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Prevalence of Bacterial Infections among Cage-Cultured Marine Fishes at the Eastern Province of Saudi Arabia.

Mohamed A Hassan[1]*, WS Soliman[2], Mahmoud A Mahmoud[3], Sami Shabeeb Al-Shabeeb[4], and Peer Mohamed Imran[4].

[1]Fisheries Research Center, Eastern Province, Kingdom of Saudi Arabia and Faculty of Veterinary Medicine, Bani-Suif University, Egypt
[2]Department of Hydrobiology, Veterinary Research Division, National Research Center, Dokki, Egypt.
[3]Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.
[4]Fisheries Research Center, Eastern Province, Kingdom of Saudi Arabia.

ABSTRACT

A total number of 480 fishes of different species namely Twobar Seabream (*Acanthopagrus bifasciatus*), Sobity Seabream (*Sparidentex hasta*), Red Sea Seabream *(Diplodus noct)*, Brown-spotted Grouper (*Epinephelus coioides*), Rabbit Fish (*Siganus canaliculatus*), Gilthead Seabream (Sparus aurata), were randomly collected from two private marine fish farms located at Arabian Gulf, Eastern Province, Saudi Arabia during a period of October 2013 until September 2014. The prevalence of bacterial infections in different seasons of the year and species susceptibility for antibiotics were detected. Bacteriological examination revealed the overall infection with different types of bacteria was 39.16% and they were related to Gram-negative bacteria. *A. hydrophila* were the most prevalent isolated bacteria represented by 41.48 % followed by *Ph. damselae* (20.21%) and *V. vulnificus* (19.68%). Water analysis revealed that, the severe clinical signs of infected fish appeared on fishes reared in cages showed high level of free ammonia and low dissolved water oxygen concentration. The most prominent clinical signs of diseased fish was external haemorrhages, ulcerations, corneal opacity and partial exophthalmia accompanied with peri- orpital haemorrhage. Fish mortalities have been observed in some cages of *Sparidentex hasta* infected with *Ph. damselae* or *V. vulnificus* in summer months only. The degree of susceptibility of the most prevalent isolates causing mortality (*Ph. damselae*, and *V. vulnificus*) towards 8 different types of antibiotics were recorded. Gross and histopathological lesions of infected fishes with Photobacterium were carried out.

Keywords: Marine fishes, Arabian Gulf, Saudi Arabia, Bacterial infections, Seasonal Variation, Antibiotic sensitivity test, Histopathology.

Corresponding author

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INTRODUCTION

Aquaculture is the fastest growing industry around the World with about 80 million tones being produced annually [1]. Aquaculture is therefore an emerging industrial sector which requires continued research with scientific, technical developments, and innovations [2]. Bacteria, the major group of pathogens, pose one of the most significant threats to successful fish production throughout the World [3]. Bacterial diseases are responsible for heavy mortalities in both culture and wild fishes throughout the world and most of the causative microorganisms are naturally occurring opportunist pathogens which invade the tissue of a fish host, thereby rendered such susceptible to infection. Among all other bacteria, Aeromonad, Pseudomonad and Edwardsiella are the major bacterial fish pathogens, which are widely distributed in aquatic organisms in nature [3].

Available literature about bacterial infections among Arabian Gulf fish is limited. *Vibrio anguillarum*, *V. ordain*, *V. carchariae*, *V. damsela*, and three other *Vibrio* spp. were isolated from diseased silvery black porgy cultured in floating cages in Kuwait in August 1987. Moreover, *S. agalactiae* caused severe mortality among cage-cultured European seabream *Sparus auratus* occurred in Kuwait Bay during August and early September 2001 [4]. Recently, [5] reported *Vibrio harveyi* from diseased shrimp from hatchery unit in Iran Shrimp Research Center.

In Japan, Photobacteriosis is one of the most economically important diseases of cultured fish, causing severe losses in cultured yellowtail juveniles [6]; [7]. In Europe and the Mediterranean, Photobacteriosis was first reported in 1991 in juvenile gilthead sea bream in the northwest of Spain [8]. Simultaneously, epizootic outbreaks were reported from France [9] in a population of sea bass (*Dicentrarchus labrax*) and Italy [10] in a population of gilthead sea bream. Other outbreaks were reported from sea bass in Greece [11], gilthead sea bream in Portugal (Baptista et al. 1996), sea bass in Turkey [12], and gilthead sea bream in Malta [13]. In 1994, Photobacteriosis caused 50% mortality in a population of market-size hybrid striped bass cultured in Israel [14]. However, *Photobacterium damselae* was originally described as a new pathogenic Vibrio species causing ulcers in *Chromis punctipinnis* [15]. Motile Aeromonas septicemia (MAS) caused by mesophilic *A. hydrophila* affects a wide variety of primarily freshwater and marine fish species. In the Southeastern United States, outbreaks of MAS resulting in industry-wide losses of food sized catfish totaling over 8 million pounds. However, *A. hydrophila* is also found as part of the normal intestinal flora of fish [16] and is thought to be opportunistic, causing disease only when a fish is stressed or injured [17].

