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Original Article

Aerobic bacteria and fungi from skin lesions of fish in Khartoum state

Walaa Hassan Ibrahim, Abdel-Hafeez Hassan Nimir, Suleiman Mohamed El-Sanousi and Yassir Adam Shuaib

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AFFILIATIONS

- Walaa Hassan Ibrahim
Ministry of Animal Resources and Fisheries, White Nile State, Kosti, the Sudan.

- Abdel-Hafeez Hassan Nimir
- Suleiman Mohamed El-Sanousi
Faculty of Veterinary Medicine, University of Khartoum, P.O. Box 13314 (Shambat) Khartoum North, the Sudan.

- Yassir Adam Shuaib
College of Veterinary Medicine, Sudan University of Science and Technology, P.O. Box 204 (Hilat Kuku), Khartoum North, the Sudan.

CORRESPONDENCE

Walaa Hassan Ibrahim
Ministry of Animal Resources and Fisheries, White Nile State, Kosti, the Sudan.
E-mail: al.goon86@gmail.com

ABSTRACT

Objective: This cross-sectional study was conducted from April to July 2014 in Khartoum state, the Sudan, to investigate aerobic bacteria and fungi of skin lesions of fish in 3 different areas in Khartoum.
Material and methods: A total of 50 samples were collected from the skin lesions of different types of fish including *Synodontis* species (n=17), *Tilapia niloticus* (n=15), *Labeo niloticus* (n=10), *Hydrocynus* species (n=4), and *Clarias* species (n=4). Liquid, semi-solid, and solid culture media like nutrient broth, blood agar, MacConkey agar, sabouraud dextrose agar (SDA), and Simmon's citrate medium were used for the isolation and identification of bacteria and fungi. Besides, Gram staining and biochemical characterization were also conducted.
Results: Culturing of the collected samples revealed growth of bacteria from all (100%), and growth of fungi could be found from 32% samples. A number of 188 bacteria were isolated, mainly *Staphylococcus* species, *Bacillus* species, *Aeromonas* species, *Pseudomonas* species, and *Vibrio* species. Besides, 16 fungi could be identified containing *Aspergillus niger*, *A. flavus*, *A. fumigatus*, and *Phycomycete*.
Conclusion: Fishes with skin lesions are harboring many pathogenic bacteria and fungi and may act as a source of zoonotic infections and can transmit several pathogens to workers in fish industry and consumers. Therefore, thorough and strict routine inspection of fish is recommended to ensure safety and that there are no serious risks to consumers.

KEYWORDS

Bacteria, Fish, Fungi, Skin lesions, Sudan

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INTRODUCTION

Due to the big amount of surface and underground water resources in the Sudan, there exist a hug wealth of fish too (MARF, 2001a, b; Saaed, 2004; De Young, 2006). In the last few years, Sudanese peoples' interest in fish consumption is noticeably rising as in other parts of the world. This is because of the unstable and escalating prices of red meat and because of the general believe that regular fish consumption is a possible practice for health improvement (Goja, 2013; Deliens et al., 2014). Consequently, there is an increase in fish trade and investments. However, if the fish habitats are contaminated with pathogens, consumption of these fishes may impose risk to humans (Goja, 2013).

Fishes are susceptible to a wide variety of pathogens especially when they are physiologically unbalanced or nutritionally deficient and subjected to stresses, i.e. poor water quality, and over stocking (Plumb, 1997; Goja, 2013; Rowe et al., 2014). Infected fishes show many disease symptoms but in case of skin problems the symptoms are mainly ulcerative and hemorrhagic skin patches (Bruno and Wood, 1994; Adeyemo, 2003; Haj-Ali, 2010; Hassan et al., 2010).

