antibiotics (Rağbetli *et al.,* 2016). The occlusion, high humidity, and raised temperature of the skin at the prosthesis site leads to changed bacterial flora in several particular organisms and types of species. The unnatural environment for the stump skin of a tight-fitting occlusive plastic socket makes the stumps extra vulnerable to infection (Köhler, 1989).

In Uganda, several amputees have failed to appreciate the use of prostheses as a perfect answer to their challenge of lacking a limb or limbs. This is attributed to itches and sores associated with their use (Orthopedic report, Kiruddu General Referral Hospital, 2017). The affected individuals have abandoned the prosthetic devices due to the discomfort they pose. Therefore, this research sought to identify the bacteria occurring on stumps of amputees using upper and lower extremity prostheses.

Skin problems of the stump in limb amputees are observed frequently in clinical practice. This has led to a significant number of amputees abandoning prostheses. This is attributed to problems including mechanically-induced ones like epidermoid cysts, calluses, and verrucous hyperplasia. Allergic reactions such as stump edema, eczema, allergic contact dermatitis, and rash, and bacterial or fungal infections have also been reported. However, the bacterial species causing these infections of the stump in limb amputees attending Kiruddu General Referral Hospital have not been studied extensively.

2 METHODOLOGY

Study design

This research was conducted as an experimental study to investigate the bacteria present on the stumps of amputees and the effect of prostheses on the number of species of bacteria found.

Study site

Samples were collected from Mulago National Referral Hospital (MNRH) and Kiruddu General Referral Hospital (KGRH), Kiruddu, Salama road, Makindye – Kampala, Uganda. The samples were immediately transported to the microbiology laboratory at the University of Kisubi for analysis.

Study population

The study population comprised of amputees who had applied for prostheses and were attending

Mulago National Referral Hospital and KGRH.

Sample size determination

A total of 30 patients were recruited into the study due to the scarcity of patients receiving orthopedic services at the two study sites.

Sample population

The sample size was estimated using the formula n=4pq/L2 (Martin 1988).

Where: n = sample size required

p =estimated number of amputees

q =1-p

L=desired errors (required precision).

Estimated value of p = 81%

Desired error = 10% =0.1

$$n = \frac{4 \times 0.81(1-0.81)}{0.12}$$

n = 62

Thus a sample size of sixty-two amputees was targeted and due to limitations of funds and time, the study approached 30 participants.

Inclusion criteria

These included amputees who had been clinically examined, monitored, trained on how to use prostheses, and finally qualified to acquire one. Only adult participants who consented to be part of the study were included.

Exclusion criteria

Amputees who did not complete clinical examinations or the training were not eligible for the study. Amputees who were already using prostheses were also not eligible for the study. Those who did not consent to participate in the study were not considered and those who consented but did not return for the second examination three weeks after starting to use the prostheses were eliminated from the study. Minors were kept out of this study unless if their parents or guardians consented.

Sample collection

Samples were obtained by swabbing stumps of amputees before acquiring prostheses and three weeks after starting to use them. Swab samples were collected from different sites including the Transfemoral stumps and the Trans-tibial stumps using sterile swabs. The swabs were well labeled with the patient identification number and immediately transported in a cool box at 4°C to the microbiology laboratory at the University of Kisubi for analysis.

Laboratory methods

Sample processing

Swabs were immersed in peptone water immediately after collection to allow amplification of organisms.

Media preparation

Preparation of MacConkey Agar

MacConkey agar (with salt) was prepared by dissolving 44.4g of the powder in 1.0 liter of distilled water, and then autoclaved at 121°C (15psi) for 15 minutes. The media was prepared according to the manufacturer's instructions (Mast Group Ltd., Merseyside, U.K.). Sterility testing was done by incubating the media at 37°C overnight.

Preparation of Blood Agar

MacConkey agar was prepared by dissolving blood agar base powder in 1.0 liter of distilled water, and then autoclaved at 121°C (15psi) for 15 minutes. The base was allowed to cool to 55oC in a water bath. Human blood (6%) was then added to the base and thoroughly mixed without foaming, and poured onto plates, then allowed to cool. This procedure followed the manufacturer's instructions (Mast Group Ltd., Merseyside, U.K.). Sterility testing was done by incubating the media at 37oC overnight and stored at 4oC until required for use in seven days.

