OCCURRENCE OF HUMAN PATHOGENIC BACTERIA IN LAKE VICTORIA SHORE WATER AND OREOCHROMIS NILOTICUS AT KASENYI LANDING SITE, WAKISO DISTRICT IN UGANDA: A CROSS-SECTIONAL STUDY.

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Abstract

Background:

As of 2008; human pathogenic bacteria were being identified in lake water and fish and by 2017; it was still a persistent problem in the aquatic environment up to date. Sources attributed to their presence in lake water and fish include; surface run-off from land, sewage discharge, sewage overflow, run-off of domestic and wildlife animal waste, and direct waste deposition by grazing animals among others. Mitigation for aquatic ecosystems degradation over the years has been attempted through field and/or laboratory-based bacteriological monitoring of lake surface water quality.

Methods:

A cross-sectional laboratory-based survey was undertaken at the Kasenyi landing site. Thirtyone (n=31) Lake Victoria shore water samples and thirty (n=30) Oreochromis niloticus samples were collected and bacteriologically examined for Staphylococcus species, Enterococcus species, and Enterobacteria. Data were analyzed using Microsoft Excel 2013 software to compute the chi-square and p-values.

Results:

Citrobacter freundii was the most occurring human bacterial contaminant in water at 71% (22/31) while Klebsiella pneumoniae was the least occurring human bacterial contaminant at 6% (2/31). In the Orechromis niloticus organ samples; the most occurring was Enterococcus species at 77.5% (93/120) while the least occurring was Citrobacter freundii at 39% (47/120). There was no statistically significant relationship between the occurrence of bacteria in the Lake Victoria shore water and in the Oreochromis niloticus organs.

Conclusion:

Citrobacter freundii had the highest occurrence in the Lake Victoria shore water while Enterococcus species had the highest occurrence in all the four Oreochromis niloticus organs (i.e. skin, intestine, gills, and muscle). A chance-based relationship between the occurrence of bacteria in the Lake Victoria shore water and in the specific Oreochromis niloticus organs was established.

Recommendation:

A larger sample size research to evidence the potential sources of human pathogenic bacteria in the Lake Victoria shore water and the fish at the Kasenyi landing site.

Keywords: Water, Oreochromis, Citrobacter, Enterococcus, Staphylococcus, Submitted: 2023-05-26 Accepted: 2023-06-01

1. Introduction.

Lake Victoria is the world's second-largest and the largest by surface area in Africa and Uganda; it had over five hundred fish species before the 1960s but a decline has been experienced over the years to less than three hundred fish species altogether due to exotic species introductions, eutrophication, and ecosystem degradation; which could translate into misfortune for millions of people currently dependent onto it (https://www.lv bcom.org and https://blogs.worldbank.org). To try mitigating for aquatic ecosystems bacterial degradation; field and/or laboratory-based bacteriological monitoring of lake surface water quality has been attempted over the past years via the isolation and identification of bacteriological parameters which have always reflected the status quo of the aquatic habitat (Nurul et al., 2016; and Meron et al., 2020). The sources attributed to the introduction and subsequent presence of the diverse populations of human pathogenic bacteria into lake water have included; surface runoff from land, sewage discharge, sewage overflow, run-off of domestic and wildlife animal waste, direct waste deposition by grazing animals among others, overpopulation, soil erosion, and aesthetic practices such as swimming; among others (https ://www.lvbcom.org, https://thefishsite.com, and https://www.pca.state.mn.us).

As of 2008; pathogenic bacteria such as *Escherichia coli*, *Salmonella species*, and *Vibrio species* among others were being identified in lake water (Abhirosh et al., 2008), and by 2017; the same pathogenic bacterial groups were still a persistent problem in water (Vincy et al., 2017). Of recent; *Escherichia coli* and *Pseudomonas species* have still been detected in lake shore water at a lagoon in the Amazon (Rondón-Espinoza et al., 2022).