Hence, our study was applied for isolation and identification of the most prevalent bacteria that causing septicaemic diseases in cage-cultured marine fishes of some private farms in Eastern Province of Saudi Arabia which lead to economic losses and try to decrease these losses by applying preventative and treatment measures using the drug of choice depending on culture sensitivity test.

MATERIAL AND METHODS

Fish Farms and sampling procedures:

A total number of 480 fishes of different species were randomly captured as a part of on-going monitoring program from two private marine fish farms with floating cage system at the Eastern province of Saudi Arabia from October 2013 until September 2014. Farm I is situated in North of Dammam city. Six fish species, Twobar Seabream (*Acanthopagrus bifasciatus*), Sobity Seabream (*Sparidentex hasta*), Red Sea Seabream (*Diplodus noct*), Brown-spotted Grouper (*Epinephelus coioides*), Rabbit Fish (*Siganus canaliculatus*) and Gilthead Seabream (*Sparus aurata*) were the main cultured fish species in 67 square Cages measured 5 x5 m with depth 4 m / cage. Farm Π is situated in South of Dammam city. Only one fish species, Gilthead Seabream (*Sparus aurata*) is cultured in this farm. It had 10 circular Cages, diameter of the circle measured 12 m with depth 5 m/cage.

360 fish were examined from Farm I and 120 fish from Farm Π. Fish species, numbers of fishes, average body weights, and average lengths from each farm are shown in (Table 1). Some of these fishes were apparently healthy and others were clinically diseased showing external lesions.

Transportation of fish samples for bacterial isolation

The fishes were transferred as quickly as possible to the Fisheries Research Centre in Qatef in a special vessels supplied with oxygen for applying clinical and laboratory examination based on morphological and biochemical characteristics. Clinical and PM examination were carried out using methods described by [18].

Bacteriological examination:

Samples from liver, spleen and kidney from fishes were cultured onto general and selective media; brain heart agar, tryptic soy agar and tryptic soy broth (Oxoid) supplemented with 2% (w/v) NaCl, and thiosulphate-citrate-bile salt-sucrose agar (TCBS, Oxoid). Aeromonas agar base medium supplemented with ampicillin and pseudomonas agar medium with adding 2 % NaCl. All the inoculated media were incubated at 28 ºC for 24-48 hours. Further identification of bacterial isolates carried out using API system to identify purified isolates to genus or species level, basic tests including Gram's stain, motility, morphology, oxidase test, catalase, glucose oxidation-fermentation, amylase, gelatinase, lipase, indole, H2S production, and nitrate reduction, were performed following the criteria described in the Bergey's Manual of Systematic Bacteriology [19]. The presumptive vibrio species were confirmed by their growth in different concentrations of NaCl and by their sensitivity to a vibriostatic agent O/129 (Oxoid Limited, Thermo Fisher Scientific). Further identification was performed using the commercial API 20E, API 20NE STREP, (Biomérieux, France) were used as shown in fig 3 (A,B).

Water Samples

10 water samples were collected under complete aseptic conditions in sterile bottles transferred to lab. for applying different physical, chemical and bacteriological examinations. They were obtained from different locations within each aquaculture facility and stored according to standard methods described by [20] and [21] Temperature, dissolved Oxygen (DO), pH and salinity were measured on spot while un-ionized ammonia (NH3), nitrites and nitrates were measured in laboratory according to methods adopted from [21].

Identification of the isolates

Pure cultures of the isolates were identified by biochemical characterization following the criteria proposed by those described in the Bergey's Manual of Determinative Bacteriology, [19]. Final confirmation of each strain was achieved using the analytical profile index of API20-E and API20-NE system [18].