Inoculation on blood agar and MacConkey agar

The organisms were transferred from peptone water to solid media by streaking. Blood agar (6%) and MacConkey (with salt) agar were streaked and incubated at 37oC for 24 hours.

Identification of isolated organisms

After 24 hours, the plates were inspected for bacterial growth. The bacteria were identified according to colon size, shape, and hemolysis on blood agar. On MacConkey agar, lactose fermenters or non-lactose fermenters were identified. The suspected colonies were further subcultured to obtain pure colonies that were subjected to Gram staining and biochemical tests.

Confirmatory tests of the isolates

Several biochemical tests were used to confirm the presence of the isolates.

Preparation of biochemical tests

Preparation of triple sugar iron agar (TSA)

This was prepared by suspending 65g in 1.0 liter of distilled water, mixed thoroughly, and distributed into test tubes. The test tubes were plugged with cotton wool and aluminum foil and autoclaved at 121°C for 15 minutes. They were allowed to set as slopes. The procedure followed the manufacturer's instructions (OXOID LTD, Basingstoke, and Hampshire, England). Sterility testing was done by incubating the media at 37oC overnight. These were kept at 4oC until use in seven days. Formation of a yellow butt and a yellow slant, with the production of a colourless gas,

was monitored as a positive test for E.coli on triple sugar iron agar (Cheesbrough, 2006).

Kligler Iron Agar (KIA)

This was prepared by suspending 53.5g in 1.0 liter of distilled water, and heated until was completely dissolved. It was distributed into test tubes which were later plugged with cotton wool and aluminum foil and autoclaved at 121°C for 15 minutes, allowed cooling and solidifying as slopes. The procedure followed the manufacturer's instructions (OXOID LTD, Basingstoke, Hampshire, England). Sterility testing was done by incubating the media at 37oC overnight. The samples of water were inoculated into the KIA and incubated at 37oC overnight. The expected positive result for *E.coli* on KIA would be an acidic butt, an acidic slant with gas production, and no hydrogen sulphide production (Cheesbrough, 2006). The results would be interpreted as an alkaline slant-acid butt (red/yellow) to indicate fermentation of dextrose only. The Acid slant-butt (yellow/yellow) would indicate fermentation of dextrose and lactose. An alkaline slant-alkaline butt (red/red) indicates that neither dextrose nor lactose was fermented (non-fermenter). Cracks split, or bubbles in the medium would indicate gas production, while a black precipitate in the butt would indicate hydrogen sulfide production (Cheesbrough, 2006). The expected observation for *P.aeruginosa* would be a red butt and red slant. The media was quality controlled using *E.coli* (ATCC).

Sulphur Indole Motility (SIM) media

It was prepared by suspending 36.23g into 1.0 liter of distilled water and mixed thoroughly until completely dissolved. It was distributed it into test tubes that were plugged with cotton wool and aluminum foil. The medium was autoclaved at 121°Cfor for 15 minutes. It was allowed cooling and solidifying. The medium was prepared following the manufacturer's instructions (TULIP diagnostics (P) LTD, India). Sterility testing was done by incubating it at 37oC overnight. The media were inoculated and labeled accordingly. Growth was observed around the area of inoculation without gas production hence hydrogen sulphide negative. Motility would be positive for *E.coli*. Also of three drops of Kovac's reagent, it would turn pink and this is an appositive test for indole test. This procedure is as stated by Cheesbrough (2006).

Triple sugar iron test (TSI)

Principle and procedure

TSI agar contained three sugars, glucose, sucrose and lactose, and also ferrous iron (iii) sulphate. Also, it had an indicator phenol red that changed color according to the pH change. For the lactose non-fermenters, they broke down the glucose producing acid that lowered the pH turning the indicator to yellow in the butt. After breaking down the glucose the organisms broke down the amino acids that were present in the medium raising the pH that turned the indicator red in the slant. For the lactose fermenters, they broke down all the three sugars producing enough acid that turned the color of the indicator in the butt and the slant to yellow. The hydrogen sulphide produced by some organisms reacted with iron (iii) sulphate that turned the media black.

