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HYGIENIC QUALITY OF NEWLY CAUGHT FRESH FISH (With 11 Tables)

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(Received at 27/3/1997)

التقييم الصحي للأسماك السليمة صحياً المصطاده حديثاً

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أشتمل هذا البحث على فحص ١٥٠ عينة (٧٥ من كل من القرموط والبلطى) وكانت عينات كل نوع من الأسماك مقسمة الى ثلاثة مجموعات. المجموعه الأولى مشتمله على أسماك طازجه حديثه الأصطياد وسليمه من الناحيه الصحيه وتم فحص العضلات كيميائياً وميكروبيولوجياً مجرد وصولها إلى المعمل. بينما كانت المجموعه الثانيه مشتمله على أسماك طازجه حديثه الاصطياد سليمه من الناحيه الصحيه ولكن أجرى الفحص عليها عند درجة حرارة صفر° لمدة أربع ساعات أما عينات المجموعه الثالثه فقد كانت مشتمله على عينات أسماك تم جمعها من الأسواق المختلفه. وقد بينت نتائج الفحص الآتى: متوسط الأس الهيدروجيني للمجموعه الأولى (٢٠ عينة من كل من القرموط والبلطى (٧٠٠ ± ٠.٢٨) - (٧٠٥ ± ٠.٢٩٥) و (٦٥٦ ± ٠.٥٥) - (٦٩١ ± ٠.٢٣) لعينات المجموعه الثانيه، (٧٠٢٨ ± ٠.٣٨) - (٧٠٧٠ ± ٠.٣١) لعينات المجموعه الثالثه على التوالي. بينما كان متوسط العد الكلى للميكروبات المحببه للبروده (٧٨٠ ± ٠.٨٦ X ١٠^٢) و (٣٢٢ ± ٠.٢٦ X ١٠^٢) - (٧٨٠ ± ٠.٣١ X ١٠^٢) و (٥٦٠ ± ٠.٢٢ X ١٠^٢) - (٦٨٤٠ ± ٠.٠٤ X ١٠^٢) و (٣٧٢ ± ٢.٨٦) على التوالي. وكذلك متوسط العد الكلى للميكروب الكروى العنقودى الذهبى فى نوعى الأسماك فى المجموعات الثلاثه هى :- (٤٤٠ ± ٠.١ X ١٠^٢) و (١٠٤ ± ٠.٠٤ X ١٠^٢) - (٤٨٠ ± ٠.١٧ X ١٠^٢) و (٩٢٢ ± ٠.١٥٢ X ١٠^٢) - (٦٨٠ ± ٠.٢٥ X ١٠^٢) و (١٨٨٠ ± ٠.٣٢ X ١٠^٢) على التوالي. وكان متوسط العد الكلى للمكورات المعويه (٤٨٠ ± ٠.١٣ X ١٠^٢) (٣٢٢ ± ٠.٠٩ X ١٠^٢) - (٤٢٤ ± ٠.٣٨ X ١٠^٢) و (٢٨٠ ± ٠.٢٨ X ١٠^٢) - (٧٠٠ ± ٢٩ X ١٠^٢) و (٣٤٠ ± ٠.٢١ X ١٠^٢) على التوالي. هذا وقد تم عزل وتصنيف أنواع عديده من الميكروبات المعويه والمسببه لفساد ماده الغذائيه بنسب متفاوتة. وقد تم مناقشة الأهميه الصحيه والاقتصاديه لهذه الميكروبات المعزوله ومدى تأثيرها على الصحه العامه للمستهلك وكذا الأشرطات الصحيه الواجب الأخذ بها لحماية المنتج.