On the other hand; fish has been one of the main foods for humans over the centuries and has been characterized by a high nutritional value and easy digestibility (Leisner et al., 2001). This nutritional value does not only serve the needs of the human but also those of microorganisms like bacteria among which human pathogens may be found such as; *Enterobacteriaceae spp*, *Vibrionaceae spp*, *Aeromonadaceae spp*, *Salmonella spp*, *Pseudomonas spp*, *Escherichia spp*, *Shigella spp*, *Klebsiella spp*, *Staphylococcus spp*, *Streptococcus spp*, and *Enterococcus spp* among others (Adam and Tobaias., 1999; Cheesbrough, 2006; Petronillah et al., 2014; Gultepe et al., 2017; Meron et al., 2020; and Rondón-Espinoza et al., 2022).

The presence of such organisms in fish has been linked to serious economic losses, degradation of the nutritional value, and human infections (Getu, Misganaw, and Bazezew, 2015; Abelti, 2016; and Cheesbrough, 2006). Further still; the bacterial population profiles associated with fish tend to mirror the bacterial quality and/or nature of the habitat and thus can be based on laboratory-based bacteriological analysis to deduce the bacterial quality of the aquatic environment as has been done in the past years with several fish species including Oreochromis niloti*cus* which is one of the three main commercial fish species along with Lates niloticus and Rastrineobola argentea (Petronillah et al., 2014; Nurul et al., 2016; and NaFIRRI, 2013). Currently; there has been no study done at the Kasenyi landing site to demonstrate the occurrence profiles of human pathogenic bacteria in the Lake Victoria shore water and in the landed Oreochromis niloti*cus*; a gap that this study aimed at.

2. Methodology.

2.1. Study design.

This was a two-month cross-sectional study in which Lake Victoria surface water samples were collected from the shores at Kasenyi landing site and the fresh Oreochromis niloticus was purchased from the vendors at the site in February and March 2023. These were then transported on the cold chain to the microbiology laboratory at the Faculty of health sciences of the University of Kisubi for bacteriological analysis.

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2.2. Study area.

The study was carried out at Kasenyi trading center and landing site which is located 8km off Abaita Ababiri along the Entebbe-Kampala highway in Entebbe Municipality, Katabi sub-county, Wakiso District in Uganda.

2.3. Units of Analysis.

The study employed two units of analysis that are: the surface shore water samples and the Oreochromis niloticus fish specimen. These were then subjected to bacteriological analysis for the isolation and identification of the human bacterial pathogens i.e. Staphylococcus species, Enterococcus species, and Enterobacteria species.

2.4. Sample size determination.

The sample size for this particular study was determined based on the central limit theorem (http://sphweb.bumc.bu.edu/otlt/MPHModules/ QuantCore/PH717_Probability/PH717_Probab ility8.html) which considers an amount of, n > 30, to be a sufficiently large sample to represent a large population. This was applied independently to both units of analysis i.e. the water samples and the fish specimens.

2.5. Water Sampling and bacteriological analysis.

Ten (10) 100 ml water samples would be randomly collected along different points on the Lake Victoria shore water every two weeks intervals. These would be transported on a cold chain within two hours of collection to the microbiology laboratory at the faculty of health sciences of the University of Kisubi for bacteriological analysis of Staphylococcus species, Enterococcus species, and Enterobacteria species. The pour plate method was used to inoculate 10 ml of each water sample onto three different selective agar media and subsequent overnight incu-Mannitol salt agar (Conbation at 37°C i.e. dalab Chapman medium, Batch number: 206042) for Staphylococcus species; Bile esculin azide agar (Conda pronadisa, Cat: 1372.00, Batch number: 702142) for Enterococcus species and Mac Conkey bile salts agar (Oxoid limited, Ref:

CM0007, Lot number: 2966689) for Enterobacteria species. Biochemical tests were further performed for the identification of Enterobacteria species i.e. carbohydrate sugar fermentation and hydrogen sulphide gas production using triple sugar iron agar medium (Oxoid limited, Ref: CM0277, Lot:1155946); citrate utilization test using Simmon's citrate agar medium (Becton, Dickinson and Company, Ref: 266540, Lot: 3105010); hydrgen sulphide gas production, indole production, and motility test using SIM medium and Kovac's reagent (Conda pronadisa, Cat: 1514.00, Batch: 802012).