TSI tubes were be inoculated with a long straight wire. The well-isolated test

colony recovered from a gar plate was touched with

the end of the inoculating needle

which was then stabbed into

the deep of the tube. When the inoculating wire was removed from the deep of the tube, the slant surface was streaked with a back and forth motion. Inoculated tubes were placed into the incubator at 350C for 18 to 24 hours.

Citrate Utilization test

Principle and procedure

The techniques were used to assist in the identification of *enterobacteria.* The test was based on the ability of an organism to use citrate as the only source of carbon and ammonia as its only source of nitrogen. In the presence of citrate utilizing microbe, sodium citrate that was contained in the medium was broken down and that resulted in a change in pH that caused the color of the indicator to change to blue.

The agar was inoculated with a single colony from the subculture using a long straight wire. The well-isolated test colony recovered from a gar plate was touched with

the end of the inoculating needle

which was then stabbed into the deep of the tube. When the inoculating wire was removed from the deep of the tube, the slant surface was streaked with a back and forth motion. Inoculated tubes were placed into the incubator at 350C for 18 to 24 hours

SIM test (Sulphur indole motility)

SIM medium was a semisolid a gar that was used to determine hydrogen sulphide production, indole formation, and motility.

SIM medium was used to differentiate members of the family *Enterobacteriaceae.*

Haziness that spreads from the stab line indicated a positive test for motility.

A red color development after the addition of Kovacs reagents indicated indole production. A black precipitate indicated hydrogen sulphide production.

Urease test

The Urease test identified those organisms that were capable of hydrolyzing urea to produce ammonia and carbon dioxide. It was primarily used to distinguish urease positive protease from other *Enterobacteriaceae.*

Principle and procedure

Many organisms especially those that infested the urinary tract

had a urease enzyme which was able to split urea

in the presence of water

to release ammonia and carbon dioxide. The ammonium carbonate which

turned the medium alkaline to form

the indicator phenol red from its

original orange-yellow color

to bright pink. No color change was observed in the absence of urease-producing bacteria.

Using a sterile wire loop, a single colony of the bacteria was streaked on the surface of the urea agar slant. The tube was left loosely capped to allow entry of oxygen. The tube was then incubated for 48 hours to 7 days at 350C. The color of the medium changed from light orange to magenta if the organism produced the enzyme urease.

3 RESULTS

A total of thirty (30) subjects were recruited into this research however only twenty-six (26) completed the exercise. The 26 were divided into two groups, 22 candidates were recruited into the experimental group and 04 into the control group. Members in both groups had their stump surfaces examined for the types of bacterial present at the time of prosthesis fitting. Members in the experimental group used the prostheses while those in the control group did not use the prostheses and both groups were re-examined three weeks later. The results were as indicated in Table 4.1.

It was observed that at the time of prosthesis fitting, each amputee had a mean value of 2.32±0.0 of bacterial species at the stump surface. The mean value of 3.05±0.0 bacterial species were isolated from the amputee stumps three weeks after the use of the prostheses. There was an increase in the mean number of species of bacteria that were found on the stumps of amputees after starting to use prostheses. In the control group, each amputee had a mean value of 2.75±0.0 species of bacteria at the time of prosthesis fitting which decreased slightly three weeks later to a mean of 2.0±0.0.This implies that the use of prostheses generally increased the number of strains of bacteria found on the stumps of the amputees.

This study observed that the mean number of strains of bacteria found on the stumps of amputees increased after starting to use prostheses. In a period of three weeks, it short from approximately 2.32 strains per stump to about 3.05 strains per stump (Figure 4.1).

Statistical analysis using a paired mean sample t-test was used to test the hypothesis

H_O: The mean number of species of bacteria before and after use of prostheses is the same.

H_I: The mean number of species of bacteria before and after use of prostheses is different.

