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ZOONOTIC SIGNIFICANCE OF SOME AETIOLOGICAL AGENTS ISOLATED FROM FISH

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SUMMARY

One hundred and forty freshwater fish samples, 55 from Oreochromis niloticus (Tilapia nilotica or Bolti), 45 from Mugil cephalus (Bouri) and 40 from Clarias lazera (Armout catfish), were collected from various markets and shops at Dakahlia and Gharbia Provinces. Skin, gills, intestine and muscles from each sample were examined for the presence of some pathogenic and potentially pathogenic bacteria and fungi of public health importance. The most predominant bacterial isolates from Orechromis niloticus, Mugil cephalus and Clarias lazera respectively were: E.coli (8.8%, 9.0% and 10.4%), Salmonella spp. (1.7%, 0.0% and 1.8%), Proteus spp. (38.5%, 18.1% and 39.8%), Klebsiella pneumoniae (13.8%, 14.7% and 13.4%), Citrobacter freundi (0.0%, 10.9% and 0.0%), Enterobacter spp. (14.3%, 2.8% and 6.3%), Shigella flexneri type 6 (8.0%, 11.9% and 9.3%), Pseudomonas spp. (6.9%, 7.1% and 9.7%), Staphylococcus spp. (34.6%, 26.5% and 36.9%) and Streptococcus spp. (65.4%, 73.5% and 63.1%). Meanwhile, the predominant fungi isolated from the examined Bolti, Bouri and Armout catfish were: Aspergillus spp. (51.5%, 66.6% and 65.0%), Penicillium spp. (17.2%, 5.7% and 10.0%), Mucor spp. (15.8%, 22.0% and 20.8%), Rhizopus spp. (3.9%, 5.7% and 4.2%), Rhodotorulla mucolignosa (15.9%, 16.7% and 11.3%), Torulopsis spp. (21.8%, 22.2% and 15.5%) and Candida spp. (62.3%, 61.1% and 73.2%), respectively.

The zoonotic importance of these isolates was discussed.

INTRODUCTION

The great, rapidly increasing and expanding human population in the world, demand greatly continuous sources of animal proteins for their requirements. Shortage of food has created a new

interest in harvesting aquatic life as a more substantial, compensate and continual food protein resource than in the past.

Recently, the Egyptian government has embarked on a programme of intensive fishing of all available water sources, in a trial to provide the consumer with valuable source of protein, being tasty, palatable, easily digested, of high nutritive value and reasonable costs. However, fish may contract many pathogens of epidemic importance from contaminated water of rivers and lakes constituting a potential health hazard to both handlers and consumers (AI-Wakeel et al., 1982).

Fish may harbour many pathogens including bacteria and fungi, occasionally of epidemic and zoonotic character. These pathogens are not only pathogenic to fish itself but also may be pathogenic or potentially pathogenic to man. Such infections have been arisen from unhygienic handling, processing, storage distribution, preparation for consumption or have been associated with fresh waterfish taken imported from polluted areas. The incidence of fish -borne microorganisms infecting man results from some food habits of the people by consuming raw, inadequately or partially cooked (grilled), slightly salted or improperly, smoked fish (WHO, 1968; Lawson, 1970 and El-Monla, 1981).

So, this work was done to study the role of fish collected from various markets and shops in Dakahlia and Gharbia Provinces in transmitting pathogenic and potentially pathogenic bacteria and fungi of zoonotic importance.

MATERIAL AND METHODS

1. Material:

A total of 140 fish samples were collected from various local fish etail markets and different shops at Dakahlia and Gharbia Provinces-Egypt. The samples were 55 Oreochromis niloticus (Tilapia nilotica or Bolti) , 45 Mugil cephalus (Bouri) and 40 Clarias lazera (Armout catfish). Each sample was put in a cean plastic bag, labelled and transferred immediately on ice box in cooling container to the laboratory with the minimum time of delay for bacteriological and mycological examinations (Syme, 1996).

2. Methods:

2.1. Bacteriological examination of fish:

2.1.1. Surface: The surface of the examined fish (the scales) were removed aseptically from 4 cm^2 area of the body surface after flaming with a piece of cotton fitted to a glass rod moistened with ethylacohol. A sterile swab was rolled over the surface of each examined fish group and immersed in a tube containing peptone water (2%).