2.6. Fish Sampling and bacteriological analysis.

Ten (10) fresh whole fish specimens would be randomly purchased from available vendors at the landing site for every two weeks interval. These would be transported on a cold chain within two hours of collection to the microbiology laboratory at the faculty of health sciences of the University of Kisubi for bacteriological analysis of Staphylococcus species, Enterococcus species, and Enterobacteria species. Approximately two grams (2g) for each of the fish organs including skin, muscle, intestine, and gills were primarily inoculated by immersing in 10ml of peptone water and incubated overnight at 37C. This was followed by secondary inoculation by streaking on three different selective agar media and subsequent overnight incubation at 37°C i.e. Mannitol salt agar (Condalab Chapman medium, Batch number: 206042) for Staphylococcus species; Bile esculin azide agar (Conda pronadisa, Cat: 1372.00, Batch number: 702142) for Enterococcus species and Mac Conkey bile salts agar (Oxoid limited, Ref: CM0007, Lot number: 2966689) for Enterobacteria species. Biochemical tests were further performed for the identification of Enterobacteria species i.e. carbohydrate sugar fermentation and hydrogen sulphide gas production using triple sugar iron agar medium (Oxoid limited, Ref: CM0277, Lot:1155946); citrate utilization test using Simmon's citrate agar medium (Becton, Dickinson and Company, Ref: 266540, Lot: 3105010); hydrogen sulphide gas production, indole production, and motility test using SIM medium and Kovac's reagent (Conda pronadisa, Cat: 1514.00, Batch: 802012).

2.7. Data analysis

Statistical analysis was carried out using the Microsoft Excel 2013 computer software program to compute pie charts and bar graphs to describe the occurrence profiles while the relationship between the occurrence profiles of bacteria in water and that in fish was computed using the P-value and Chi-square test at 0.05 level of significance.

3. Results.

- 3.1. Occurrence of human pathogenic bacteria in Lake Victoria shore water at Kasenyi landing site.
 - 3.1.1. The overall occurrence of bacterial isolates in Lake Victoria shore water.

From the thirty-one (n=31) water samples; a total of seventy-nine bacterial isolates (nbiw=79) across four groups that is; two bacterial species (i.e. Citrobacter freundii and Klebsiella pneumoniae) and two bacterial genera (i.e. Staphylococcus species and Enterococcus species) were obtained [figure 1]. The most occurring was Citrobacter freundii accounting for 58% (46/79) followed by Enterococcus species at 23% (18/79) with the least being Klebsiella pneumoniae at 3% (2/79).

3.1.2. Percentage occurrence of individual bacterial isolates in Lake Victoria shore water.

From the thirty-one water samples (n=31); Citrobacter freundii was the most occurring human bacterial contaminant at 71% (22/31; p=.019) while Klebsiella pneumoniae was the least occurring human bacterial contaminant at 6% (2/31; p=1.239E-06) [figures 2, 3, 4 and 5].

3.2. Occurrence of human pathogenic bacteria in Oreochromis niloticus at Kasenyi landing site.

Overall occurrence profiles of human pathogenic bacteria in *Oreochromis niloticus*

From the one hundred and twenty (n=120) fish organ samples; a total of two hundred and ten bacterial isolates (n_{bif} =210) across three groups that is; one bacterial species (i.e. *Citrobacter freundii*) and two bacterial genera (i.e. *Staphylococcus species* and *Enterococcus species*) were obtained [figure 6]. The most occurring was *Enterococcus species* accounting for 77.5% (93/120; p=1.692E-09) followed by *Staphylococcus species* at 58% (70/120; p=.068) with the least being *Citrobacter freundii* at 39% (47/120; p=.018).

3.3. Oreochromis niloticus organ-based occurrence profiles.

Thirty samples were analyzed for each organ i.e. skin (nskin=30), intestine (nintestine=30), gills (ngills=30), and muscle (nmuscle=30): For the skin; the highest occurrence rate was attributed to Enterococcus species (80%; 24/30; p=.001) while the least occurrence was for Citrobacter freundii (23%; 7/30; p=.003) [figure 7]. For the intestines; the highest occurrence was still for Enterococcus species (70%; 21/30; p=.028)while the least occurrence was for Staphylococcus species (27%; 8/30; p=.011) [figure 8]. For the gills; the highest occurrence was observed for Enterococcus species (73%; 22/30; p=.011) while the lowest occurrence was observed for Citrobacter freundii (37%; 11/30; p=.144). For the muscle; still, Enterococcus species were obtained as the highest occurring (87%; 26/30; p=5.904E-05) while the lowest occurrence was obtained as Citrobacter freundii (40%; 12/30; p=.273).