Statistical analysis with comparison of a paired mean sample t- test at 95% confidence interval gave a computed t-value of 1.134 and a tabulated t-value of 2.080 at α = 0.05 and v = 21. Since the computed t-value, (1.134) was less than the tabulated t-value (2.080) at α = 0.05 and v = 21, the null hypothesis is not rejected. This test therefore showed that although there was an increase in the number of species of bacteria found on the stumps of amputees, the observed increase was not statistically significant.

H_O: Mean number of bacterial species at time of prosthesis fitting and 3 weeks after is the same.

H_I: Mean number of bacterial species at time of prosthesis fitting and 3 weeks after is different.

Comparison of a paired mean sample t- test at 95% confidence interval gave a computed t-value of 1.834 and a tabulated t-value of 2.353 at α = 0.05 and v = 3. Since the computed t-value, (1.834) was less than the tabulated t-value (2.353) at α = 0.05 and v = 3, the null hypothesis is not rejected. This test therefore confirmed that for the control group,

Table 1. Illustrates the frequency of occurrence of the different specie s of bacteria found on the stumps of amputees before using the prosthesis and three weeks after.

	EXPERIMENTAL GROUP		CONTROL GROUP	
Bacterial species isolated	Frequency of each strain at the time of prosthesis fitting	Frequency of each strain 3 weeks after using the prosthesis	Frequency of each strain at would be time of prosthesis fitting	Frequency of each strain 3 weeks later
S. aureus	18	17	03	03
S.epidemidis	12	17	02	02
E.coli	14	09	03	01
Non-hemolytic strepto-cocci	05	15	02	01
Strep.pyogens	02	09	01	01
TOTAL	**51**	**67**	**11**	**08**

Table 2. shows a s ummary of statistics

Treatment	Mean ± 0.0
Before prosthesis After prosthesis	2.32 3.05
Time of prosthesis Three weeks later	2.75 2.0

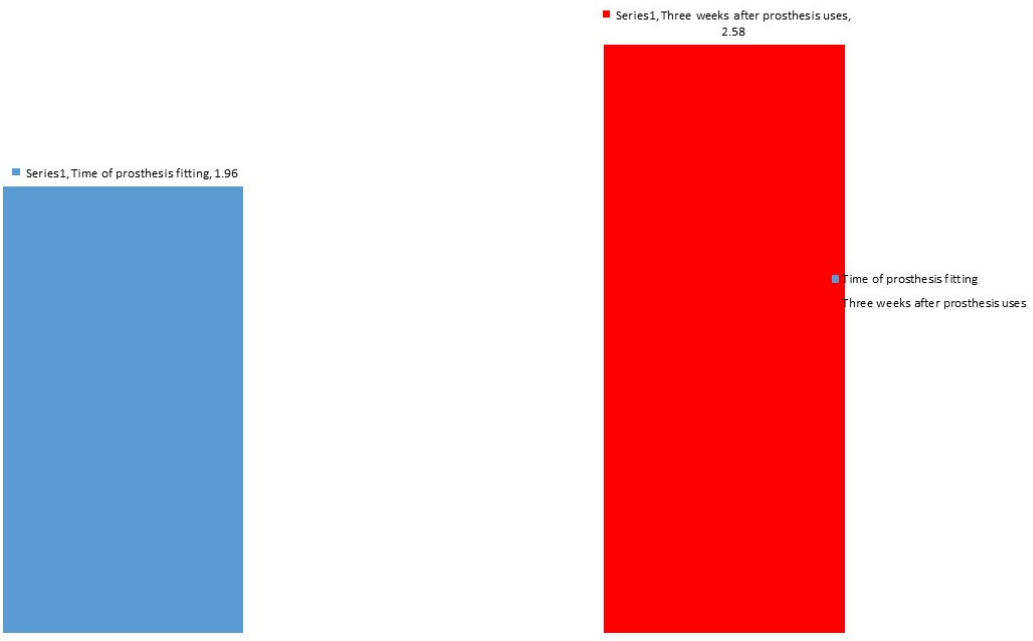

Chart 1. Comparing the number of strains of bacteria on the stump surfaces of amputees before and after using the prostheses.