2.1.2. Gills: A loopful from the gills was aseptically taken and cultivated on plates of nutrient, blod, MacConkeys and enterococcus selective differential agar (Cruickshank et al., 1975).

2.1.3. Intestinal contents: The intestinal tract was carefully dissected out and opened by sterile scissors and forceps. A loopful from the

intestinal content was taken and inoculated on plates of nutrient, blood, MacConkey's and enterococcus selective differential agar.

2.1.4. Muscles: Five grams of fish muscles were bacteriologically examined in each case, according to the method described b Wittfogel (1956) and Sedik (1977).

All inoculated plates and peptone water were incubated at 37°C for 24 hours A loopful from the incubated pepone water was taken and streaked on the previously mentioned solid media (Cruikshank et al., 1975).

Identification of the isolated Gran negative and positive bacteria was carried out according to Cherry et al., (1972); Edwards and Ewing (1972); Cruickshank et al. (1975) and Balley and Scott (1978).

2.2. Mycological examination of fish:

2.2.1. Skin: A sterile cotton swab was rolled over the surface of each examined fish after removing the scales and immersed in a tube containing Sabouraud's dextrose broth.

2.2.2. Gills: A loopful from the gills was aseptically taken and cultivated on plates of Sabouraud's dextrose agar with Chloramphenicol (250 mg/L) to inhibit any bacterial growth.

2.2.3. Intestine: A loopful from the intestinal content was taken and inoculated on plates of Sabouraud's dextrose agar.

2.2.4. Muscles: 5 grams of fish muscle were transferred under aseptic conditions to sterile homogenizer flask containing Sabouraud's dextrose broth, then the contents were homogenized for 2.5 minutes. The mixture was left for 3 days at 22.25°C and then loopful was taken and cultivated on Sabouraud's dextrose agar plates (Brown and Dorn, 1977).

Identification of the recovered moulds was carried out according to Samson (1979, while the isolated yeasts were identified according to Lodder and Kreger-Van-Rij (1970).

RESULTS AND DISCUSSION

The economic value of aquaculture and the need for its enhancement are now universally acknowlefged. Studies on fish microorganisms are of special significance because fish may constitute a health hazard to man if it is found in polluted evironment. The pollutants come almost from human or animal excreta . Therefore, it may harbour many serious pathogens including bacteria and fungi. So, fish may act as a carrier or a vehicle for many pathogens of public health importance.

The data presented in Tables (1,2 & 3) revealed, isolation of Proteus vulgaris and Proteus rettgeri from Oreochromis niloticus, Mugil cephalus and Clarias lazera at an incidence of (11.0%, 9.9%), (8.1%, 10.0%) and (13.4%, 9.7%) respectively. The obtained results were similar to those obtained by Laila et al. (1986); Mousa et al., (1987) and Abdel-Rahman (1989), but higher than those recorded by Ez-Eldin (1978).

Table 1. Number and percentage of identified *bacteria* isolated from marketed Oreochromis niloticus (Tilapia nilotica or Bolti).

Bacterial isolates	Distribution of Mo, in fresh water fish (N=55)								Total	%
	skin		Gills		Intestine		Muscles			
	No.	%	No.	%	No.	%	No.	%		
G. -ve bacteria:										
Proteus mirabilis	20	16.1	11	9. 8	7	8. 5	5	11. 1	43	11. 8
Proteus vulgaris	19	15. 3	12	10. 7	5	6.1	4	8. 9	40	11. 0
Klebsiella pneumoniae	16	12. 9	16	14.3	10	12. 2	8	17. 8	50	13. 8
Shigella flexneri type 6	15	12. 1	6	5. 4	5	6. 1	3	6. 7	29	8. 0
Escherichia coli	13	10. 5	7	6. 3	9	10. 9	3	6.7	32	8.8
Serratia sp.	12	9. 7	10	8. 9	3	3. 7	4	8. 9	29	8. 0
Proteus rettgeri	8	6. 5	10	8. 9	11	13. 4	7	15. 5	36	9. 9
Enterobacter cloacae	8	6. 5	8	7. 1	13	15. 9	6	13. 3	35	9. 6
Pseudomonas sp.	5	4. 0	14	12. 5	4	4. 9	2	4. 4	25	6. 9
Enterobcter aerogens	5	4. 0	7	6. 3	5	6. 1	-	0. 0	17	4. 7
Proteus morganii	3	2. 4	9	8. 0	6	7. 3	3	6.7	21	5. 8
Salmonella typhimurium	-	0. 0	2	1. 8	4	4. 9	-	0. 0	6	1. 7
Total	124	100.0	112	100.0	82	100.0	45	100.0	363	100.0
G.+ve bacteria:										
Streptococcus intermediate	8	57. 1	1	5. 6	5	41.7	3	37. 5	17	32.7
Streptococcus faecium	4	28. 6	8	44. 4	3	25.0	2	25. 0	17	32. 7
Staphylococcus albus	2	14. 3	4	22. 2	-	0. 0	3	37. 5	9	17. 3
Staphylococcus aureus	-	0.0	5	27. 8	4	33. 3	-	0. 0	9	17. 3
Total	14	100.0	18	100.0	12	100.0	8	100.0	52	100.0

