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BACTERIA OF SOME TRADITIONALLY PROCESSED FRESHWATER FISH IN SUDAN.

Reem A. Mohamedahmed[1*], Nazik M. A. Hammad[1], Zuheir N. Mahmoud[2]

[1]Department of Botany, Faculty of Science, University of Khartoum, Sudan.

[2]Department of Zoology, Faculty of Science, University of Khartoum, Sudan.

*Corresponding Author

ABSTRACT

Nineteen products of traditionally processed *Alestes* spp., *Hydrocynus* spp., *Clarias* spp., *Labeo* spp., *Oreochromis niloticus* and *Synodontis* spp., freshwater fish were studied for their bacteria species according to (Bergey's manual). *Enterobacter aerognenes*, *Escherichia coli*, *Klebsilla pneumoniae* (gram negative) and *Staphylococcus* sp. and *Micrococcus* spp. (gram positive) were recorded. The bacterial count differs with respect to media and dilution used. Mature wet salted *Hydrocynus* spp. (S12) and *Alestes* spp. (S6); *Alestes* each cultured in PCA and *Hydrocynus* spp. (Maloha) (S15) cultured in PCA+ showed uncountable bacterial number. *Enterobacter aerognenes* and *E. coli* co-existed in isolates I16 from 8 days old *Alestes* spp. and isolate I93 from mature *Alestes* spp. Potato Dextrose Agar showed no bacterial growth in the 7 dilutions used, and for the 19 fish samples. *Enterobacter aerognenes E. coli* and *K. penumoniae* isolates responded positively to lactose test but with different levels to citrate test. Only *K. pneumonia* responded to Urea's test.

Keywords: Bacteria, Processed, Fish, Sudan.

INTRODUCTION

Fish is one of the main sources of animal protein in human diet (Lunven, 1982). This renewable resource provides countries with food security, livelihoods and economic growth if sustainably managed and added value considered. In Sudan, the various aquatic resources fulfill the local demand on fish (El Tom, 1989; El Hag *et al*., 2012).

Fish usually spoils more rapidly by bacteria (Ghaly *et al*., 2010) and its preservation is a necessity to supply an acceptable commodity (Ghaly *et al.*, 2010; El Hag *et al*., 2012). The most important method of fish preservation in Africa is fish fermentation, drying and smoking used

ISSN: 2455-6939

Volume: 05, Issue: 05 "September-October 2019"

alone or combined (Essuman, 1992). In the Sudan, nearly 30% of the total fish landings are consumed cured either by salting, fermentation or sun drying (FAO, 1981). Salting is a traditional method of processing fish to lower the water available to support microbial growth **(**Bakhiet and Khogalie, 2012; Ali and Saeed, 2015)

Traditional drying by exposing fish to the action of sun and wind, is cheap, effective, can be performed by fishers and their families and the product can easily be transported to market **(**Essuman, 1992; Kasozi *et al*., 2016). Dried fish has a shelf life of several years as safe food. In Sudan salting is applied to relatively lean fish and the Sudanese consumer prefers little fat in the salted products (Dirar, 1993).

The objective of this study is to investigate bacteria of some traditionally processed fish in Sudan.

MATERIAL AND METHODS

Sample collection

Salted *Alestes* spp., *Hydrocynus* spp., *Clarias* spp., *Labeo* spp., *Oreochromis niloticus* and *Synodontis* spp., Table1 were collected from fish retailers. Under sterile conditions, tissue samples from each specimen were made into paste and kept in sterile jars at 4^0C till analysis.

Culturing of Bacteria

Preparation of fish stock solution and culturing of the microorganisms followed Waksman (1961). The Plate count agar, Nutrient agar, Eosin Methylene Blue agar, Potato Dextrose Agar and Mannitol salt agar were prepared and used for growth and culturing bacteria from the fish samples stock solution. After preparation, all media were sterilized by autoclaving at 121°C for 15 min and the incubation protocol followed Tournas *et al*. (2001).