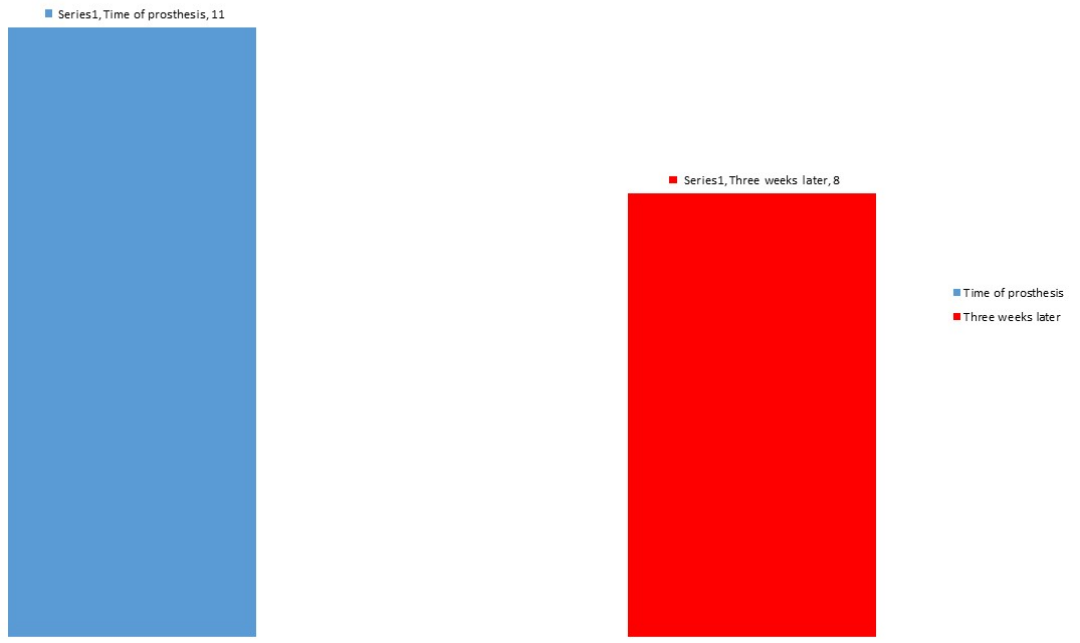

Chart 2. 2: A bar chart comparing the number of strains of bacteria found on the stumps of amputees in the control group at the time of prosthesis fitting and three weeks after.

there was no significant change in the mean number of species of bacteria found on the stumps of the amputees, meaning that any increase noticed in the experimental group would be due to the use of prostheses.

4 DISCUSSION

The use of prostheses showed favored the presence of six species of bacteria on the skin surface of stumps of amputees possibly due to modifications in local micro-environmental factors introduced at this site including warmth and wetness. E*scherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Clostridium tetani,* and the other non-hemolytic *streptococci* have also been found in other studies to be present either on the skin as pathogens or in prosthetic joint infections (Petkovšek *et al.,* 2017; Mohamad *et al.,* 2016). *E. coli* can be non-pathogenic; however, some *E.coli* strains have been reported to possess typical virulence determinants including adhesins such as P fimbriae, iron acquisition systems such as *aerobactin*, host defense avoidance mechanisms such as capsule, and toxins such as hemolysin (Petkovšek *et al.,* 2017).

The isolation of *E.coli* from the skin surfaces of stumps of amputees is of concern based on the unknown extent of its impact at such sites. The presence of *Staphylococcus epidermidis* Coagulase Negative *staphylococci* (CoNS) on the skin is most often regarded as contamination (Mohamad *et al.*, 2016); however, it can cause opportunistic infections. *Staphylococcus epidermidis* can cause biofilm-associated infections on indwelling medical devices (Nguyen *et al.*, 2017). Therefore, the role of *S. epidermidis* in infections during prostheses uses needs to be understood. CoNS preferentially colonize moist areas including armpit, groin, anterior nares, conjunctiva, toe webs, and perineal area (Mohamad *et al*., 2016). Once the host epithelial barrier is compromised, CoNS such as *S. epidermidis* can cause serious infections including bacterial sepsis and foreign body-related infections, with S. epidermidis being the most significant species in that regard (Mohamad, 2016; Nguyen, 2017).