Table 2. Number and percentage of identified *bacteria* isolated from marketed Mugil cephalus (Bouri).

Bacterial isolates	Distribution of Mo, in fresh water fish (N=45)								Total	%
	skin		Gills		Intestine		Muscles			
	No.	%	No.	%	No.	%	No.	%		
G. -ve bacteria:										
Shigella flexneri type 6	14	17. 5	4	8. 9	5	11. 6	2	4. 7	25	11. 9
Klebsiella pneumoniae	12	15. 0	7	15. 6	5	11. 6	7	16.2	31	14.7
Providencia sp.	11	13. 8	5	11.1	4	9. 3	2	4. 7	22	10. 4
Escherichia coli	9	11. 2	3	6.7	5	11. 6	2	4. 7	19	9. 0
Citrobacter freundi	8	10. 0	6	13. 3	3	7. 0	6	14.0	23	10. 9
Proteus vulgaris	6	7. 5	1	2.2	6	14. 0	4	9. 3	17	8. 1
Proteus rettgeri	5	6. 2	5	11.1	8	18. 6	3	6.9	21	10.0
Serratia sp.	5	6. 2	4	8.9	1	2.3	4	9. 3	14	6. 6
Pseudomonas sp.	4	5. 0	3	6.7	4	9. 3	4	9. 3	15	7. 1
Enterobcter aerogens	3	3. 8	2	4.4	-	0. 0	1	2. 3	6	2. 8
Arizona sp.	3	3. 8	5	11.1	2	4. 7	8	18. 6	18	8. 5
Total	80	100.0	45	100.0	43	100.0	43	100.0	211	100.0
G.+ve bacteria:										
Streptococcus pyogens	6	50. 0	3	30.0	4	50. 0	3	75. 0	16	47.0
Streptococcus faecium	4	33. 3	4	40.0	1	12. 5	-	0. 0	9	26.5
Staphylococcus aureus	2	16. 7	3	30.0	3	37. 5	1	25. 0	9	26.5
Total	12	100.0	10	100.0	8	100.0	4	100.0	34	100.0

Table 3. Number and percentage of identified *bacteria* isolated from marketed Clarias lazera (Armout catfish).

Bacterial isolates	Distribution of Mo, in fresh water fish (N=40)								Total	%
	skin		Gills		Intestine		Muscles			
	No.	%	No.	%	No.	%	No.	%		
G. -ve bacteria:										
Shigella flexneri type 6	12	14. 6	7	8. 6	3	4. 2	3	8. 8	25	9. 3
Klebsiella pneumoniae	12	14. 6	13	16. 1	7	9.7	4	11. 8	36	13. 4
Proteus vulgaris	10	12. 2	12	14. 8	8	11. 1	6	17. 7	36	13. 4
Serratia sp.	10	12. 2	8	9. 9	5	6. 9	2	5. 9	25	9. 3
Escherichia coli	9	11. 0	5	6. 2	11	15. 3	3	8. 8	28	10. 4
Proteus mirabilis	9	11. 0	5	6. 2	9	12. 5	5	14.7	28	10. 4
Pseudomonas sp.	7	8. 5	10	12.3	4	5. 6	5	14. 7	26	9. 7
Proteus rettgeri	5	6. 1	7	8. 6	10	13. 9	4	11. 8	26	9. 7
Proteus morganii	4	4. 9	6	7. 4	6	8. 3	1	2. 9	17	6. 3
Enterobcter aerogens	4	4. 9	6	7. 4	6	8. 3	1	2. 9	17	6. 3
Salmonella enteritidis	-	0. 0	2	2. 5	3	4. 2	-	0. 0	5	1. 8
Total	82	100.0	81	100.0	72	100.0	34	100.0	269	100.0
G.+ve bacteria:										
Streptococcus pyogens	6	50. 0	3	17. 7	3	27. 3	2	33.3	14	30.5
Streptococcus faecium	4	33. 3	5	29. 4	5	45. 4	1	16.7	15	32.6
Staphylococcus albus	2	16. 7	6	35. 2	-	0. 0	2	33.3	10	21.7
Staphylococcus aureus	-	0. 0	3	17. 7	3	27. 3	1	16.7	7	15.2
Total	12	100.0	17	100.0	11	100.0	6	100.0	46	100.0