Table 1: Fish species, locations, number, weight and length of examined fish

Fish species	locations	number	Weight (g)	Length (cm)
Acanthopagrus *bifasciatus*	Farm I	60	347- 407	27-29
Sparidentex hasta		60	200 - 404	20- 26
Diplodus noct		40	155 - 190	19 - 22
Epinephelus coioides		60	1324 - 1495	41 - 45
Siganus canaliculatus		80	124 - 232	20 - 25
Sparus aurata	Farm I	60	249- 461	23 -31
	Farm П	120	137 - 375	20 - 28

Antibiotic sensitivity test

An antibiotic susceptibility test was conducted according to the Kirby-Bauer disk diffusion method, [22]. The following Commercially available antibacterial disks, obtained from Oxoid (Basingstoke, Hampshire, United Kingdom) were used to determine the susceptibility patterns of the isolates against 8 different anti-bacterial (dose/disk): ampicillin (AM10 ug), amoxicillin with clavinilic acid (AMC 30ug), Neomycin (N30ug), Erythromycin (E15ug), trimethoprim-sulphamethoxazole (SXT25ug), nalidixic acid (NA 30ug), nitrofurantoin

(F300ug) and chloramphenicol (C30ug). Drug sensitivity tests were performed using Mueller–Hinton agar and broth (Oxoid) supplemented with NaCl at a final concentration of 2% and pH of 7.2. Petri dishes were streaked with an inoculums prepared from 48-h colonies on TSAs. Interpretation of the results was made in accordance with the standard measurement of inhibitory zones in millimetres (mm) as sensitive (S), intermediary sensitive (I) and resistant (R).

Histopathological examination:

Tissues specimens from liver, spleen, kidney and intestine were taken from diagnostic diseased fish samples and were fixed at 10% formal saline, processed by conventional method sectioned at 4 um and stained with Haematoxylin and Eosin [23].

RESULTS

Clinical examination of fish

Clinical examinations together with bacteriological examinations of the randomly collected fishes from both farms revealed that the bacterial infections in this study take one of two forms. In form I, diseased fish exhibit no apparent external clinical signs except rare individuals that display slight hemorrhagic areas around the head and gills. This form usually associated with fish infected with *Aeromonas hydrophila or vibrio alginolyticus or Pseudomonas spp.* In form П, diseased fish have a prominent external clinical signs mainly in fish infected with *Ph. damselae* or *V. vulnificus*. Most diseased cases showed haemorrhage and ulcers in the mouth with corneal opacity as shown in fig.1 (A,B), also some fishes showing haemorrhagic patches all over the body specially at the abdominal region and at the base of the fins with anal inflammation and haemorrhage as shown in fig.1 (C, D and E). Other fishes can show partial exophthalmia with haemorrhage of the conjunctiva and iris, fig.1 (F). All the previous clinical signs expressing what is called "haemorrhagic septicaemia" in most diseases caused by Gm –ve bacteria especially Photobacteriosis and Vibriosis. Fish mortalities have been observed in some cages of *Sparidentex hasta* infected with *Ph. damselae* or *V. vulnificus in* summer months only.

Postmortem examination

Naturally infected marine fishes showing congestion and enlargement of all internal organs especially liver, spleen, kidney and intestine with branchial haemorrhage and in some cases pale liver and gills which was pathognomonic for septicaemic diseases (fig 2, A,B,C,D,E and F). In addition, exophthalmia and ascites was demonstrated in acute cases especially fish of form II.

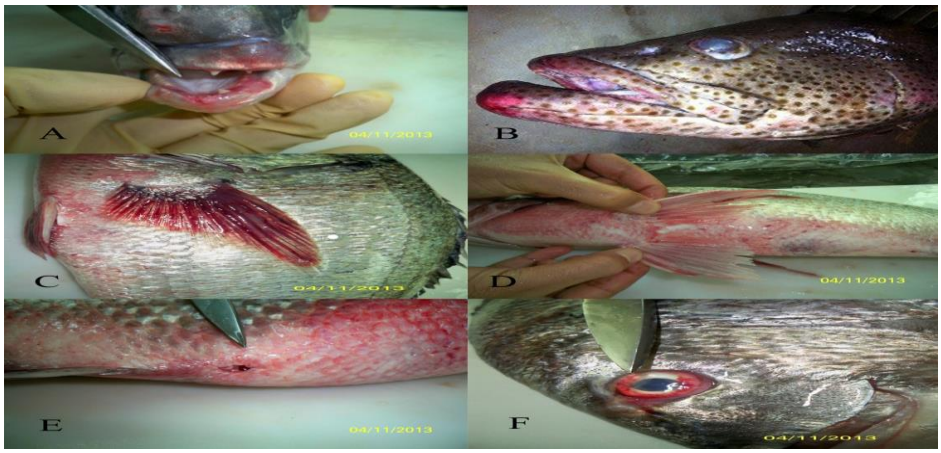

Figure 1: (A) Naturally infected Sobity fish showing ulcers at the mouth. (B) Naturally infected grouper fish showing haemorrhagic patches at the mouth parts with corneal opacity. (C) Naturally infected sea bream fish showing sever haemorrhagic pectoral fin. (D) Naturally infected Sobity fish showing sever haemorrhagic patches scattered all over the body. (E) Naturally infected Sea bream fish showing haemorrhagic vent and at the ventral aspect of the body. (F) Naturally infected Sobity fish showing partial exophthalmia with haemorrhage of the conjunctiva and iris.