For microscopic characterization, the bacterial cells were gram-stained according Collins *et al*. (1995). Colony colour, shape, elevation, margin, consistency and transparency were determined under a stereo-microscope and the descriptive terminologies of Bergeys *et al*. (1989); Prescott *et al*. (1993); were adopted. The biochemical tests followed Prescott *et.al*. (1993), Collee *et al*. (1996) and Collins *et al*. (1995).

ISSN: 2455-6939

Volume: 05, Issue: 05 "September-October 2019"

Table 1: Fish sample ID, Sources and processing.

Fish species	Sample code	Processing status
Alestes spp.	S1	8 days old wet salted
	S2	9 days old wet salted
	S3	10 days old wet salted
	S4	13 days old wet salted
	S5	25 days old wet salted
	S6	Mature wet salted
	S7	Mature wet salted
Hydrocynus spp.	S8	8 days old wet salted
	S9	12 days old wet salted.
	S10	Mature wet salted
	S11	Mature wet salted
	S12	Mature wet salted
Labeo spp.	S13	Mature wet salted
Alestes and *Hydrocynus* spp.	S14	Mature watery salted (Maloha)
	S15	
Synodontis spp.	S16	Mature mandeshe paste
	S17	
Oreochromis niloticus	S18	Dry salted (Kajake)
Clarias spp.	S19	Dry salted (Kajake)

ISSN: 2455-6939

Volume: 05, Issue: 05 "September-October 2019"

RESULTS

Bacteria spp.

The study revealed the presence of three gram negative bacteria species *Enterobacter aerognenes*, *Escherichia coli*, *Klebsilla pneumoniae* (Plate 1) and gram positive *Staphylococcus* spp. and *Micrococcus* spp. (Plate 2) in the different processed fish species. *Enterobacter aerognenes* and *E. coli* co-existed in isolates I_{16} from 8 days old *Alestes* spp. and I_{93} from mature *Alestes* spp. *Enterobacter aerognenes*, *E. coli* and *K. penumoniae* were found in different isolates from 8, 13, 25 days fermented *Alestes* spp., mature mandeshe of *Synodontis* spp., and 12 days old wet salted *Hydrocynus* spp. Eight days old wet salted *Hydrocynus* spp. (S8) and 10 days old wet salted *Alestes* spp. (S3) showed no bacterial load (Table 1). Mature wet salted *Hydrocynus* spp. (S12) and *Alestes* spp. S6; *Alestes* each cultured in PCA and *Hydrocynus* spp. (Maloha) (S15) cultured in PCA+ showed uncountable bacterial number. Sixty six (34%) of the isolates were rod and gram negative and 126 (66%) were cocci and gram positive bacteria. *Enterobacter aerognenes E. coli* and *K. pneumonia* isolates responded positively to lactose test. Only *K. pneumonia* responded to Urea's test.

Klebsilla

ISSN: 2455-6939

Volume: 05, Issue: 05 "September-October 2019"