3.3.1. Overall relationship.

Overall; chi-square (X^2) and p-value analysis revealed statistical significance for the occurrence profiles of Klebsiella pneumoniae $(X^2=8.549, p=.036)$ and no statistical significance for the occurrence profiles of Citrobacter freundii $(X^2=0.034, p=.998)$, Staphylococcus species $(X^2=0.003, p=.999)$, and Enterococcus species $(X^2=0.039, p=.998)$ as shown in table 1.

3.3.2. Oreochromis niloticus organ-based relationship.

As shown in tables 2, 3, 4 and 5; chi-square (X^2) and p-value analysis revealed no statistically



Figure 1: Overall occurrence of bacterial isolates in the lake shore water

B	ACTERIA	P-value	X ² (df=3)	CV (df=3, α=0.05)
1	Citrobacter freundii	0.99831678	0.034451183	7.814727903
2	Klebsiella pneumoniae	0.035935609	8.548586205	7.814727903
3	Staphylococcus species	0.999957216	0.002959594	7.814727903
4	Enterococcus species	0.997964375	0.039142514	7.814727903

Key: P-value = probability value; X^2 = chi-square value; df = degrees of freedom; CV = critical value; α = level of statistical significance.

significant relationship between the occurrence profiles of all the bacteria isolated from the Lake Victoria shore water and from the specific Oreochromis niloticus organs investigated. The relationship was thus entirely bound to chance whereby for the skin, gills and muscle; the chance-based relationship was highest (X^2 =0.007, p=.999; X^2 =0.017, p=.999; and X^2 =0.023, p=.999) for Staphylococcus aureus [tables 2, 4 and 5] respectively; and highest (X^2 =0.013, p=.999) for Citrobacter freundii only in the intestines [table 3]. Further still; the chance-based relationship was similarly lowest $(X^2=4.742, p=.192)$ for Klebsiella pneumoniae amongst all the four Oreochromis niloticus organs [tables 2 to 5].

4. Discussion.

4.1. Occurrence of human pathogenic bacteria in Lake Victoria shore water at Kasenyi landing site.

From this study; thirty-one water samples (n=31) were analysed; *Citrobacter freundii* was



Figure 2: Occurrence profile for Citrobacter freundii in the lake shore water



Figure 3: Occurrence profile for Klebsiella pneumonia in the lake shore water



Figure 4: Occurrence profile for Staphylococcus species in the lake shore water.

	Table 2: Relationship	p between bacteria in Lake Victoria shore water and onOreochromis niloticus skin
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B	ACTERIA	P-value	X ² (df=3)	CV (df=3, α=0.05)
1	Citrobacter freundii	0.975227287	0.214427414	7.814727903
2	Klebsiella pneumoniae	0.191717112	4.741712273	7.814727903
3	Staphylococcus species	0.999837158	0.007220926	7.814727903
4	Enterococcus species	0.997947996	0.039353861	7.814727903

Key: P-value = probability value; X^2 = chi-square value; df = degrees of freedom; CV = critical value; α = level of statistical significance.

Table 3: Relationship between bacteria in Lake Victoria shore water and inOreochromis niloticus intestines

B	ACTERIA	P-value	X ² (df=3)	CV (df=3, <i>a</i> =0.05)
1	Citrobacter freundii	0.999628209	0.012533586	7.814727903
2	Klebsiella pneumoniae	0.191717112	4.741712273	7.814727903
3	Staphylococcus species	0.999082124	0.022942188	7.814727903
4	Enterococcus species	0.999433464	0.016610451	7.814727903

Key: P-value = probability value; X^2 = chi-square value; df = degrees of freedom; CV = critical value; α = level of statistical significance.



Figure 5: Occurrence profile for Enterococcus species in the lake shore water.