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Figure 2: (A): Naturally, infected Sobity fish showing sever congested kidney. (B)): Naturally infected Sobity fish showing sever congested gills with haemorrhage.(C) Naturally infected Sobity fish showing sever congestion and haemorrhagic enteritis.(D) Naturally infected sea bream fish showing swelling of the liver with congested and enlarged kidney. (E) Naturally infected Siganus fish showing enlarged and congested liver with petecheal haemorrhage. (F) Naturally infected Sobity fish showing enlarged and congested spleen.

Bacteriological examination

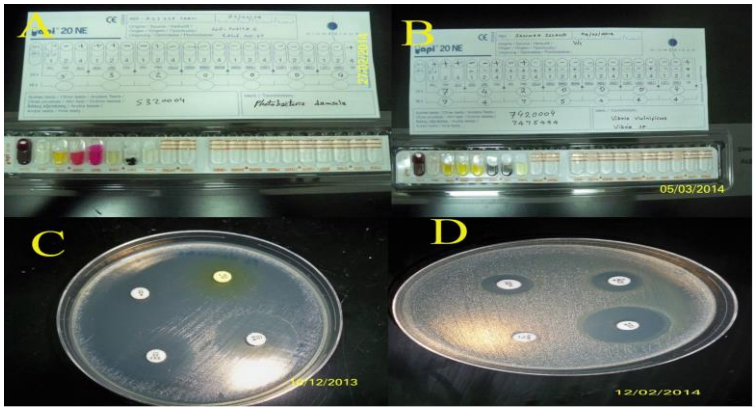

Figure 3: (A) API-NE strip for identification of *Ph. damselae.*(B) API-NE strip for identification of *V. vulnificus*.(C) Antibiotic sensitivity test of *Ph. damselae. (D)* Antibiotic sensitivity test of *V. vulnificus.*

Bacteriological examination of the randomly collected fishes from both farms in this study revealed that 39.16% of the examined fish were infected with 5 types of Gram-negative bacteria, *A. hydrophila, Ph. damselae, V. vulnificus*, *V. alginolyticus* and *Pseudomonas spp.* Prevalence of bacterial infections for fish collected from Farm I and Farm Π was 45% and 21.66% respectively as shown in table 2.

Table 2: Prevalence of bacterial infections in Fish Farm I and Fish Farm Π

Locality / M.O	Farm I			Farm Π		
	No. Examined	No. Infected	%	No. Examined	No. Infected	%
A. hydrophila	360	70	19.44	120	8	6.66
Ph. damselae		38	10.55		0	0.0
V. vulnificus		25	6.94		12	10.0
V. alginolyticus		22	6.11		6	5.0
Pseudomonas spp.		7	1.94		0	0.0
Total	360	162	45.00	120	26	21.66

A. hydrophila was the most prevalent isolated bacteria represented by 41.48 % followed by *Ph. damselae* (20.21%), *V. vulnificus* (19.68%), *V. alginolyticus* (14.89%) and Pseudomonas spp. (3.72%) as shown in table 3.

Moreover, the total prevalence of *A. hydrophila* among *Acanthopagrus bifasciatus*, *Sparidentex hasta, Diplodus noct, Epinephelus coioides, Siganus canaliculatus* and *Sparus aurata* were 33.33%, 32.14%, 100%, 27.27%, 61.11% and 34.11% respectively, while total prevalence of *Ph. damselae* among *Acanthopagrus bifasciatus, Sparidentex hasta, Epinephelus coioides* and *Siganus canaliculatus* were 16.66%, 42.85%, 72.72% and 38.88% respectively. Further, the total prevalence of *V. vulnificus* among *Acanthopagrus bifasciatus* and *Sparus aurata* were 27.77% and 37.64% respectively, while the total prevalence of the same fish species with *V. alginolyticus* were 22.22% and 28.23% respectively. *Pseudomonas spp* infected only *Sparidentex hasta with prevalence* 25%. As shown in table3.

The highest prevalence of bacterial infections was found in *Sparus aurata* (47.22%) followed by *Sparidentex hasta* (46.66%), *Diplodus noct* (42.5%), *Epinephelus coioides* (36.66%), *Acanthopagrus bifasciatus* (30%) and *Siganus canaliculatus* (22.5%) as shown in table 3

Table 3: Prevalence of bacterial infections in the examined fishes.