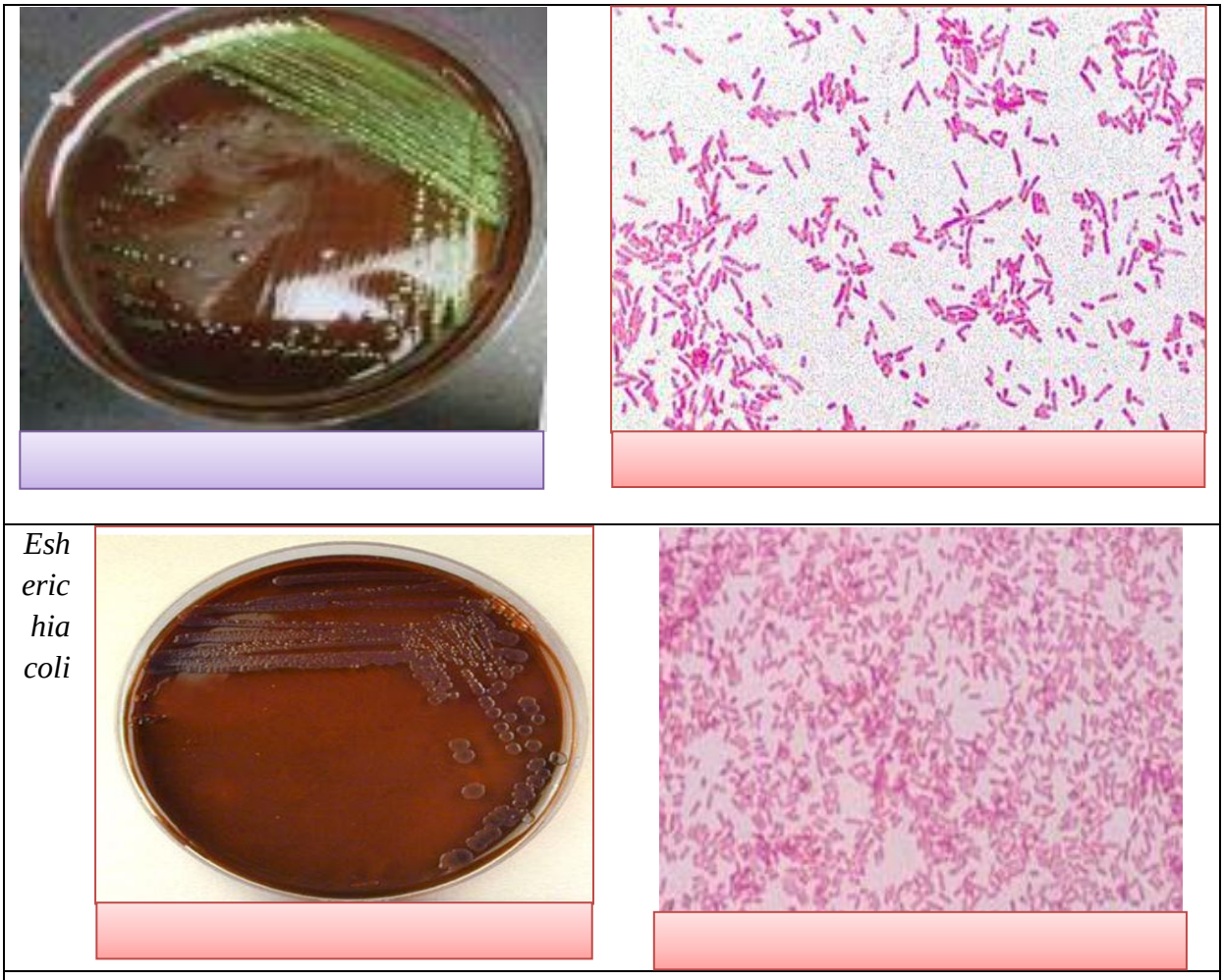

Plate 1. Gram negative bacteria

ISSN: 2455-6939

Volume: 05, Issue: 05 "September-October 2019"