Figure 6: **Key**: Series 1 – Positive results; Series 2 – Negative results. Overall occurrence profiles of human pathogenic bacteria in Oreochromis niloticus organs



Figure 7: Key: Series 1 – Positive results; Series 2 – Negative results. Occurrence profiles of human pathogenic bacteria on the skin of Oreochromis niloticus



Figure 8: **Key**: Series 1 – Positiveresults; Series 2 – Negative results Occurrence profiles of human pathogenic bacteria in the intestines of Oreochromis niloticus



Figure 9: **Key**: Series 1 – Positive results; Series 2 – Negative results Occurrence profiles of human pathogenic bacteria in the gills of Oreochromisniloticus

Table 4: Relationship between bacteria in Lake Victoria shore water and inOreochromis niloticus gills

B	ACTERIA	P-value	X ² (df=3)	CV (df=3, α=0.05)
1	Citrobacter freundii	0.997001468	0.050792384	7.814727903
2	Klebsiella pneumoniae	0.191717112	4.741712273	7.814727903
3	Staphylococcus species	0.999433464	0.016610451	7.814727903
4	Enterococcus species	0.999082124	0.022942188	7.814727903

Key: P-value = probability value; X^2 = chi-square value; df = degrees of freedom; CV = critical value; α = level of statistical significance.

Table 5: Relationship between bacteria in Lake Victoria shore water and inOreochromis niloticus muscle

B	ACTERIA	P-value	X ² (df=3)	CV
1	Citrobacter freundii	0.998740957	0.028353625	7.814727903
2	Klebsiella pneumoniae	0.191717112	4.741712273	7.814727903
3	Staphylococcus species	0.999082124	0.022942188	7.814727903
4	Enterococcus species	0.995999117	0.061693661	7.814727903

Key: P-value = probability value; X^2 = chi-square value; df = degrees of freedom; CV = critical value; α = level of statistical significance.



Figure 10: Key: Series 1 – Positiveresults; Series 2 – Negative results Occurrence profiles of human pathogenic bacteria in the gills of Oreochromisniloticus

the most occurring human bacterial contaminant at 71% (22/31) while *Klebsiella pneumoniae* was the least occurring human bacterial contaminant at 6% (2/31) [figures 2, 3, 4 and 5].

These findings are similar to those of Banciu et al. (2021) who identified *Citrobacter freundii* in surface waters of their study site and such could be attributed to soil surface run-off based on the fact that Kasenyi landing site lacks any form of physical barriers between shore waters and the bare land neighboring the shore.

Further still; these findings are different from those of Rondón-Espinoza et al., (2022), Gultepe et al. (2017), and Bisimwa et al. (2022) who identified no such bacteria as *Citrobacter freundii*, *Klebsiella pneumoniae*, *Staphylococcus species*, and/or *Enterococcus species*. This difference has been attributed to the difference in the scope of bacteriological analysis exhibited between this current study and the former who never comprehensively considered human pathogenic bacteria in their scope.

4.2. Occurrence of human pathogenic bacteria in Oreochromis niloticus at Kasenyi landing site.

From this study; thirty samples were analyzed for each organ i.e. skin (nskin=30), intestine (nintestine=30), gills (ngills=30), and muscle (nmuscle=30): For the skin; the highest occurrence rate was attributed to Enterococcus species (80%; 24/30) while the least occurrence was for Citrobacter freundii (23%; 7/30) [figure 7]. For the intestines; the highest occurrence was still for Enterococcus species (70%; 21/30) while the least occurrence was for Staphylococcus species (27%; 8/30) [figure 8]. For the gills; the highest occurrence was observed for Enterococcus species (73%; 22/30) while the lowest occurrence was observed for Citrobacter freundii (37%; 11/30) [figure 9]. For the muscle; still, Enterococcus species were obtained as the highest occurring (87%; 26/30)while the lowest occurrence was obtained as Citrobacter freundii (40%; 12/30) [figure 10].

These findings are similar to those of Rondón-

Espinoza et al., (2022) who identified Staphylococcus aureus in their research and Nurul et al. (2016) who isolated Enterococcus faecalis in their work, and such similarity could be attributed to the similarity in the bacteriological scope of analysis between the earlier researchers and this current study; Further still; the presence of Staphylococcus species in the organs of Oreochromis niloticus could be linked to the bathing practices that are common along the shore and thus introducing the Staphylococcus species into the Lakeshore water. In the same regard; the presence of Enterococcus species in the organs of Oreochromis niloticus highlights a possibility of faecal contamination in Lake Victoria even in areas that may not necessarily be at or near the Kasenyi landing site. Additionally; the presence of Citrobacter freundii in the organs of the Oreochromis niloticus could still be attributed to the soil surface runoff based on the fact that the Kasenvi landing site lacks any form of physical barriers between shore waters and the bare land neighboring the shore. It is also the researchers' thought that this may be the case even in other areas that are bordering the shores of Lake Victoria other than the Kasenvi landing site.