NO. Fish species	No of ex. fish	No of inf. Fish	%	*Ph. damselae*		*V. vulnificus*		*V. alginolyticus*		*A. hydrophila*		*Pseudomonas spp.*	
				No	%	No	%	No	%	No	%	No	%
Acanthopagrus *bifasciatus*	60	18	30	3	16.66	5	27.77	4	22.22	6	33.33	0	0
Sparidentex hasta	60	28	46.66	12	42.85	0	0	0	0	9	32.14	7	25
Diplodus noct	40	17	42.5	0	0	0	0	0	0	17	100	0	0
Epinephelus coioides	60	22	36.66	16	72.72	0	0	0	0	6	27.27	0	0
Siganus canaliculatus	80	18	22.5	7	38.88	0	0	0	0	11	61.11	0	0
Sparus aurata	180	85	47.22	0	0	32	37.64	24	28.23	29	34.11	0	0
Total	480	188	39.16	38	20.21	37	19.68	28	14.89	78	41.48	7	3.72

Water samples examination

The values for physico-chemical parameters of the water samples taken from the two farms were not significantly varied. Water temperature during the sampling period ranged from 16.8 ± 0.7°C to 30.2 ± 1.0°C. Dissolved oxygen varied from 4.5 to 5.4 mg/L. The cage water pH ranged from 7.1 to 8.4. The salinity was 38 ± 2.0 to 42 ± 0.8 ppt. The value of NH3, NO2 and No3 were 1.4, 0.96 and 1.7 mg/l respectively. The improper values of water quality parameters in both investigated fish farms demonstrated that the nitrogenous waste products may be significantly accused for predisposing for these bacterial infections, where suppression of immunity occur and so responsibility of fish to be highly susceptible for infection. The values in both farms recorded for NH3, NO2 and NO3 were far from the optimum recommended levels (the recommended marine high reliability trigger value.)

Epizootiology

Studying the epizootiology of bacterial infection in the randomly collected fishes from both farms revealed that susceptibility of fish to be infected with isolated bacteria in this study is various from one fish species to another.

Acanthopagrus bifasciatus was the most susceptible species to be infected with different types of bacteria which infected with 4 types of bacteria, followed by *Sparidentex hasta &* Sparus aurata (infected with

3 types of bacteria), *Epinephelus coioides & Siganus canaliculatus* (infected with 2 species of bacteria), while the lowest susceptible species to be infected with different types of bacteria was *Diplodus noct* (infected with only one type of bacteria) as sown in table 3.

Epinephelus coioides was the most susceptible species to be infected with *Photobacterium damselae* where the infection rate reached (72.72%), followed by *Sparidentex hasta* (42.85%), *Siganus canaliculatus (38.88%)*, *Acanthopagrus bifasciatus (16.66%),* while *Diplodus noct* & *Sparus aurata* not susceptible to be infected with the same bacteria.

From sex fish species only two fish species, *Acanthopagrus bifasciatus & Sparus aurata* were susceptible to be infected with *Vibrio vulnificus & Vibrio alginolyticus*.

All sex fish species in this study were susceptible to be infected with *A. hydrophila*, the most susceptible was *Diplodus noct* (100%) followed by *Siganus canaliculatus* (61.11%), Sparus aurata (34.11%), *Acanthopagrus bifasciatus* (33.33%), *Sparidentex hasta* (32.14%) and *Epinephelus coioides* (27.27%).

Only one fish species, *Sparidentex hasta was* susceptible to be infected with *Pseudomonas spp.*

Seasonal prevalence

Studying the seasonal prevalence of bacterial infection among the randomly collected fishes from both farms revealed that, the highest total prevalence of bacterial infections was recorded in the summer season (45%), followed by autumn (41.66%), then spring (38.33%). On the other hand the minimal prevalence of infection was recorded in winter (31.66%). As shown in table 4.

The highest prevalence of bacterial infection among the naturally infected marine fishes in winter season was recorded for *A. hydrophila* (23.33%) while the lowest one (1.66%) was recorded for *Ph. damselae &* V. *vulnificus*. For spring season, the highest prevalence of bacterial infection (15%) was recorded for *A. Hydrophila*, while the lowest (6.66%) was recorded for *Ph. Damselae*. On the other hand, *Pseudomonas spp* not recorded. The highest prevalence of bacterial infection (14.16%) in summer season was recorded for *Ph. Damselae*, while the lowest (9.16%) was recorded for v*. vulnificus*. *Pseudomonas spp,* not recorded in summer season. The highest prevalence of bacterial infection (16.66%) in autumn season was recorded for *A. Hydrophila* while the lowest (1.66%) was recorded for *Pseudomonas spp*.

The Prevalence of different types of bacterial infections in the different seasons is illustrated in table (4).