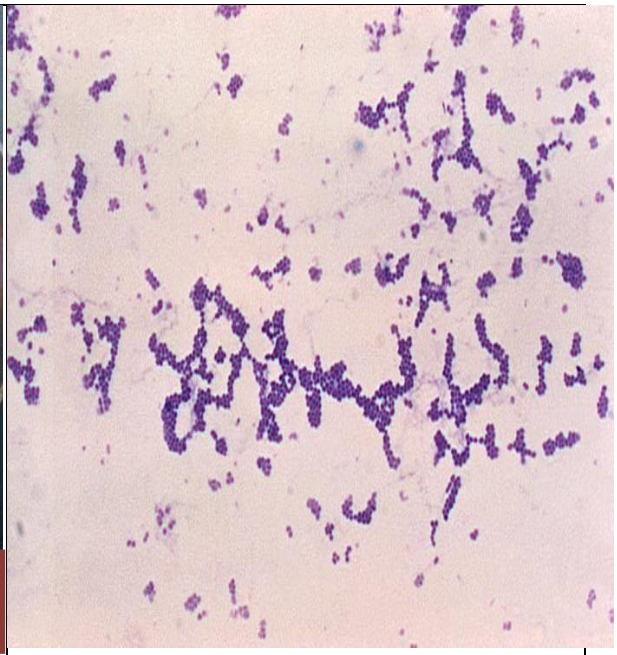

Plate 2. Gram positive bacteria**.**

Out of the five agar media used (Plate count agar; PCA; Plate count agar, PCA+; Potato Dextrose Agar, PDA; Mannitol salt agar, MAS and Eosin Methylene Blue agar, EMB), PDA showed no bacterial growth in the 7 dilutions used, and for the 19 fish samples (Table 2). Only those media with positive results were listed in Table 2 as appropriate.

The bacterial cultural efficiency differs with respect to medium dilution and sample status and most of the bacterial count concentrated between dilution 10^{-1} and 10^{-2}.

ISSN: 2455-6939

Volume: 05, Issue: 05 "September-October 2019"

Table 2: Total counting of bacteria Samples colony count using different media Un= Uncountable bacterial number

Fish sample	Medium	Colony Count / Dilution						
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
S1	PCA	2	4	0	0	0	0	0
	PCA+	1	3	0	0	0	0	0
	MSA	3	5	0	0	0	0	0
	EMB	0	7	0	0	0	0	0
S2	PCA	Un	5	Un	Un	0	0	0
	PCA+	Un	Un	4	0	0	0	0
	MSA	Un	Un	0	0	0	0	0
S3	All media	0	0	0	0	0	0	0
S4	PCA	Un	2	1	0	0	0	0
	PCA+	Un	Un	1	0	0	0	0
	MSA	9	2	0	0	0	0	0
	EMB	5	0	0	0	0	0	0
S5	PCA	Un	Un	4	3	0	0	0
	PCA+	0	2	4	0	2	2	1
	MSA	1	0	0	0	0	0	0
	EMB	0	0	1	3	0	2	1
S6	PCA	Un	Un	Un	Un	Un	Un	Un
	PCA+	Un	Un	Un	Un	Un	9	0
	MSA	Un	0	0	0	0	0	0

ISSN: 2455-6939

Volume: 05, Issue: 05 "September-October 2019"

S7	PCA+	9	2	0	0	0	0	0
	MSA	3	0	0	0	0	0	0
	EMB	5	0	0	0	0	0	0
S8	All media	0	0	0	0	0	0	0
S9	PCA	3	2	0	0	0	0	0
	PCA+	5	3	0	0	0	0	0
	MSA	Un	Un	0	0	0	0	0
S10	PCA	Un	0	0	0	0	0	0
	PCA+	5	1	1	0	0	0	0
S11	PCA	Un	0	0	0	0	0	0
	EMB	8	5	0	0	0	0	0
S12	PCA	Un	Un	Un	Un	Un	Un	Un
	PCA+	Un	0	0	0	0	0	0
	MSA	Un	0	0	0	0	0	0
S13	PCA	6	0	0	0	0	0	0
	MSA	Un	0	0	0	0	0	0
S14	PCA	Un	Un	0	0	0	0	0
	PCA+	7	0	0	0	0	0	0
S15	PCA	4	0	0	0	0	0	0
	PCA+	Un	Un	Un	Un	Un	Un	Un
S16	PCA	6	5	0	0	0	0	0
	PCA+	9	8	0	0	0	Un	0

ISSN: 2455-6939

Volume: 05, Issue: 05 "September-October 2019"

	MSA	Un	0	0	0	0	0	0
S17	PCA+	2	2	1	0	0	0	0
	EMB	Un	Un	0	0	0	0	0
S18	PCA	0	Un	Un	Un	Un	0	0
	MSA	1	1	0	0	0	0	0
S19	PCA	4	0	0	0	0	0	0
	PCA+	Un	Un	0	0	1	0	0
	MSA	3	0	0	0	1	0	0