Clostridium tetanae infections follow injuries including abscesses, parenteral drug use, and traumatic and surgical infected wounds. There is a need to investigate whether *C. tetanae* infection can cause localized tetanus upon entry through abscesses due to prostheses use. *Streptococcus* pyogens also isolated in this study can invade the skin and soft tissues and in severe cases leaves infected tissues or limbs destroyed (Madeleine *et al.,* 2009). Also, *Staphylococcus aureus* occurs as both a

commensal and a human pathogen. Approximately 30% of the human population is colonized with *S. aureus*. As a pathogen, *S. aureus* can because skin, soft tissue, pleuropulmonary, and device-related infections among others.

The use of prostheses generally increased the number of species of bacteria found on the skin surface of stumps of amputees (Figure 4.1). However, the increase was not statistically significant. No remarkable change in the mean number of the types of bacteria that were found on the stump surfaces of patients who did not use the prostheses. This implies that the observed increase in bacteria was truly due to the use of the artificial limbs in the experimental category. When the stump skin of an amputee is placed in a tight plastic socket the skin gets warm and wet by perspiration. This decreases the physical strength of the skin and increases the number of bacteria at this site. This interface is not a suitable part for friction resulting from movements as compared to the sole which is well developed for high repeated loads (Drago, 2013).

Some amputee patients complain about the discomfort caused by prostheses which start with itches and later advance into sores. This was also observed in this study and four candidates abandoned the prosthesis because of the itches and sores. This research shows that bacterial multiplication while using prostheses can be attributed to the presence of prostheses. No bacterial infection was reported for three participants during the study. This required investigation of host factors and the role of cleaning and cleaning agents used. However, this research did not investigate the role of cleaning and cleaning agents, and neither did it investigate the role of host factors predisposing to skin infections during prosthetic use including advanced age, prior arthroplasty and underlying joint disease, poor nutritional status, obesity, diabetes mellitus, malignancy remote infections, prior native joint infections and advanced HIV disease (Petkovšek *et al*., 2017). Further studies of larger strain collections and comparisons are needed, especially as in this study the determination of possible bacterial pathogens before amputation, after amputation, before use of prostheses, and after starting to use them could not be done. Additional studies should also be performed to confirm the significance of the detected bacterial strains in skin infections during the use of limb prostheses.

5 CONCLUSIONS AND RECOMMENDATIONS

The use of prostheses generally increased the number of species of bacteria found on the skin surface of stumps of amputees three weeks after starting to use the artificial limbs. However, the increment was statistically insignificant. An increase in bacterial load was observed and it was attributed to the use of prostheses.

Recommendations

The prevalence of each isolated bacterial species should be determined in order to assess the relative significance of each genus in disease causation at amputation sites during the use of prostheses.

Additional studies should also be performed to confirm the significance of the detected bacterial strains in skin infections during the use of limb prostheses.

6 Limitations of the study

Due to the scarcity of orthopedic service centers, only a few patients could be recruited into the study limiting the sample size to thirty patients.

7 ACKNOWLEDGEMENT

I acknowledge the efforts of Mr. Kizito Muwonge and Mr. Asiimwe Albert who have tirelessly supported me during this research. I am thankful to my friends for their collaboration and support for the completion of this course of study. i also thank the microbiology laboratory at the University of Kisubi for having allowed this research to be carried out.