Edwardsiella tarda, *Aeromonas* species, *Pseudomonas* species and many other pathogenic bacteria have been isolated from diseased fishes and often these bacteria are fatal if not treated early enough (Yagoub, 2009; Goja, 2013; Kar, 2015; Sebastião et al., 2015). Besides to that, fungal infections are common among fish populations and can lead to heavy economic losses as well (Scarfe et al., 2005; Ramaiah, 2006). Most fish fungal infections are caused by the fungi of the family *Saprolegniaceae*, *Phycomycetes*, *Aspergillus* and *Penicillium* (Yagoub, 2004; Gozlan et al., 2014). This study was aiming at identifying the bacterial and fungal causative agents of fish skin infections in Khartoum state.

MATERIALS AND METHODS

Study area and samples collection: A cross-sectional study was carried out from April to July 2014 in Khartoum state. Three areas were investigated, namely; El-Shagarah Center for Fish Research, Almawrda Fish Market, and El-Haj Yousif Fish Market.

A total number of 50 samples were collected from the skin lesions of different types of fish including *Synodontis* species (n=17), *Tilapia niloticus* (n=15), *Labeo niloticus* (n=10), *Hydrocynus* species (n=4), and *Clarias* species (n=4). Samples were collected as described by Buller (2004). For investigation of bacteria, a sterile cotton swab was rubbed all over the lesion, while for fungi a piece of tissue from the lesion was taken using a sterile scalpel blade. All samples were placed in an ice container and transported to the laboratory of the Department of Microbiology, Faculty of Veterinary Medicine, University of Khartoum, and were cultured within 2 h of collection.

Culture media and reagents: Different types of culture media including liquid, semi-solid, and solid media, and chemicals, and reagents were used for isolation and identification of bacteria and fungi and all were either bought from companies ready-to-use or prepared according to Barrow and Feltham (2003) and Ochei and Kolhatkar (2000). The culture media were peptone water, nutrient broth, glucose phosphate peptone broth, nutrient agar, blood agar, MacConkey agar, sabouraud dextrose agar (SDA), starch agar, nutrient gelatin media, urea agar, Simmon's citrate medium, Hugh and Leifson's (O-F) medium, motility medium, and Arginine media. Chemicals and reagents included Gram solution, crystal violet, Lugol's iodine, decolorizing reagent, counter stain, Tetra methyl-p-phenylenediamine dihydrochloride, and Alpha-naphthol solution, as well as Voges-Proskauer (VP) test, Methyl red, Kovac's reagent and Nessler's, Andrade's indicator, Neutral red, Phenol red, Bromothymol blue, and Lead acetate paper.

Culturing: Collected samples were first inoculated into liquid media and incubated at 37°C for 24 h, after that all were streaked onto blood and MacConkey agars and incubated aerobically at 37°C for 24 h also. Further incubation was continued for another 24 h and if no growth was evident, then the plates were discarded and the sample was considered negative. All cultures were examined by naked eye for growth, colony morphology, and any changes in the medium. Purification of isolates was done by sub-culturing of a single well separated colony onto blood agar or nutrient agar and finally pure cultures were stored at 4°C.

Identification of isolates: Gram staining was carried out according to Barrow and Feltham (2003). The prepared slides were examined microscopically under oil emersion objective lens. Biochemical tests including catalase test, oxidase test, motility test, and oxidation fermentation (O/F) test were conducted. Other biochemical tests were sugar fermentation test, gelatin hydrolysis, Arginine hydrolysis, starch hydrolysis, slide and tube coagulase test, Indole production test, H_2S production test, urease test, citrate utilization test, Voges-Proskaur (VP) test, and Methyl red test.

Fungal investigations: According to Barrow and Feltham (2003), samples were also cultured onto sabouraud dextrose agar media and incubated at 22°C for 1-2 weeks, during which period the plates were

examined daily. Identification of isolated fungi was done by examining the mold in wet mount by transporting a portion of a colony to a drop of Lacto phenol cotton blue (LPCB) stain on sterile slide. A cover slip was applied on the preparation and examined microscopically.