Cultural characteristics of bacterial isolates

From the different cultural media 192 bacterial isolates were obtained. Those with circular or irregular shape constituted 94.3% and 5.7%, respectively. The size was either small (83.9%) or large (16.1%). Entire margin amounted to (83.3%) and the undulated isolates to (16.7%). Elevation of the isolates was either raised (65.6%), or convex (32.8%) or flat (1.6%). Opaque opacity was found in (87.0%) isolates and (13.0%) were translucent. The consistency of the isolates was mostly smooth (93.8%); the viscous, rough and mucoid constituted (4.2%), (1.6%) and (0.5%), respectively. Chromogenisis of the bacterial isolates was cream (47.9%) white (46.4%) and yellow (5.7%) in colour.

Biochemical test of Gram negative

Enterobacter aerognenes E. coli and *K. pneumonia* isolates responded positively to lactose test. Only *K. pneumonia* responded to Urea's test in 12 out of 63 tests. Cases of positive response to citrate test were 18 in *E. aerognenes*, 12 in *K. pneumonia* and 1in *E. coli*.

Enterobacter aerognenes constituted 60%, *Escherichia coli* 24% and *Klebsilla pneumonia* 16% of all fish samples. The highest records for *E. aerognenes* was in mature fermented mandeshe; for *E. coli* was in (8days) fermented *Alestes* spp. and Maloha (*Alestes* and *Hydrocynus* spp.). The highest *K. pneumonia* record was in mature *Alestes* spp. The percentage of gram negative bacteria for each fish sample was given in Fig. 1.

ISSN: 2455-6939

Volume: 05, Issue: 05 "September-October 2019"