SUMMARY

150 samples of *Clarias lazera* and *Tilapia nilotica* (75 each) were collected randomly from kafr El Sheikh Fisheries and Fish markets and examined for determination of pH and the bacteriological status. Each type consisted of three groups; newly caught fresh fish, newly caught fresh fish stored at 0°C for 4 hours, and fish obtained from different fish markets (25 each). The pH values of *Clarias lazera* and *Tilapia nilotica* in group I, II, and III were 7.00 ± 0.028 and 7.05 ± 0.095 ; 6.56 ± 0.055 and 6.91 ± 0.023 ; and 7.28 ± 0.038 and 7.07 ± 0.031 , respectively. Results of bacteriological examination of *Claria lazera* and *Tilapia nilotica* group I, II and revealed that the average psychrotrophic count of their muscles were $0.78 \pm 0.022 \times 10^2$ and $0.56 \pm 0.028 \times 10^2$; $1.42 \pm 0.053 \times 10^2$ and $1.14 \pm 0.043 \times 10^2$; and $660.0 \pm 26.46 \times 10^2$ and $336.0 \pm 22.27 \times 10^2$ /g, respectively. While the average Enterobacteriaceae count were $0.49 \pm 0.086 \times 10^2$ and $0.32 \pm 0.026 \times 10^2$; $0.78 \pm 0.031 \times 10^2$ and $0.56 \pm 0.022 \times 10^2$; and $68.40 \pm 2.98 \times 10^2$ and $37.2 \pm 2.86 \times 10^2$ /g, respectively. On the other hand, the average *Staphylococcus aureus* count were $0.144 \pm 0.01 \times 10^2$ and $0.104 \pm 0.004 \times 10^2$; $0.148 \pm 0.017 \times 10^2$ and $0.192 \pm 0.152 \times 10^2$; and $1.068 \pm 0.025 \times 10^2$ and $1.88 \pm 0.032 \times 10^2$ /g, respectively. Moreover, the average total Enterococci count were $0.148 \pm 0.013 \times 10^2$ and $0.132 \pm 0.009 \times 10^2$; $0.424 \pm 0.038 \times 10^2$ and $0.280 \pm 0.028 \times 10^2$; and $7.00 \pm 0.29 \times 10^2$ and $3.40 \pm 0.21 \times 10^2$ /g, respectively. The following bacterial species could be isolated and identified from group I, II and III of *Clarias lazera* and *Tilapia* in different percentages were: Achromobacter (58.3% and 55.6%), Flavobacterium (25.0% and 27.8%) and Micrococci (16.6% and 16.7%) & Pseudomonas sp. (67.6% and 85.7%), Micrococci (11.3% and 4.29%), Achromobacter (8.45% and 4.29%), Flavobacterium (5.63% and 2.86%) and Alcaligenes (7.04% and 2.86%) as well as Pseudomonas sp. (61.98% and 68.4%), Micrococci (12.4% and 10.3%), Alcaligenes (8.26% and 3.42%), *Escherichia cloi* (6.61% and 5.13%), Proteus sp. (4.13% and 6.84%), Acintobacter (2.48% and 0.85%), Serratia (1.65% and 3.42%) and Achromobacter (1.65% and 0.85%), respectively. The public health significance as well as the economic importance of the isolated and identified bacteria were discussed.

Key words: Fresh Fish - Hygienic quality

INTRODUCTION

Bacteriology of newly caught fresh fish muscle do not take a sufficient studies since many years ago. When Anderson (1907) used the microscopic examination for detection of the routes through which the bacteria attack fish muscles, he found that bacteria in gutted fish proceed from the kidney (which was not usually removed), along the cardinal vein which lies beneath the backbone in the caudal region of the fish and breaking up the corpuscles and finally entering the tail flesh as a process associated with the development of red colour around the bone. Reay and Shewan (1949) found that even in stall fish (particular large species as a cod) muscle samples could be aseptically removed from the center of the fish were frequently found to be sterile. Hjorth-Hansen (1913) mentioned that the pH act as a most important factor in control the process of growth and multiplication of bacteria and it was ranged between 7.05 to 7.33 in newly caught fish. Shonberg (1937) and (1957) reported that the fish muscles of healthy and newly caught fish were considered sterile. He also explained that fish become contaminated due to its adverse environment and unhygienic handling resulting in presence of great numbers of bacteria in the flesh of weakly decomposed fish. Also Lerke *et al.* (1965) reported similar results and revealed that the diseased weakened fish contains considerable numbers of bacteria in their muscles. Recently, Huss *et al.* (1974) recorded counts of bacteria lower than of the quoted in fish caught in unpolluted water while higher counts were recorded in fish caught in polluted water and poor hygienic standards on board-ship during initial handling. Jay (1986) and Hayes (1992), reported similar principals in which, it was generally accepted that, the flesh of newly caught fish was sterile but bacteria were found in variable numbers in three sites on the fish, slime coat on the skin, gills and the intestines. From all the previously mentioned reviews, this research was planned about the hygienic quality of the muscles of newly caught healthy fish.

MATERIAL and METHODS

Collection of samples:

Two types of fish (75) namely *Clarias lazera* and *Tilapia nilotica* (75 each) were collected from Kafr El Sheikh Fisheries and Fish markets. Each type comprised three groups and each group composed of 25 samples according to the condition of examination. The newly caught eventually still

alive fish represented group I. The newly caught fish stored at 0 °C for 4 hours before examination represented group II. Fish which were collected from different markets represented group III. All samples were collected and transported as quickly as possible into the laboratory under complete aseptic condition and prepared for the following investigation.