RESULTS

Culture of the 50 specimens of the investigated fish lesions revealed growth of bacteria from all (100.0%) and growth of fungi from some of (32.0%). Isolates of bacteria were 188 and of fungi were 16.

Gram-positive bacteria: Gram stained smears from the cultures and biochemical tests (**Table 1** and **2**) showed that 78.7% (n=148) of the detected bacteria were Gram-positive cocci and rods. *Staphylococcus* species and *Bacillus* species were detected with *Staphylococcus auricularis* (n=17) and *Bacillus badius* (n=11) being the most isolated micro-organisms. *Micrococcus lylae* (n=7) was the most frequent among the detected 6 species of *Micrococcus*. Besides, *Corynebacterium mycetoides*, *C. pseudotuberculosis*, and *C. pseudodiphtheriticum* were isolated 3, 2, and 1 times, respectively. With 5 strains, *Streptococcus uberis* was the most frequent *Streptococcus* species. In addition, *Listeria innocua*, *L. ivonovii*, *Kurthia zopfii*, *Leuconostoc* species, *Stomatococcus mucilaginosus*, *Aerococcus* species, and *Gemella haemolysans* were each found between 2 to 9 times (**Table 4**).

Gram-negative Bacteria: Table 3 depicted the characters and biochemical tests of the Gram-negative isolates (21.3%; n=40). *Aeromonas sobria*, *A. salmonicida*, *Pseudomonas pseudoalcaligenes*, *Ps. paucimobilis*, *Vibrio cholera*, *V. cincinnatiensis*, *V. damsel*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Serratia marcescens*, *Providencia rettgeri*, and *Yersinia pseudotuberculosis* were isolated between 1 and 6 times (**Table 5**).

Fungi: *Aspergillus* species (n=12; 75.0%) and *Phycomycete* species (n=4; 25.0%) were found in the samples of lesions of fish skin. *Aspergillus niger* (n=5; 31.3%), *A. flavus* (n=4; 25.0%), and *A. fumigatus* (n=3; 18.7%), were isolated and identified (**Table 6**).

DISCUSSION

Aquatic creatures, like fish, could potentially be harboring many infectious zoonotic micro-organisms that are able to cause health problems in humans. Hunt et al. (2008), Igbinosa et al. (2012), Waltzek et al. (2012), Haenen et al. (2013), and Harper and Erickson (2016) indicated that consumers, fishermen, aquarium workers, and salesmen normally glean these zoonoses either by- i) direct contact, or ii) by ingestion of raw or undercooked aquatic products, and lately, their incidence is escalating. Moreover, infectious diseases are very important in fish industry, because of their consequential heavy economic losses, especially in aquacultures (Haenen et al., 2013; Lafferty et al., 2015). These diseases are commonly caused by pathogens that are either indigenous; originate from the aquatic environment itself or exogenous; occur due to contamination (Haenen et al., 2013). Skin infections in fish are mainly caused by bacteria, parasites and *Saprolegnia* species, but also by oomycetes and fungi (Cutuli et al., 2015).

Many aerobic Gram positive and gram negative bacteria have been isolated from skin lesions of different types of fish in this study. These findings were similar to the findings of Buller (2004), Yiagnisis and Athanassopoulou (2011), Haenen et al. (2013), Tanekhy (2013), and Saad and Atallah (2014) who were able to isolate aerobic bacteria from different types of fish from different places around the world. Other workers from the Sudan, Egypt, and Nigeria like Selma (2006), Hassan et al. (2010), Ajayi (2012), and Goja (2013) have indicated that indicated that many bacteria can colonize intact healthy or injured and diseased skin of fish and internal organs. Their ability to do so, is underlined by many factors like ability to express virulence factors to invade the host and produce pathological effects. Other important factors would be environmental hygiene and immunity and resistance of the fish.