A References:

1). Salawu. A, C. Middleton, A. Gilbertson, K. Kodavali, & Neumann. V. (2006). “Stump Ulcers and Continued Prosthetic Limb Use.” Sage Journals, Volume: 30 issue: 3, page(s): 279-285. https://doi.org/10.1080/03093640600836139

2). Meulenbelt, H. E., Geertzen, J. H., Jonkman, M. F., & Dijkstra, P. U. (2011). Skin problems of the stump in lower limb amputees: 1. A clinical study. Acta dermato-venereologica, 91(2), 173–177. https://doi.org/10.2340/00015555-1040

3). RaLbetli C, Mehmet. P, Yasemin. B, Huseyin . G, & Nesrin .C.(2016). “Evaluation of Antimicrobial Resistance in Staphylococcus aureus Isolates by years.” Hindawi Publishing CorporationInterdisciplinary Perspectives on Infectious DiseasesVolume,

Article ID 9171395, 4 pages. http://dx.doi.org/10.1155/2016/9171395

4). Köhler. P, Lindh L., & Björklind. A, (1989) "Bacteria on stumps of amputees and the effect of antiseptics." Digital resources foundation for the orthotics and prosthetics community. PP:149-151 Vol:13https://doi.org/10.3109/03093648909079424PMid:2514413

5). Mohamad M, Deabate L, Belaieff W, Bouvet C, & Zingg M, (2016). Prosthetic Joint Infections Due to Coagulase-Negative Staphylococci, Int J Infect; 3(1):e32883. doi: 10.17795/iji-32883.https://doi.org/10.17795/iji-32883

6). Petkovsek Z, Elersic K, Gubina M, Zgur-Bertok D, Starcic & Erjavec M. (2009). Virulence potential of Escherichia coli isolates from skin and soft tissue infections. Journal of Clinical Microbiology. 2009 Jun;47(6):1811-1817. DOI: 10.1128/jcm.01421-08

PMid:19357208 PMCid:PMC2691073

7). Nguyen TH, Park MD & Otto M (2017) Host Response to Staphylococcus epidermidis Colonization and Infections. Front. Cell. Infect. Microbiol. 7:90. doi: 10.3389/fcimb.2017.00090

8). Nguyen, T. H., Park, M. D., & Otto, M. (2017). Host Response to Staphylococcus epidermidis Colonization and Infections. Frontiers in cellular and infection microbiology, 7, 90. https://doi.org/10.3389/fcimb.2017.00090

9). Cunningham M. W. (2000). Pathogenesis of group A streptococcal infection. Clinical microbiology reviews, 13(3), 470-511. https://doi.org/10.1128/cmr.13.3.470-511.2000

PMid:10885988 PMCid:PMC88944

10). Drago. L, Signori .V, Vecchi. E , Vassena. C , Palazzi.E , Cappelletti. L , Romanò. D , & Romanò. C.L, (2013) "Use of dithiothreitol to improve the diagnosis of prosthetic joint infections." Journal of Orthopaedic research.https://doi.org/10.1002/jor.22423

PMid:23817975

11). Buikema, K., & Meyerle, J. (2014). Amputation stump: Privileged harbor for infections, tumors, and immune disorders, Clinics in Dermatology. 32, 670 - 677.https://doi.org/10.1016/j.clindermatol.2014.04.015PMid:25160109

12). Cheesebrough, M. (2009). District laboratory practice in tropical countries. Cambridge university press. New York.

13). Cunningham, M. W. (2000). Pathogenesis of Group A Streptococcal Infections. Clinical Microbiology Reviews, 470-511.https://doi.org/10.1128/CMR.13.3.470

PMid:10885988 PMCid:PMC88944

14). Drago, D. A. (2013). Living Safely, Aging Well: A Guide to Preventing Injuries at Home. JHU Press.

15). Gillespie, & H, S. (2014). Medical microbiology illustrated. Butterworth-Heinemann.

16). Lewis, P. R. (2012). Manufacturing defects in polymeric medical devices. Durability and Reliability of Medical Polymers. 225.https://doi.org/10.1533/9780857096517.2.183https://doi.org/10.1533/9780857096517.2.225

17). Tillander, J., Hagberg, K., & Hagberg, L.,(2010). Osseointegrated titanium implants for limb prostheses attachments: infectious complications. Clinical Orthopaedics and Related Research®,. 468 (10), 2781-2788.https://doi.org/10.1007/s11999-010-1370-0PMid:20473597 PMCid:PMC2939339