I- Determination of the pH (Pearson, 1973):

Ten grams of fish meat were aseptically cutted into small pieces and placed into sterile beaker containing 10 ml of distilled water. The mixture was then left at room temperature for 10 minutes with frequent agitation. The pH value of the meat extract was determined using pH meter (D.D. 620 digital pH meter). The obtained results were recorded.

II- Bacteriological examination:

The obtained samples were prepared and the decimal serial dilutions were done according to ICMSF (1978). The Standard Plate Count technique (SPC) was applied for detection of psychrotrophic count using SPC agar at 7°C for 10 days, (ICMSF, 1978) and the total Enterobacteriaceae count using Violet Red Bile Glucose agar (VRBG) was determined according to the methods adopted by (Gork, 1976). *Staphylococcus aureus* was determined by plating on Baird Parker agar (ICMSF, 1978). Also suspected colonies of *Staphylococcus aureus* were purified and identified according to Cruickshank *et al.* (1975). Isolation and identification of Enterococci were done according to the technique recommended by (Efthymiou and Joseph, 1974) on Enterococcus Selective Differential agar. While colonies isolated from (SPC) and (VRBG) agar plates were purified and identified according to the developed scheme recommended by Vanderzant and Nickelson (1969).

RESULTS and DISCUSSION

1- pH of fish muscle sample:

The achieved results available in Table (1) indicate that the average values of pH in fish muscle samples of *Clarias lazera* and *Tilapia nilotica* of group I, II and III were 7.00 ± 0.028 and 7.05 ± 0.095 with an overall mean of 7.03 ± 0.02 ; 6.56 ± 0.055 and 6.91 ± 0.023 with an overall mean of 6.73 ± 0.039 ; and 7.28 ± 0.038 and 7.07 ± 0.031 with an overall mean of 7.26 ± 0.024 , respectively.

Moreover, a significant variance appeared at the level of the type of fish samples. While the variance appeared highly significant at level of sampling group ($P < 0.05$), but the interactions between the sampling type and

group appeared only significant ($P < 0.01$), (Table, 2). Nearly similar results were outlined by El-Morshidy and Hafez (1986), Mahmoud, (1990) and (1994), and Hasan (1991). They recorded pH values of fish muscle as 6.5-6.9 immediately after caught for hake and skate, and 7.1-7.2 for newly caught halibut (the difference attributed to the fish species). pH 6.06 of *Tilapia* was recorded directly after caught, then after there was a sharp increase and stabilized at 6.35 depending upon the time of catching. On the other hand, other pH values recorded were 6.2-6.8 for fresh fish, 6.8-7.5 for suspected fish and 7.5 or above for fish in incipient spoilage.

II- Bacteriological examination:

1- Total psychrotrophic count:

From the results tabulated in Table (3) it was found that the average psychrotrophic count of muscle of *Clarias lazera* and *Tilapia nilotica* of group I, II and III were: $0.78 \pm 0.022 \times 10^2$ and $0.56 \pm 0.028 \times 10^2$ and $1.14 \pm 0.043 \times 10^2$ with an overall mean of $1.28 \pm 0.039 \times 10^2$; and $680 \pm 26.46 \times 10^2$ and $336.0 \pm 22.27 \times 10^2$ with an overall mean of $508 \pm 29.94 \times 10^2/\text{g}$, respectively.

From Table (4) a moderately significant variance was observed at the level of sample type while there was a highly significant variance at the level group of the samples. In the same time the interaction between the type and group of samples appeared moderately significant variance Table (4). Nearly similar results obtained by Hobbs (1983), Jay (1986) and Hayes (1992).

2- Total Enterobacteriaceae count:

The presented results in Table (5) reveal that the average Enterobacteriaceae count of muscle samples of *Claria lazera* and *Tilapia nilotica* in case of group I, II and III were $0.496 \pm 0.086 \times 10^2$ and $0.32 \pm 0.026 \times 10^2$ with an overall mean of $0.408 \pm 0.023 \times 10^2$; $0.784 \pm 0.031 \times 10^2$ and $0.564 \pm 0.022 \times 10^2$ with an overall mean of $0.674 \pm 0.025 \times 10^2$; and $68.40 \pm 2.98 \times 10^2$ and $37.20 \pm 2.86 \times 10^2$ with an overall mean of $52.80 \pm 1.00 \times 10^2/\text{g}$, respectively, (Table 5). Moreover, from Table (6) it was found that the analysis of variance appeared moderately significant ($P < 0.05$) at the level of the type of fish, also the same degree of significance appeared at the level of sampling group. While it is appeared highly significant concerning the interaction between the type of sample and its group, (Table, 6). Nearly similar results were reported by Brock *et al.* (1984), Gofarsh *et al.* (1985) and Govczyco and Pekphlen (1985).