Table 4: Seasonal prevalence of bacterial infections in the examined marine fishes.

Type of M.O / Season	*Ph. damselae*			*V. vulnificus*		*V. alginolyticus*		*A. hydrophila*		*Pseudomonas spp.*		*Total*	
	No. Exa.	No. Inf.	%	No. Inf.	%	No. Inf.	%	No. Inf.	%	No. Inf.	%	No. Inf.	%
Winter	120	2	1.66	2	1.66	1	0.83	28	23.33	5	4.16	38	31.66
Spring	120	8	6.66	11	9.16	9	7.5	18	15	0	0	46	38.33
Summer	120	17	14.16	14	11.66	11	9.16	12	10	0	0	54	45.00
Autumn	120	11	9.16	10	8.33	7	5.83	20	16.66	2	1.66	50	41.66
Total	480	38	7.91	37	7.70	28	5.83	78	16.25	7	1.45	188	39.16

Histopathological examination

The histopathological examination of infected fishes with *photobacterium damselae* revealed that the most pathological lesions were in liver, kidneys, intestine and spleen. In liver, the lesions characterized by focal aggregation of melanophores in the area of hepatopancrease (Fig. 4A).In some cases, the lesions were of prominent chronic lesions where newly formed bile ductules were noticed in the hepatic tissue, in such cases, fibrous connective tissue proliferation was noticed (Fig. 4B). Early granulomatous reaction formed of central

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area of necrotic tissue infiltrated with mono nuclear inflammatory cells was not uncommon in the area of hepatopancrease (Fig. 4C&D). Clusters of bacterial colonies were sometimes appeared in the area of necrosis (Fig. 4E). Areas of hemorrhages with abnormal shape of extravasated red blood cells were also noticed in the hepatic tissue (Fig. 4F).

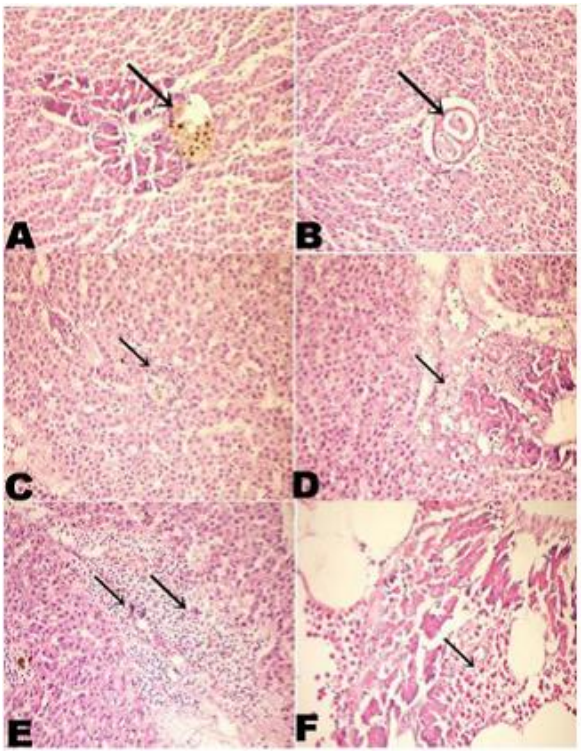

Figure 4: Liver of Sobity Seabream (*Sparidentex hasta*) fish infected with photobacterium damselae showing (A),focal aggregation of melanophores.(B), newly formed bile ductules formation and fibrous connective tissue proliferation (arrow). (C), early granuloma formation (arrow), the granulome formed of necrotic cells, macrophages and melanophores. (D), mononuclear inflammatory cells infiltration and clusters of bacterial colonies (arrow). (E), focal areas of haemorrhage.Notice: bnormal shapes of RBCs . (H&E stain x 400).