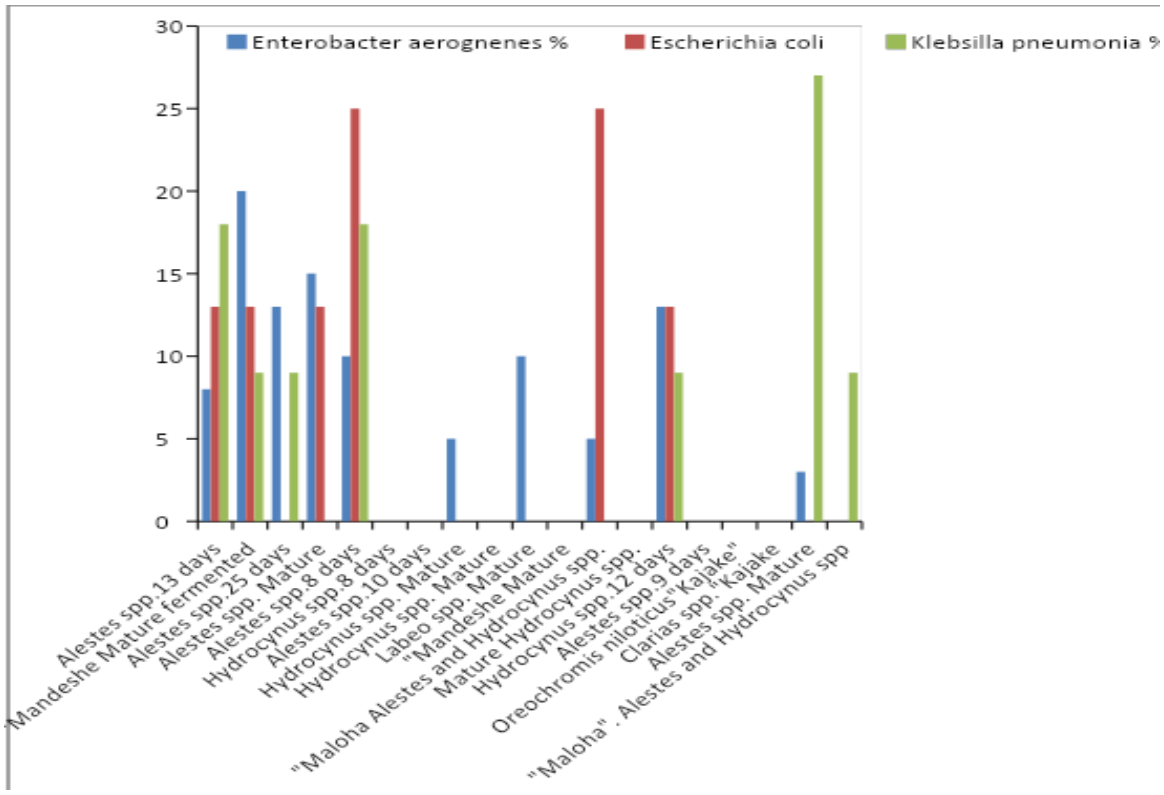

Fig. 1: Percentage of gram negative bacteria for each fish samples

Characterization of Gram positive bacteria

The total percentage of *Micrococcus* spp. was 69% and of *Staphylococcus* spp. was 31% of all fish samples. The highest *Staphylococcus* spp. % was in *Alestes* spp. (25 days) and the highest *Micrococcus* % was in *Alestes* spp. (8 days).

ISSN: 2455-6939

Volume: 05, Issue: 05 "September-October 2019"