Staphylococcus species was the most frequent species among all isolated bacteria in the present study. This was different from the observation of Süheyla and Osman (2004) who mentioned that staphylococci are the second most frequently isolated bacteria from clinical specimens after *Enterobacteriaceae*. Nonetheless, it was in agreement with the findings of Majumder et al. (2001), Yiagnisis and Athanassopoulou (2011), Carbajal-González et al. (2011), and Ali (2014) who were able to isolate *Staphylococcus* species from healthy and diseased fish. The ability of the members of the genus *Staphylococcus* to establish themselves, colonize their hosts and survive could be explained by their ability to express and/or possess one or more potential virulence factors. These factors are adherence factors or exotoxins and include i) surface proteins that promote colonization of host tissues, ii) invasins that promote bacterial spread in tissues like leukocidin, kinases, and hyaluronidase, iii) inhibition of phagocytic engulfment by surface factors such as capsule and protein A, iv) biochemical properties that enhance survival in phagocytes like carotenoids and catalase production, v) immunological disguises, vi) hemolysins, leukotoxin, leukocidin toxins for membrane-damaging

Table 1: Characters and biochemical reactions of the isolated *Staphylococcus* species, *Streptococcus* species, *Gemella haemolysans*, *Leuconostoc* species, *Aerococcus* species, *Micrococcus* species, and *Stomatococcus* species from skin lesions of fish in Khartoum state, the Sudan (April to July 2014).

Ch & BT	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Shape	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Gram reaction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Motility	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-
O/F	F	F	F	F	F	F	F	ND	ND	ND	ND	ND	ND	-	-	O	-	-	-	F
Coagulase	-	-	-	-	-	-	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
VP	d	+	+	d	+	+	+	+	+	-	-	+	d	-	+	-	-	-	-	+
Arginine hydrolysis	-	-	+	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Hemolysis	ND	ND	ND	ND	ND	ND	ND	α	β	β	α	α	α	ND	ND	ND	ND	ND	ND	ND
Starch hydrolysis	ND	ND	ND	ND	ND	ND	ND	d	d	+	d	-	-	ND	ND	ND	ND	ND	ND	ND
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	d	d	+	-	+
Sucrose	d	+	+	-	-	+	-	+	+	+	d	+	+	-	+	-	-	d	-	+
Mannitol	-	-	+	+	-	d	-	+	-	-	-	d	d	ND	ND	ND	ND	ND	ND	ND
Lactose	-	-	-	d	-	-	+	+	d	+	-	d	d	ND	ND	ND	ND	ND	ND	ND
Fructose	+	+	+	+	+	+	-	ND	ND	ND	ND	ND	ND	-	+	-	d	+	-	+
Mannose	-	+	+	-	+	-	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Trehalose	+	+	-	+	-	+	+	+	+	-	d	+	+	ND	ND	ND	ND	ND	ND	ND
Sorbitol	ND	ND	ND	ND	ND	ND	ND	+	-	+	-	-	d	ND	ND	ND	ND	ND	ND	ND
Ribose	ND	ND	ND	ND	ND	ND	ND	+	+	d	-	d	d	ND	ND	ND	ND	ND	ND	ND
Arabinose	ND	ND	ND	ND	ND	ND	ND	-	-	-	-	d	-	ND	ND	ND	ND	ND	ND	ND
Aerobic growth	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	+	+	+	+	+	+	+
Pigmentation	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	C	Y	Or	R	Y	Y	-

Ch & BT=Characters and biochemical test, 1=*Staphylococcus auricularis,* 2=*Staph. lugdunensis,* 3= *Staph. capitis,* 4= *Staph. kloosii,* 5=*Staph. saccharolyticus,* 6=*Staph. warneri,* 7=*Staph. caprae,* 8=*Streptococcus uberis,* 9=*Strept. agalactiae,* 10=*Strept. zooepidermicus,* 11=*Gemella haemolysans,* 12=*Leuconostoc* species, 13=*Aerococcus* species, 14=*Micrococcus lylae,* 15=*M. kristinae,* 16=*M. nishinomiyaensis,* 17=*M. roseus,* 18=*M. varians,* 19=*M. luteus,* and 20=*Stomatococcus mucilaginosus.* +=positive, -=negative, O=oxidative, d=different, F=fermentative, S=sphere, C= cream, Y=yellow, Or=orange, R=red, α=green zone around colonies on blood agar, β=clear, colorless zone around colonies on blood agar, and ND=not done.