Moreover, there are many reports about the public health significance of Enterobacteriaceae, Edwards and Ewing (1972), Sydney *et al.* (1978), Banwart (1979) and Collins (1984). Members of this family of bacteria are of

potential public health importance as it causes disease for human during lowering of their resistance. Also this group contains most members of food poisoning microorganisms as Enteropathogenic and Enterotoxigenic *Escherichia coli*, Salmonella, Shigella and other members cause infection for human as Klebsiella, Enterobacter, Serratia, Acintobacter and Proteus spp. Furthermore, this group is used as indicator for the degree of water pollution.

3- Total Staphylococcus aureus count:

From Table (7) the presented results show that the average Staphylococcus count in muscle samples of *Clarias lazera* and *Tilapia nilotica* of group I, II and II were $0.144 \pm 0.01 \times 10^2$ and $0.104 \pm 0.004 \times 10^2$, with an overall mean of $0.124 \pm 0.016 \times 10^2$; $0.148 \pm 0.016 \times 10^2$ and $0.192 \pm 0.152 \times 10^2$, with an overall mean of $0.170 \pm 0.011 \times 10^2$; and $1.068 \pm 0.25 \times 10^2$ and $1.88 \pm 0.032 \times 10^2$, with an overall mean of $1.128 \pm 0.016 \times 10^2/g$, respectively. Also, the analysis of variance appeared non significant and low significant in case of the variance between the type and interaction between the type and group of the samples. While appeared highly significant in case of the variance between groups of the samples, (Table 8). Nearly similar results were reported by Nickelson *et al.* (1980), Brock *et al.* (1984) and Lakshmanan *et al.* (1984). Concerning the public health importance, *Staphylococcus aureus* is considered one of the most important food poisoning microorganisms, besides it can cause disease in either human or animals (Collins, 1984).

4- Total Enterococci count:

The achieved results in Table (9) reveal that the average Enterococci count in muscle samples of *Clarias lazera* and *Tilapia nilotica* of group I, II and III were $0.148 \pm 0.013 \times 10^2$ and $0.132 \pm 0.009 \times 10^2$, with an overall mean of $0.140 \pm 0.008 \times 10^2$; $0.424 \pm 0.038 \times 10^2$ and $0.280 \pm 0.028 \times 10^2$, with an overall mean of $0.352 \pm 0.025 \times 10^2$; and $7.00 \pm .29 \times 10^2$ and $3.40 \pm 0.21 \times 10^2$, with an overall mean of $5.20 \pm 0.312 \times 10^2/g$, respectively. Moreover, the analysis of variance between the types of samples and the interaction between types and groups of samples appeared moderately significant, while it appeared highly significant between the samples group, (Table, 10). Nearly similar results were reported by Collins (1984), Jay (1986) and Hayes (1992). Enterococci, are considered as potential pathogens and cause disease problems in humans. They are used by most investigators as an indicator of faecal pollution especially in the frozen food stuffs because of their resistance to freezing temperatures more than the Coliform group. Also, such organisms contaminate the food through the

main source of infection which is the gastrointestinal tract of man and animals.

5- Isolated and identified bacteria:

The presented results in Table (11) show that from the examined samples of *Clarias lazera* and *Tilapia nilotica*, the following Gram-negative bacteria could be recovered at high rates of frequency as *Achromobacter* (58.3% and 55.6%), *Flavobacterium* (25% and 27.8%) and *Micrococci* (16.6% and 16.7%) from group I. *Pseudomonas* sp. (67.6% and 85.7%), *Achromobacter* (8.45% and 4.29%), *Flavobacterium* (5.63% and 2.86%), *Alcaligenes* (7.04% and 2.86%) and *Micrococci* (11.3% and 4.29%) from group II and *Pseudomonas* sp. (61.98% and 68.4%), *Micrococci* (12.4% and 10.3%), *Alcaligenes* (8.26% and 3.42%), *Escherichia coli* (6.61% and 5.13%) and *Proteus* sp. (4.13% and 6.84%) from group III. Nearly similar results were reported by Gorczyca and Pekkohlen (1985), Gennari *et al.* (1988) and Hayes (1992). The public health significance of these isolated microorganisms was explained by Banwart (1979) and Collins (1984). They stated that most of them cause disease problems for human and animals. As well as they play an important role in spoilage, damage of food stuffs and great economic losses.