Proteus organisms have been isolated fro cases of cystitis, pyelitis and are also considered as a secondary invador in wound infections and diseases of mucous membrane in man (Soltys, 1963). Proteus organisms are considered as a potential pathogen in cases of food poisoning and gastroenteritis in man (Jenning., 1975). So the zoonotic iportance of Proteus in food posses a potential health hazard and spoilage as well as enteric infection in man (Banwart, 1981).

Klebsiella species were isolated from Oreochronic niloticus, Mugil cephalus and Clarias lazera at an incidence of 13.8%, 14.7% and 1.4% respectively (Tables, 1,2 &3). These results were higher than those obtained by Mousa et al. (1978), but nearly similar to those recorded by Nabil (1975) and Laila et al. (1986). Klebsiella was reported to be associated with urinary tract infections in man (Cruickshank et al., 1970) and Baily and Scott (1978). Moreover, Klebisella are considered as an oppotunistic pathogen, involved several syndromes including pneumonia and upper repiratory tract infection (Banwart, 1981).

Shigella flexeneri type 6 was isolated from Orechromis niloticus, Mugil cephalus and Clarias lazera at percentages of 8.0%, 11.9% and 9.3% respectively. These results were higher than those recorded by Laila et al. (1986), but lower than those obtained by Mousa et al., (1978). It is implicated in cases of food-borne gastroenteritis and excreted in the faeces of infected man and animals (Banwart, 1981).

The incidence percentages of Escherichia coli isolated from Oreochromis niloticus, Mugil and Clarias lazera were 8.8%, 9.9% and 10.4% respectively (Table 1,2&3). These results were similar to those obtained by Nabila (1975) and Laila et al. (1986), but lower than those recorded by Ez-Eldin (1978). Mousa et al. (1987) and Abdel-Rahman (1989). In adition, E.coli could be isolated from skin, gills, intestine and muscles of Oreochromis niloticus, Mugil cephyalus and Clarias lazera at rates of (10.5%, 11.2% & 11.0%), (6.3%, 6.7% & 6.2%) , (10.9%, 11.6% & 15.3% (and (6.7%, 4.7% & 8.8%) respectively (Tables 1,2 and 3). In fish E.coli not only causes spoilage but the mere presence of enteropathogenic or enterotoxigenic strains is considered as indicator organisms for faecal contamination (Banwart, 1981).

Moreover, in human-being, this organism induces fatal intestinal infections in infants, peritonitis, gall bladder infections, haemorrhagic colitis (severe abdominal pain, watery stools followed by frankly bloodly diarrhoea) and urinary tract infections, it is also found in the majority of the abscesses and fistulates involving the perineal region of man (Abraham et al., 1983; Stephen et al., 1983 and Gyles, 1986).

The occurrence of Salmonella typhimurium and enteritidis in the examined fish samples was at an incidence rate of 1.7% from Oreochromis niloticus Table (1) 1.8% from Clarias lazera respectively, whereas it could not be isolated from Mugilcephalus. The organism was isolated from gills and intestine at perentages of 1.8% and 4.9% respectively (Table 1). These results were in agreement with those obtained by Lotfi

Table 4. Number and percentage of identified *fungi* isolated from marketed Oreochromis niloticus (Tilapia nilotica or Bolti).