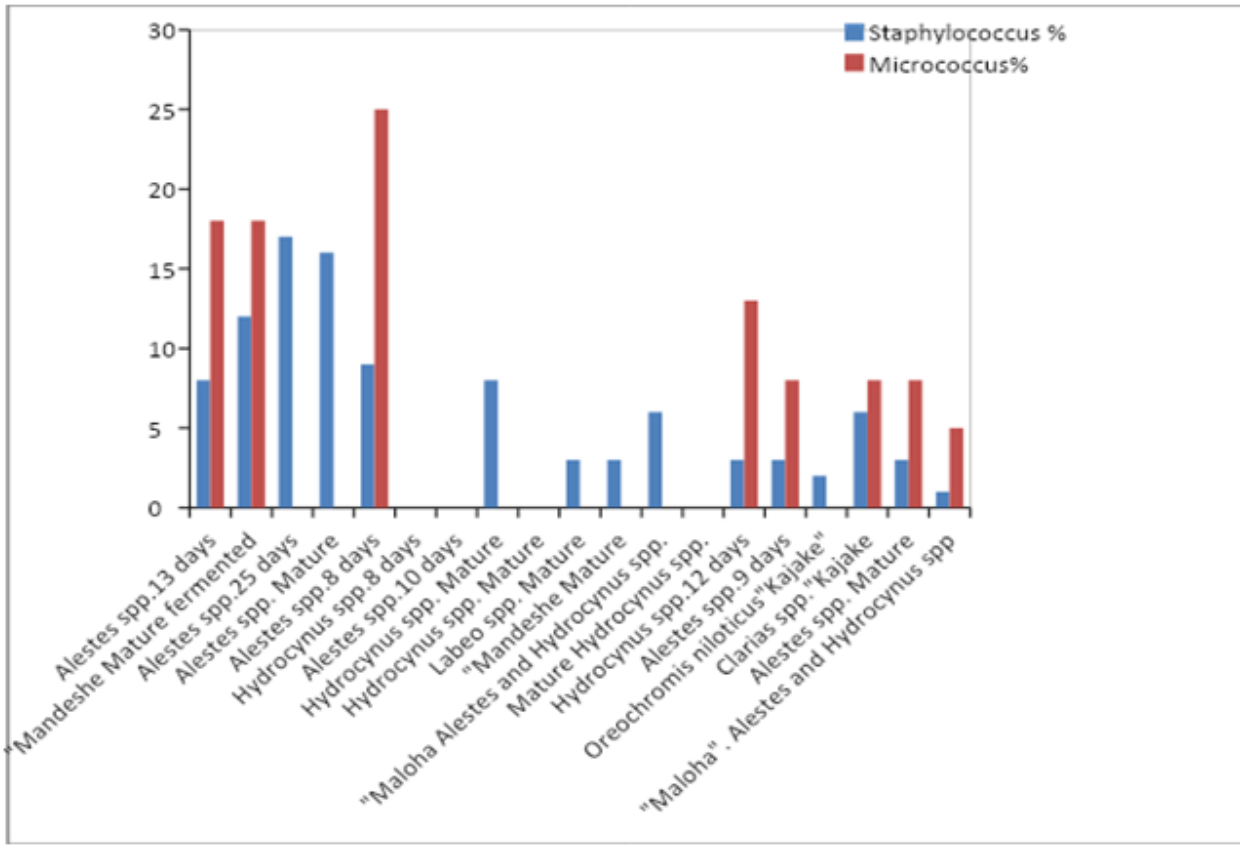

Fig. 2: Percentage of gram positive bacteria for each fish samples

Ninety isolate of *Staphylococcus* spp. under aerobic conditions responded positively to Catalase and to Glucose fermented reactions. To both reaction and under the same conditions 13 *Micrococcus* spp. isolates reacted positively to Catalase and negatively to Glucose fermented reactions. Under facultative conditions 26 *Micrococcus* spp. isolates reacted positively to Catalase and negatively to Glucose fermented reactions. The percentage number of Gram-positive bacteria for each fish sample was given in Fig. 2.

DISCUSSION

According to Anihouvi *et al.* (2006, 2007) a wide range of microorganisms including Gram-positive and Gram-negative bacteria; are involved in fish fermentation. In the present study *E. aerognenes*, *E. coli*, *K. penumoniae* and gram positive *Staphleococcus* spp. and *Micrococcus* spp. were isolated from the differently fermented and dried fish. Tahra *et al*., (2015) isolated from salted fish *Staphylococcus* spp. and *Micrococcus* spp. Bacteria isolated from Egyptian salted fish included *Micrococcus* spp., *Bacilli*, *Proteus vulgaris*, *P. mirabils* and *Aeromonas liquefaciens* (El-Tahan *et al*., 1998). Lee *et al.* (1993) isolated *Micrococcus* spp., *Pseudomonas*

spp., *Aerococcus* spp., and *Vibrio* spp. from the salt-fermented fishery products. Abd-Allah (2011) reported that the microbial load which was detected in Egyptian fermented salted *Mugil cephalus* fishes (Fesseikh) consisted of different bacterial flora like *Staphylococcus* spp. Suleiman *et al*., (2014) isolated bacteria from Kejeik samples and identified it to the family Enterobacteriaceae level.

The quality of salted and sun dried fish are adversely affected by microbial contamination, and determination of microbiological quality of processed dried fish product is important for protecting consumer's health (Lilabati *et al*., 1999).

Kasozi *et al.* (2016) studied the characteristics of traditionally dry-salted fish product collected from West Nile Region, Uganda and found that *E.coli* can be used to determined and estimate the microbial quality Aremu *et al.* (2013) found the shelf life was three months for salted *Hydrocynus forskalii* and its fermentation resulted in significant ($p<0.05$) reduction in total *Staphylococcus* spp., *Micrococcus* spp. and yeast-mould count. According to El Hag *et al*. (2012) *Staphylococcus* sp. was generally present in all the samples of fish and could contribute to keeping the quality of such products, and inhibit some pathogens. The fermentation may contribute positively to the flavor development of the product. According to Anihouvi *et al*., 2006 and Mensah, 1997, during fermentation of fish amines, ammoniac, acetic acid and lactic acid are produced organic acids. These compounds are responsible for the characteristic odour of fermented fish products and the acidity controls the growth of spoilage organisms and contributes to the extended shelf life of the product.. Some microorganisms of treated salted fish enhance nutritive value of the fish product (Beddows, 1985). El Hag *et al*. (2012) reported *Staphylococcus xylosus*, *Staphylococcus lentus* and *Staphylococcus saprophyticus* from salted *Alestes* spp.

Salting is one of the oldest methods of fish preservation. It extracts water from the fish flesh to a level that slow down microbial growth and enzymatic activities (Mabesa and Babaan 1993). The use of salt in fish processing may either be by dry salting (krenching) or wet salting (Essuman,1992).

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ISSN: 2455-6939

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