Table 2: Characters and biochemical reactions of the isolated *Bacillus* species, *Corynebacterium* species, *Listeria* species and *Kurthia* species from skin lesions of fish in Khartoum state, the Sudan (April to July 2014).

Ch & BT	1	2	3	4	5	6	7	8	9	10	11	12	13
Shape	R	R	R	R	R	R	R	R	R	R	R	R	R
Gram reaction	+	+	+	d	d	+	+	+	+	+	+	+	+
Spore shape	O	O	O	O	Ro	O	O	ND	ND	ND	ND	ND	ND
Spore position	S	C	C	C	T	S	T	ND	ND	ND	ND	ND	ND
Growth at 50 °C	d	+	-	+	-	-	d	ND	ND	ND	ND	ND	ND
Motility	+	+	+	+	+	-	+	-	-	-	+	+	+
Utilization of citrate	-	+	-	-	d	d	+	ND	ND	ND	ND	ND	ND
Urease	-	d	+	-	d	d	-	-	+	+	ND	ND	-
Indole	-	-	-	-	-	-	-	ND	ND	ND	ND	ND	ND
VP	-	d	-	+	-	+	+	-	-	-	+	+	-
Starch hydrolysis	-	+	+	-	-	+	-	-	+	-	ND	ND	-
Oxidase	d	-	+	-	+	d	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+
O/F	ND	ND	ND	ND	ND	ND	ND	F	F	-	F	F	-
H_2S	ND	ND	ND	ND	ND	ND	ND	-	-	-	-	-	D
Gelatin liquefaction	ND	ND	ND	ND	ND	ND	ND	-	d	-	ND	ND	-
Glucose	ND	ND	ND	ND	ND	ND	ND	+	+	-	+	+	-
Maltose	ND	ND	ND	ND	ND	ND	ND	-	+	-	-	+	+
Sucrose	ND	ND	ND	ND	ND	ND	ND	-	d	-	d	d	-
Salicin	ND	ND	ND	ND	ND	ND	ND	-	-	-	+	+	+
Trehalose	ND	ND	ND	ND	ND	ND	ND	d	-	-	ND	ND	ND
Xylose	ND	ND	ND	ND	ND	ND	ND	-	-	-	-	+	-

Ch & BT=Characters and biochemical test, 1=*Bacillus badius,* 2=*B. licheniformis,* 3=*B. lentus,* 4=*B. sterothermophilus,* 5=*B. sphaericus*, 6=*B. mycoides*, 7=*B. pumilus*, 8=*Corynebacterium mycetoides*, 9=*C. pseudotuberculosis*, 10=*C. pseudodiphtheriticum*, 11=*Listeria innocua*, 12=*L. ivonovii*, and 13=*Kurthia zopfii.* R=Rod, +=Positive, -=Negative, O=Oval, S=Subterminal, D=different, Ro=Round, C=Central, T=Terminal, F=Fermentative, and ND=Not done.

Table 3: Characters and biochemical reactions of the isolated *Aeromonas* species, *Vibrio* species, *Pseudomonas* species, *Klebsiella pneumoniae , Proteus mirabilis, Serratia marcescens, Providencia rettgeri,* and *Yersinia pseudotuberculosis* from skin lesions of fish in Khartoum state, the Sudan (April to July 2014).