Fungal isolates	Distribution of Mo, in fresh water fish (N=55)								Total	%
	skin		Gills		Intestine		Muscles			
	No.	%	No.	%	No.	%	No.	%		
Mould sp.:										
Aspergillus niger	20	48.8	8	21.0	4	17.4	6	23.1	38	29.7
Aspergillus flavus	6	14.6	5	13.2	3	13.0	-	0.0	14	10.9
Aspergillus fumigatus	5	12.2	6	15.8	1	4.4	2	7.7	14	10.9
Penicillium	5	12.2	7	18.4	6	26.1	4	15.4	22	17.2
Unidentified sp.	3	7.3	5	13.2	3	13.0	5	19.2	16	12.6
Mucor sp.	2	4.9	4	10.5	5	21.7	8	30.8	19	14.8
Rhizopus sp.	-	0.0	3	7.9	1	4.4	1	3.8	5	3.9
Total	41	100.0	38	100.0	23	100.0	26	100.0	128	100.0
Yeast sp.:										
Rhodotorulla mucilignosa	8	28.6	2	11.1	1	7.6	-	0.0	11	15.9
Torulopsis sp.	5	17.9	5	27.8	3	23.1	2	20.0	15	21.8
Yeast-like organism:										
Candida albicans	6	21.4	6	33.3	4	30.8	3	30.0	19	27.5
Candida tropicalis	5	17.9	3	16.7	3	23.1	4	40.0	15	21.8
Candida krusei	4	14.2	2	11.1	2	15.4	1	10.0	9	13.0
Total	28	100.0	18	100.0	13	100.0	10	100.0	69	100.0

Table 5. Number and percentage of identified *Fungi* isolated from marketed Mugil cephalus (Bouri).

Fungal isolates	Distribution of Mo, in fresh water fish (N=45)								Total	%
	skin		Gills		Intestine		Muscles			
	No.	%	No.	%	No.	%	No.	%		
Mould sp.:										
Aspergillus niger	14	26. 9	12	27.3	13	37.1	9	32.1	48	30.1
Mucor sp.	13	25. 0	9	20. 5	8	22. 9	5	17.9	35	22. 0
Aspergillus flavus	12	23.1	17	38.6	8	22. 9	8	28. 6	45	28. 3
Rhizopus sp.	6	11.5	-	0. 0	2	5. 7	1	3. 6	9	5.7
Aspergillus fumigatus	4	7.7	3	6. 8	3	8. 5	3	10.7	13	8. 2
Penicillium	3	5. 8	3	6. 8	1	2. 9	2	7. 1	9	5.7
Total	52	100.0	44	100.0	35	100.0	28	100.0	159	100.0
Yeast sp.:										
Torulopsis sp.	8	32.0	3	15.8	4	18.2	1	16.7	16	22.2
Rhodotorulla mucilignosa	5	20. 0	2	10. 5	5	22.7	-	0. 0	12	16.7
Yeast-like organism:										
Candida albicans	5	20.0	5	26.3	7	31. 8	2	33. 3	19	26. 4
Candida tropicalis	4	16.0	3	15. 8	3	13.6	1	16.7	11	15.3
Candida krusei	2	8. 0	4	21.1	2	9. 1	-	0.0	8	11. 1
Candida parapsilosis	1	4. 0	2	10.5	1	4. 6	2	33. 3	6	8. 3
Total	25	100.0	19	100.0	22	100.0	6	100.0	72	100.0

Table 6. Number and percentage of identified *fungi* isolated from marketed Clarias lazera (Armout catfish).