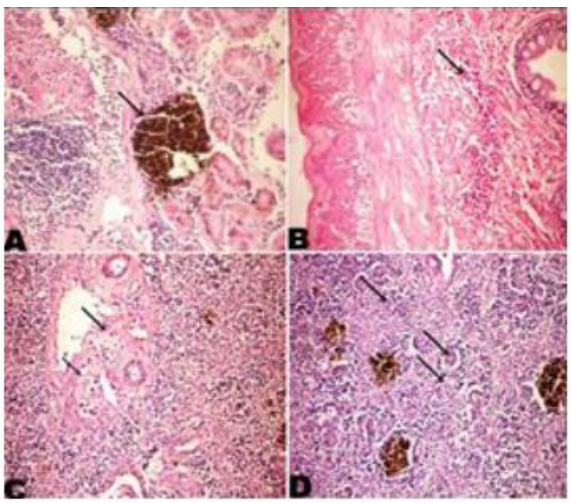

Figure 5: Tissue sections of Sobity Seabream (*Sparidentex hasta*) fish infected with photobacterium damselae showing, (A), kidneys with hyperactivity of melanomacrophage centers and coagulative necrosis of epithelial lining of some renal tubules. (B), Posterior intestine showing hyperplasia of goblet cells in epithelial lining and haemorrhage in lamina propria (arrow) and vacuolation of tunica muscularis.(C), Spleen showing capillary thrombi (arrows)and swelling of endothelial cells of blood capillaries. (D), Spleen showing thickening of wall of ellipsoidal blood capillaries (arrows). (H&E stain x 400)

In kidneys, hyperactivity of melanomacrophage centers and coagulative necrosis of epithelial lining of some renal tubules were a common findings (Fig.5A). The posterior intestine showed hyperplasia of goblet cells in epithelial lining and haemorrhage in lamina propria & vacuolation of tunica muscularis. (Fig.5B). The splenic tissue appeared with thrombi and swelling of endothelial cells of blood capillaries (Fig. 5C). The ellipsoidal wall of blood capillaries in such cases showed marked thickening.

Table 5: Assessment of selected isolates to 8 different types of antibiotics

Isolates / Antibiotics	*Ph. damselae*	*V. vulnificus*
Chloramphenicol (C30)	(33) +++	(28) +++
Trimethoprim+ sulphamethoxazole (SXT25)	(24) +++	(21) +++
Amoxicillin+ clavinilic acid (AMC30)	(17) ++	(15) +
Nitrofurantoin (F300)	(23) +++	(15) +
Nalidixic acid (NA30)	(28) +++	(22) +++
Ampicillin (AM10)	(11) +	(11) +
Neomycin (N30)	(12) +	(10) +
Erythromycin 15ug (E)	(11) +	(14) +

Antibiotic sensitivity test

The degree of susceptibility of the most prevalent isolates shown in Table 5.

Degree of sensitivity:

+++ (21mm) ….. sensitive ; **++** (16-20mm)…. intermediate ; **+** (15 mm) ……..resistant

Isolates of Photobacteria were susceptible to chloramphenicol, Nalidixic acid, sulphamethoxazole with trimethoprim and Nitrofurantoin while it was resistant to Ampicillin, Neomycin and Erythromycin as shown in Fig 3,C. On the other hand *V. vulnificus* isolates were sensitive to chloramphenicol, sulphamethoxazole with trimethoprim and Nalidixic acid and resistant to other antibiotics as shown in Fig 3,D.

DISCUSSION

For development of Saudi's marine fish aquaculture industry in Saudi Gulf coast, Fisheries Research Center in the Eastern Province of Saudi Arabia carry out surveillance and monitoring program for aquatic diseases in mariculture systems.

Extensive investments in alternative technologies and new management techniques have been proposed for improving the output and productivity of this sector. The development of intensive marine fish farming in the form of the concentration of large quantities of biomass in relatively small volume water leads-under certain conditions (combination of factors) to the emergence of diseases, which lead to losses in the population [24, 25].

Bacterial infections are the most eminent etiologies that put the live of fishes into jeopardy with consequent negative impact on growth, fecundity and productivity. There are two types of disease producing bacteria infectious to fish, obligate and facultative pathogens. The first group rarely found in the absence of a host. On contrary facultative forms are ubiquitous in aquatic systems. Many of apparently normal and healthy fish harbor lots of potential pathogens. Both types cause diseases when fish is immuno-compromised by some form of stressors [26, 27].

With regard to clinical picture together with post mortem lesions of the randomly collected fishes from both farms revealed that the bacterial infections in this study take one of two forms. In form I, diseased fish exhibit no apparent external clinical signs except rare individuals that display slight hemorrhagic areas around the head and gills. This form usually associated with fish infected with *Aeromonas hydrophila* or *Vibrio alginolyticus* or *Pseudomonas spp*. In form П, diseased fish have a prominent external clinical signs mainly in fish infected with *Ph. damselae* or *V. vulnificus*. All the recorded clinical signs in our work expressing what is called "haemorrhagic septicaemia and post mortem findings which was pathognomonic for septicaemic diseases were in accordance with results obtained by [28] and [29]. Results also were supported by those reported by [30] and [31] who mentioned that fish bacterial infections can arise as a bacteremia, which implies the presence of bacterial organisms in the bloodstream without clinical signs. Others occur as a septicemia where bacteria and its extracullare products actually exist in the circulatory system disrupting fish physiological functions and induce variety of pathological alterations that may lead to death. [32] mentioned that the clinical symptoms caused by any pathogen depend on the type of host, age of the fish and stage of disease (acute, chronic, subclinical form). Moreover, in some cases, there is no correlation between internal and external injuries. In fact, systemic diseases (eg. Pasteurellosis) with high mortality rates, causing internal damage to infected fish, but often have a healthy appearance. Conversely, other diseases with relatively low mortality cause significant physical damage, including ulcers, necrosis, exophthalmos, making the fish unfit for the market.