Ch & BT	1	2	3	4	5	6	7	8	9	10	11	12
Shape	R	R	R	R	R	R	R	R	R	R	R	R
Motility	+	d	+	+	+	+	d	-	+	+	+	d
Oxidase	+	+	+	+	+	+	+	-	-	-	-	-
Catalase	+	+	+	+	+	+	+	+	+	+	+	+
Urease	+	-	-	-	-	d	-	+	+	d	+	+
Indole	+	d	-	+	-	-	ND	-	-	-	+	-
Gas from glucose	d	d	-	-	-	ND	ND	ND	ND	ND	ND	ND
VP	d	-	+	+	+	-	ND	+	-	+	-	-
Arginine	+	+	-	-	+	ND	ND	ND	ND	ND	ND	ND
Glucose	+	+	+	+	+	-	+	+	+	+	+	+
Sucrose	+	d	+	+	-	-	ND	+	-	+	d	-
Xylose	-	-	+	-	-	ND	ND	ND	ND	ND	ND	ND
Salicin	d	d	+	-	-	ND	ND	ND	ND	ND	ND	ND
Arabinose	-	+	+	-	-	ND	ND	+	-	-	-	+
Citrate	ND	ND	ND	ND	ND	d	-	+	d	+	+	-
Gelatin hydrolysis	ND	ND	ND	ND	ND	-	-	-	+	+	-	-
H_2S	ND	ND	ND	ND	ND	-	-	-	+	d	+	-
MR	ND	ND	ND	ND	ND	ND	ND	-	d	-	+	+
Mannitol	ND	ND	+	-	+	+	+	ND	ND	ND	ND	ND
Lactose	ND	ND	+	-	-	-	-	ND	ND	ND	ND	ND
Dulcitol	ND	ND	d	-	-	-	-	ND	ND	ND	ND	ND
Salicin	ND	ND	+	-	+	d	d	ND	ND	ND	ND	ND
Trehalose	ND	ND	+	+	+	-	+	ND	ND	ND	ND	ND

Ch & BT=Characters and biochemical test, 1=*Aeromonas sobria*, 2=*A. salmonicida*, 3=*Vibrio cincinnatiensis*, 4=*V. cholera*, 5=*V. damsel*, 6=*Pseudomonas pseudoalcaligenes*, 7=*P. paucimobilis*, 8=*Klebsiella pneumoniae*, 9=*Proteus mirabilis*, 10=*Serratia marcescens*, 11=*Providencia rettgeri*, and 12=*Yersinia pseudotuberculosis.* +=positive, -= negative, d= different, R=rod, and ND=not done.

Table 4: Number and percentage of Gram-positive cocci and rods bacteria isolated from skin lesions of fish in Khartoum state, the Sudan (April to July 2014).

Microorganism	No.	%
Staphylococcus **species**		
Staph. auricularis	17	11.5
Staph. lugdunensis	9	6.10
Staph. capitis	7	4.70
Staph. kloosii	6	4.10
Staph. saccharolyticus	4	2.70
Staph. warneri	4	2.70
Staph. caprae	3	2.00
Bacillus **species**		
B. badius	11	7.40
B. licheniformis	6	4.10
B. lentus	6	4.10
B. sterothermophilus	6	4.10
B. sphaericus	3	2.00
B. mycoides	2	1.40
B. pumilus	1	0.70
Micrococcus **species**		
Micro. lylae	7	4.70
Micro. kristinae	2	1.40
Micro. nishinomiyaensis	2	1.40
Micro. roseus	1	0.70
Micro. varians	1	0.70
Micro. luteus	1	0.70
Corynebacterium **species**		
C. mycetoides	3	2.00
C. pseudotuberculosis	2	1.40
C. pseudodiphtheriticum	1	0.70
Streptococcus **species**		
Strept. uberis	5	3.40
Strept. agalactiae	2	1.40
Strept. zooepidermicus	1	0.70
Listeria **species**		
L. innocua	6	4.10
L. ivonovii	2	1.40
Other **species**		
Kurthia zopfii	9	6.10
Leuconostoc species	7	4.70
Stomatococcus mucilaginosus	6	4.10
Aerococcus species	3	2.00
Gemella haemolysans	2	1.40
Total	**148**	**100**

and lysing of cell membranes of hosts, vii) exotoxins that damage host tissues or otherwise provoke symptoms of disease, and iix) inherent and acquired resistance to antimicrobial agents (Süheyla and Osman, 2004; Otto, 2004; Ki and Rotstein, 2008).