Fungal isolates	Distribution of Mo. in fresh water fish (N= 40)								Total	%
	skin		Gills		Intestine		Muscles			
	No.	%	No.	%	No.	%	No.	%		
Mould sp.:										
Aspergillus flavus	11	33. 3	7	18. 4	5	17. 9	8	38. 0	31	25. 8
Aspergillus niger	9	27. 3	8	21. 0	8	28. 6	5	23. 8	30	25.0
Aspergillus fumigatus	6	18. 2	6	15. 8	2	7. 1	3	14. 3	17	14.2
Penicillium	4	12.1	5	13. 2	2	7. 1	1	4. 8	12	10. 0
Mucor sp.	2	6. 1	10	26. 3	10	35. 7	3	14. 3	25	20. 8
Rhizopus sp.	1	3. 0	2	5. 3	1	3. 6	1	4. 8	5	4. 2
Total	33	00.0	38	100.0	28	100.0	21	100.0	120	100.0
Yeast sp.:										
Torulopsis sp.	4	13.3	3	20.0	2	12. 5	3	20.0	11	15. 5
Rhodotorulla mucilignosa	3	10.0	2	13.3	3	18.7	-	0. 0	8	11. 3
Yeast-like organism:										
Candida albicans	11	36.7	4	26.7	5	31. 3	4	40.0	24	33. 8
Candida tropicalis	7	23.3	3	20.0	3	18.7	2	20.0	15	21.1
Candida kruseı	3	10.0	2	13.3	2	12.5	1	10.0	8	11. 3
Candida parapsilosis	2	6.7	1	6.7	1	6. 3	1	10.0	5	7. 0
Total	30	100.0	15	100.0	16	100.0	10	100.0	71	100.0

et al., (1974); Trust and Sparrow (1974); Nabila (1975) and Abdel-Rahman (1989) . On the other hand, this organism could not be isolated from the fish examined by Sedik (1977); Mahmoud (1981) and Mousa et al. (1987). Salmonella causes food poisoning characterized by fever, headache and general aching of the limbs as well as diarrhoea and vomiting in man (Bettey and Richard 1978 and Banwart, 1981).

The presence of Staphylococcus aureus in fish indicates their contamination from polluted water (Brown and Dorn 1977). Staphylococcus aureus was isolated from Oreochromis niloticus, Mugil cephalus and Clarias lazera at incidence of 17.3%, 26.5% and 15.2%, respectively (Tables 1,2 &3). These results were higher than those obtained by Ez-Eldin (1978) and Abdel-Rahman (1989). It is one of the most imortant specific microorganisms responsible for food poisoning in human-beings (Jay, 1970). It causes a variety of superfical and deep infection in most cases of pus formation (Cruickshank et al., 1975).

Aspergillus niger, fumigatus and flavus were isolated from Oreochrmis niloticus, Mugi cephalus and Clarias lazera at an incidence of (29.7%, 10.9% and 10.9%), (30.1%, 8.2% and 28.3%) and 25.0%, 14.2% and 25.8%) respectively (Tables 4,5 & 6). These results were higher than those obtained by El-Bassiouny et al., (1989) and Samira (1991). Aspergillus species have been incriminated as a causative agent in many human mycotic infections especially broncho-pulmonary aspergillosis (Jordan et al., 1971).

Rhodotorulla mucilignosa was isolated from Orechromis niloticus, Mugil cephalus and Clarias lazera at an incidence of 15.9%, 16.7% and 11.3% respectively (Table 4,5 & 6). Rhodotorulla may cause fungamia, endocarditis and mycotic keratitis in human-beings (Washingtom, 1981).

The data recorded in Tables (4,5 & 6) revealed that Candida albicans, tropicalis and krusei were isolated from Oreochromis niloticus, Mugil cephalus and Clarias lazera at percentage of (17.5%, 21.8% and 13.0%), (26.4%, 15.3 and 11.1%) and (33.8%, 21.1% and 11.3%) respectively. These results were higher than those obtained by Marzouk et al. (1990). In man,, Candida infection is responsible for the appearance of white adherent patches on the mucous membrane of the mouth (Thrush) particularly in children (Basu and Banerjee, 1991). It is also a common cause of vaginitis and vulvovaginitis in women . (Daftary et al., 1963). Moreover, the organism infects the skin either inflamed or abraded especially of the moist and warm part of the body giving rise to the cutaneous form of candidiasis (Emmons et al. 1977).

From the results achieved and public health hazard point of view, any threat to the environment will sonner or later become a threat to the health of the human race and we can conclude that, fish may be contaminated with various zoonotic pathogenic and potyentially pathogenic bacteria and fungi of health importance.

On conclusion, the different pathogens isolated during this work threaten human health. These organisms may reach the human either through direct contact with fish or through its ingestion if unproperly cooked. It should be mentioned here that Egyptians rarely consume fish raw or semi-cooked. The danger that may be inflicted on man lies mainly during handling of fish with the presence of hand abrasions or wound . So, thorough cooking of fish with correct personal care of hands during handling of fish is best advice given to safe-guard human contacts.

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