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Table (1): The average pH values of the examined muscles of *Clarias lazera* and *Tilapia nilotica* (Mean \pm SE).

Group	Type		Overall mean
	<i>Clarias Lazera</i>	<i>Tilapia nilotica</i>	
Group I	7.00 \pm 0.028	7.05 \pm 0.095	7.03 \pm 0.020
Group II	6.56 \pm 0.055	6.91 \pm 0.023	6.73 \pm 0.039
Group III	7.28 \pm 0.038	7.07 \pm 0.031	7.26 \pm 0.024
Overall mean	6.95 \pm 0.042	7.07 \pm 0.023	7.01 \pm 0.024

Table (2): Variance analysis of pH values of the examined muscles of *Clarias lazera* and *Tilapia nilotica*.

Source of variance	D.F.	S.S.	M.S.	F. Value
Correction factor	--	7366.8093	--	--
Total variance	149	13.2307	--	--
Variance between types	1	0.5886	0.5886	18.55*
Variance between groups	2	7.0464	3.5232	111.05***
Type X group interaction	3	1.0266	0.5133	16.1797*
Within (Error)	144	4.5684	0.031725	--

P < 0.05 = 4.00, D.F. 1, 144

P < 0.01 = 7.08, D.F. 1, 144

* Significant, ** Moderately significant, *** Highly significant

Table (3): The average psychrotrophic count in the examined muscles of *Clarias lazera* and *Tilapia nilotica* (Mean \pm SE).

Group	Type		Overall mean
	<i>Clarias Lazera</i>	<i>Tilapia nilotica</i>	
Group I	0.78 \pm 0.022 X 10 ²	0.56 \pm 0.028 X 10 ²	0.67 \pm 0.023 X 10 ²
Group II	1.42 \pm 0.053 X 10 ²	1.14 \pm 0.043 X 10 ²	1.28 \pm 0.039 X 10 ²
Group III	660.00 \pm 26.46 X 10 ²	336.00 \pm 22.27 X 10 ²	508.00 \pm 29.94 X 10 ²
Overall mean	341.09 \pm 38.21 X 10 ²	112.57 \pm 19.77 X 10 ²	169.98 \pm 29.95 X 10 ²

Table (4): Variance analysis of psychrotrophic count in the examined muscles of *Clarias lazera* and *Tilapia nilotica*.

Source of variance	D.F.	S.S.	M.S.	F. Value
Correction factor	--	4334116	--	--
Total variance	149	10765993	--	--
Variance between types	1	494489.48	494489.48	99.23**
Variance between groups	2	8569187.9	4284593.9	859.77***
Type X group interaction	3	984702.17	492351.08	98.80**
Within (Error)	144	717614.25	4983.4322	--

Table (5): The average Enterobacteriaceae count in the examined muscles of *Clarias lazera* and *Tilapia nilotica* (Mean \pm SE).

Group	Type		Overall mean
	<i>Clarias Lazera</i>	<i>Tilapia nilotica</i>	
Group I	0.496 \pm 0.086 X 10 ²	0.32 \pm 0.026 X 10 ²	0.408 \pm 0.023 X 10 ²
Group II	0.784 \pm 0.031 X 10 ²	0.564 \pm 0.022 X 10 ²	0.647 \pm 0.025 X 10 ²
Group III	68.40 \pm 2.98 X 10 ²	37.20 \pm 2.86 X 10 ²	52.80 \pm 1.00 X 10 ²
Overall mean	23.23 \pm 3.84 X 10 ²	12.69 \pm 2.22 X 10 ²	17.96 \pm 2.25 X 10 ²

Table (6): Variance analysis of Enterobacteriaceae count in the examined muscles of *Clarias lazera* and *Tilapia nilotica*.

Source of variance	D.F.	S.S.	M.S.	F. Value
Correction factor	--	48387.83	--	--
Total variance	149	113445.96	--	--
Variance between types	1	4159.61	4159.61	58.49**
Variance between groups	2	91035.20	45517.60	640.0***
Type X group interaction	3	8009.39	4004.695	26.31**
Within (Error)	144	10241.76	71.12	--

Table (7): The average *Staphylococcus aureus* count in the examined muscles of *Clarias lazera* and *Tilapia nilotica* (Mean \pm SE).