Table 5: Number and percentage of Gram-negative bacteria isolated from skin lesions of fish in Khartoum state, the Sudan (April to July 2014).

Microorganism	No.	%
Aeromonas **species**		
A. sobria	5	12.5
A. salmonicida	2	5.00
Pseudomonas **species**		
Ps. pseudoalcaligenes	4	10.0
Ps. paucimobilis	3	7.50
Vibrio **species**		
V. cholerae	3	7.50
V. cincinnatiensis	2	5.00
V. damsela	1	2.50
Other **species**		
Klebsiella pneumoniae	6	15.0
Proteus mirabilis	5	12.5
Serratiamarcescens	4	10.0
Providencia rettgeri	3	7.50
Yersinia pseudotuberculosis	2	5.00
Total	**40**	**100**

Table 6: Number and percentage of the isolated *Aspergillus* species and Phycomycete species isolated from skin lesions of fish in Khartoum state, the Sudan (April to July 2014).

Microorganism	no.	%
Fungi		
Aspergillus niger	5	31.3
A. flavus	4	25.0
A. fumigatus	3	18.7
Phycomycete species	4	25.0
Total	**16**	**100**

Isolation of *Bacillus* species from skin lesion of fish in this study typified the findings of Ajayi (2012) and Goja (2013). Furthermore, it was also similar to the findings of Goodwin et al. (1994) who detected *B. mycoides* from pale areas or ulcers on the dorsum and focal necrosis of epaxial muscle in channel catfish during an epizootic in a commercial culture pond in the USA. Additionally, histologic examination of the lesions revealed necrotic muscle with chains of Gram-positive bacilli and experimental infection by injection of the detected bacteria into channel catfish caused lesions that resembled those in fish during the epizootic. Turnbull (1996) and Kasing et al. (1999) indicated that *Bacillus* species are widely distributed in the environment mainly because of formation of endo-spore which is highly resistant to heat, cold, radiation, desiccation, and disinfectants, as well as, exhibiting a wide range of

physiologic abilities that allow them to live in every natural environment. The principal virulence factors of *Bacillus* species are a necrotizing enterotoxin and a potent hemolysin or cereolysin, besides to capsule and numerous enzymes and aggressins.

Micrococcus species were reported herein and this was coinciding with what has been reported from skin lesions of three fish species from several locations in India by Pal and Pradhan (1990) and Kumar and Day (1992). Likewise, *Micrococcus* species were isolated from ulcers of wild fish and mrigal carp (*Cirrhinus mrigala*) by Majumder et al. (2001) and Sharma et al. (2013). Also, Lilley et al. (1991), Kocur et al. (2006), and Bannerman and Peacock (2007) stated that *Micrococcus* species have been associated with necrotic ulcers and are most probably opportunistic pathogens in immuno-compromised fish causing secondary infections leading to death in severely ulcerated fish.