However; these findings are different from those of Meron et al. (2020), Gultepe et al. (2017) and Shinkafi and Ukwaja (2010) who never identified any of *Citrobacter freundii*, *Staphylococcus species*, and *Enterococcus species* bacteria in their study. This difference has been attributed to the difference in the bacteriological scope of analysis between the earlier researchers and this current study where the earlier researchers mainly considered bacteria that are pathogenic to fish and not to humans.

4.3. Relationship between bacterial occurrence profiles in Lake Victoria shore water and Oreochromis niloticus.

From this study; the overall relationship between the occurrence of bacteria in water and the occurrence of bacteria in all Oreochromis niloticus organs revealed a statistically significant outcome (X2=8.549, p=.036) for Klebsiella pneumoniae and thus establishing statistical evidence on the presence of the bacterium in the shore water and its complete absence in the Oreochromis niloticus organs. Further still; the analysis revealed a chance-based relationship for Citrobacter freundii (X2=0.034, p=.998), Staphylococcus species (X2=0.003, p=.999), and Enterococcus species (X2=0.039, p=.998) implying that there is a very high chance of isolating such bacteria from both the Lake shore water and the Oreochromis niloticus organs [table 1].

Additionally; analysis of the relationship between the occurrence of bacteria in the Lakeshore water and in the individual Oreochromis niloticus organs entirely revealed a chance-based outcome for all the isolated bacteria [tables 2 to 5] and thus implying that there is a very high chance of isolating such bacteria from both the Lakeshore water and the Oreochromis niloticus organs.

5. Conclusion.

Citrobacter freundii had the highest occurrence in the Lake Victoria shore water and this could be due to soil surface run-off. For the Oreochromis niloticus organs: Enterococcus species had the highest occurrence in all four organs i.e. skin, intestine, gills, and muscle and this could be linked to a possibility of faecal contamination into the Lake Victoria water. A statistically significant difference between the presence of Klebsiella pneumoniae in Lake Shore water and its absence in the Oreochromis niloticus organs has been established. Further still; there is a very high chance of isolating Staphylococcus species, Citrobacter freundii, and Enterococcus species from both the Lakeshore water and the Oreochromis niloticus organs.

6. Limitations of the Study.

The study only considered human pathogenic bacteria including Staphylococcus species, Enterococcus species, and Enterobacteria. Additionally; the level of bacterial contamination has not been established for both the Lake Shore water and the Oreochromis niloticus organs. Further still; the potential sources of bacterial contamination of the Lakeshore water and the Oreochromis niloticus organs have also not been established.

7. Recommendation.

The researchers in this current study suggest the carrying out of a larger sample size research that could evidence the: introduction of Citrobacter freundii by soil surface run-off; potential sources of faecal contamination into the Lake Victoria water; potential sources of all the isolated bacteria into the Lake Victoria shore water.

8. Data Availability.

All the raw data that was used to compile the results herewith can be availed on request provided valid reasons.

9. Conflicts Of Interest.

There are no conflicts of interest associated with this work.

10. Authors Contributions

Mr. Lujjimbirwa Fortunate was the lead author and data analyst as per this write-up; Dr. Odoki Martin was the supervisor and primary coauthor; Mr. Kasozi James, Mr. Ssentongo Vianney, Mr. Mwesigwa Phillip, and Mrs. Nagingo Patricia were the research assistants tasked with laboratory work and also secondary co-authors.

11. List Of Abbreviations And Acronyms.

%: percentage (fraction over hundred) C: degrees Celsius E: exponential et al: and others g: gram(s) i.e.: such as Km: Kilometer(s) ml: milliliter(s) n: sample size NaFIRRI: National Fisheries Resources Research Institute n_{bif}: number of bacteria isolated from fish (Oreochromis niloticus) n_{biw}: number of bacteria isolated from water n_{gills}: number of gill samples n_{intestine}: number of intestine samples n_{muscle}: number of muscle samples

n_{skin}: number of skin samples p-value: probability value SIM: Sulphur Indole Motility spp: specie(s)

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