Group	Type		Overall mean
	<i>Clarias Lazera</i>	<i>Tilapia nilotica</i>	
Group I	0.144 \pm 0.010 X 10 ²	0.104 \pm 0.004 X 10 ²	0.124 \pm 0.016 X 10 ²
Group II	0.148 \pm 0.016 X 10 ²	0.192 \pm 0.152 X 10 ²	0.170 \pm 0.011 X 10 ²
Group III	1.068 \pm 0.025 X 10 ²	1.188 \pm 0.032 X 10 ²	1.128 \pm 0.016 X 10 ²
Overall mean	0.453 \pm 0.520 X 10 ²	0.495 \pm 0.058 X 10 ²	0.474 \pm 0.039 X 10 ²

Table (8): Variance analysis of *Staphylococcus aureus* count in the examined muscles of *Clarias lazera* and *Tilapia nilotica*.

Source of variance	D.F.	S.S.	M.S.	F. Value
Correction factor	--	33.7014	--	--
Total variance	149	33.7286	--	--
Variance between types	1	0.0636	0.0636	6.674N.S.
Variance between groups	2	32.1316	16.058	1694.99***
Type X group interaction	3	0.1606	0.0803	8.43*
Within (Error)	144	1.3728	0.00953	--

Table (9): The average Enterococcus count in the examined muscles of *Clarias lazera* and *Tilapia nilotica* (Mean \pm SE).

Group	Type		Overall mean
	<i>Clarias Lazera</i>	<i>Tilapia nilotica</i>	
Group I	0.148 \pm 0.013 X 10 ²	0.132 \pm 0.009 X 10 ²	0.140 \pm 0.008 X 10 ²
Group II	0.424 \pm 0.038 X 10 ²	0.280 \pm 0.028 X 10 ²	0.352 \pm 0.025 X 10 ²
Group III	7.00 \pm 0.290 X 10 ²	3.400 \pm 0.210 X 10 ²	5.20 \pm 0.312 X 10 ²
Overall mean	2.524 \pm 0.380 X 10 ²	1.271 \pm 0.188 X 10 ²	1.897 \pm 0.218 X 10 ²

Table (10): Variance analysis of Enterococcus count in the examined muscles of *Clarias lazera* and *Tilapia nilotica*.

Source of variance	D.F.	S.S.	M.S.	F. Value
Correction factor	--	539.98106	--	--
Total variance	149	1058.9389	--	--
Variance between types	1	58.90667	58.90667	109.477**
Variance between groups	2	819.19414	409.59707	761.23***
Type X group interaction	3	103.35573	51.677865	96.043**
Within (Error)	144	77.48236	0.5380719	--

Table (11): Frequency distribution of the isolated and identified bacteria from the examined samples.

Group	Group I						Group II						Group III						Total							
	C. lazera		T. nilotica		C. lazera		T. nilotica		C. lazera		T. nilotica		C. lazera		T. nilotica		C. lazera		T. nilotica							
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%						
<i>Escherichia coli</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	6.61	6	5.13	8	3.17	6	2.17		
Acintobacter	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2.48	1	0.85	3	1.19	1	0.36		
Proteus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	4.13	8	6.84	5	1.98	8	2.89		
Serratia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1.65	4	3.42	2	0.79	4	1.44		
<i>Pseudomonas</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	75	61.98	80	68.4	123	48.81	140	50.54		
Achromobacter	35	58.3	50	55.6	6	8.45	3	4.29	2	1.65	2	1.65	1	0.85	1	0.85	2	1.65	3	4.29	2	1.65	43	17.16	54	19.49
Flavobacterium	15	25.0	25	27.8	4	5.63	2	2.86	2	2.86	1	0.83	1	0.85	1	0.85	1	0.83	2	2.86	4	3.42	20	7.94	28	10.11
Alcaligenes	0	0	0	0	5	7.04	2	2.86	2	2.86	10	8.26	4	3.42	4	3.42	15	12.4	15	12.4	15	5.95	6	2.17		
Micrococci	10	16.6	15	16.7	8	11.3	3	4.29	3	4.29	15	12.4	12	10.3	12	10.3	33	27.4	33	27.4	33	13.1	30	10.83		
Total	60	100	90	100	71	100	70	100	71	100	121	100	117	100	117	100	252	100	252	100	252	100	277	100		

Date	Description	Miles											
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