Kurthia zopfii was isolated herein as did by Selma (2006) and Carbajal-González et al. (2011). *Kurthia* species are not usually regarded as pathogens although have been isolated from meat and dairy products and occasionally from clinical materials (Keddie, 1981). Its presence maybe due to contamination during handling. *Strept. uberis*, *Strep. agalactiae, Strept. zooepidermicus* were isolated from infections of fish skin in the present study. This was in agreement with Ajayi (2012), Sharma et al. (2013), and Goja (2013). Salvador et al. (2005) who found *Streptococcus* species in samples of brain, liver, kidney and ascites liquid in fish that were showing hemorrhagic lesions on the skin and other symptoms. *Listeria innocua* and *L. ivonovii* were isolated and identified from fish skin lesions in the present study concurring the findings of Mohammed (1999) and Soliman (1999). Cutaneous listeriosis in human, although it is very rare and is mostly an occupation-associated infection, the bacteria needs a scratch or a wound to be able to infect the skin. Besides, it was generally seen in immunocompromised patients (Godshall et al., 2013). *Listeria* species-skin infection in fish is probably occurring in the same scenario of cutaneous listeriosis in human. Three different species of *Corynebacterium* were isolated from skin lesions of fish in this study. Selma (2006) was able to grow *C. xerosis* and *C. Pseudodiphtheriticum* from samples taken from fresh water fish. The pathogenicity of *Corynebacterium* species is mainly observed in immunosuppressed hosts when the bacteria exhibits their virulence factor.

Gram-negative bacteria including *Aeromonas* species, *Pseudomonas* species, *Klebsiella* species, *Proteus* species, *Serratia marcescens*, *Providencia rettgeri* and *Yersinia pseudotuberculosis* were detected in the present study. These Gram-negative bacteria were reported from skin of fish by Banu (1996), Islam (1996), Abdel Elrahman (2003), Selma (2006), Carbajal-González et al. (2011), Ajayi (2012), Sharma et al. (2013), and Cutuli et al. (2015). In early 1970s, Duijn (1973) stated that the majority of fish diseases are caused by Gram-negative bacteria. This might possibly be because of several factors that make Gram-negative bacteria able to infect their hosts and produce disease symptoms. These factors are adhesins, motility, growth and invasion, iron acquisition, extracellular products (ECPs), and endotoxins (Méndez et al., 2012). Isolation of these bacteria from fish skin lesions might otherwise be be due to water pollution or less resistance of the fish.

Four fungi have been found in this study, namely *A. niger*, *A. flavus*, *A. fumigates* and *Phycomycetes*. This was corresponding to the observations of Olufemi (1985) who indicated that a number of *Aspergillus* species were responsible of aflatoxicosis in fish. It was also in agreement with Yagoub (2004) and Sharma et al. (2013) who reported *Aspergillus* species in fish in the Sudan and from skin ulcers of fish in India. Moreover, Olufemi and Roberts (1983) found that *Aspergillus* species play a significant role as pathogens in farmed fishes. Cutuli et al. (2015) reported skin infections of tilapia by *Fusarium oxysporum* species complex. The detection of *Phycomycetes* species was in agreement with the findings of Yagoub (2004). Iqbal and Saleemi (2013) indicated that fungal infection in fish might occur because of the use of contaminated feed or alternatively decomposed feed in the aquatic environment of the fish. Certainly, infection of fish by pathogenic fungi diminishes the market value of the fish and the nutritional value of its flesh.

The detected bacteria and fungi in the present study could have been recovered from lesions of primary or secondary infections. Aly (1996) indicated that in primary infections, the causative agent usually affects the normal skin and these infections are clinically characteristic and have specific disease course and often are caused by a single pathogen. In primary infections of many fungi, they invade the keratinized tissue of the skin, because of their strong affinity to keratin. However, in secondary infections, the skin that is already diseased and because of the underlying disease, the clinical picture and course of these infections vary.

CONCLUSION

This study pointed out to that fish with skin lesions in the investigated area could perhaps be a source of zoonotic infections and transmit the detected pathogens to workers in fish industry and consumers. Hence, it is recommended to raise the awareness of workers in fish

industry and consumers about the possible biological hazards that could be contracted from fish with topical lesions. Additionally, thorough and strict inspection of fish should be a routine to ensure safety and that there are no risks to consumers.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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AUTHOR CONTRIBUTIONS

Supervision: AHN and SME
Conceptualization, Investigation and lab work: WHI
Writing of the original draft, review and editing: YAS

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