In concern to bacteriological examination of the randomly collected fishes from both farms in this study revealed that 39.16% of the examined fish were infected with 5 types of Gram-negative bacteria, *A. hydrophila*, *Ph. damselae*, *V. vulnificus*, *V. alginolyticus* and *Pseudomonas spp*. and this was nearly similar to results obtained by [33] who recorded septicemic bacterial infections such as vibrios, aeromonads, pseudomonads and photobacteria isolated from several fingerlings, juveniles, adults and brood stocks of some marine fish species. Our results are supported by those reported by [34] who declared that the main pathogenic microorganisms isolated from diseased gilt-head seabream in marine water at south western Spain were *Vibrio spp, Pseudomonas spp, P. piscicida*, *Flavobacteria* maritimus and *Aeromonas spp*. [35] described several microbial disease outbreaks in farm stocks of newly cultured sparid fish species, such as common seabream, redbanded seabream, and white seabream. The isolated bacterial strains were identified as *Vibrio spp*. and *Photobacterium damselae* subsp. *damselae*. [36] proved that, Photobacterium sp. (Pasteurella damsela) is a primary cause of sudden mortality in different types of marine fishes, namely red grouper, sea bass, sea bream and rabbit, along the sea shore of Matrouh-Saloum in Matrouh Governorate, Egypt. [37]demonstrate the most prevalent bacterial isolates that may lead to Grouper fish mortality at the East Cost Libyan area of the Mediterranean Sea. It was found that the gram negative oxidase positive bacterial group (*Pasteurella, Vibrio and Aeromonas spp.*) were the most isolated bacteria with high incidence refers especially to *Pasteurella piscicida* with an incidence of 64%. Some variability of results could be blamed to many reasons such as, different localities of isolation, diverse type of investigated fish samples and variable magnitudes of environmental stressors [38].

With respect to the most prevalent microorganism isolated in our work, *A. hydrophila* was the most prevalent isolated bacteria represented by 41.48 % followed by *Ph. damselae* (20.21%), *V. vulnificus* (19.68%), *V. alginolyticus* (14.89%) and *Pseudomonas spp*. (3.72%). Although *A. hydrophila* were the most prevalent isolates but they were mostly isolated from apparently healthy fish with slight clinical signs and without post-mortem lesions and this was disagree with previous study declared that marine fish can succumb MAS disease caused by *A. hydrophila*, as supported by [39] who isolated *A. hydrophila* from ulcer disease in Cod, Gadus morhua L., a strictly marine fish. Authors added that motile Aeromonas group especially *A. hydrophila* is considered as one of the most important pathogen responsible for haemorrhagic septicemia in a wide variety of marine water fish. Moreover, [40] isolated *A. hydrophila* from ulcers, lesions, and blood of ulcerated European flounder. The interpretation of results were demonstrated also by [41] who found that *Aeromonas hydrophila* present in freshwater and brackish environments that is frequently isolated from raw and processed seafood products. *A. hydrophila* has frequently been found in fish and shellfish. In a retail survey of seafood, motile Aeromonas were found in 66% of shellfish and 34% of finfish. Seafood probably become contaminated by *Aeromonas spp*. through the growing waters and the animals themselves, with many fish species containing *Aeromonas spp*. in their gut. [28] isolated Gram negative bacteria from marine fish by the following percentages respectively, 17.55% (*V. anguillarum*), 16.73% (*V. alginolyticus*), 15.51% (*P. piscicida*), 15.91% (Ps. fluorescens), 13.46% (S. fecalis), 11.02% (A. hydrophila) and 6.12% (A. sobria), while previous studies applied by [42] who isolated two types of bacteria from kidney and liver by Siberian sturgeon

(*Acipenser baerii*) and were identified as *Vibrio alginolyticus* and *Pasteurella spp*. according to the cell morphology, gram stain, colony morphology and metabolic reactions. They also mentioned that *V. alginolyticus* has caused large-scale mortalities in silver sea bream (*Sparus sarba*) in Hong Kong, gilt-head sea bream (*S. aurata*) in Spain, cultured black sea bream (*Mylio macrocephalus*) fry in Japan and cobia in other study. [43] demonstrated that a total of 44 vibrios belonging to five different species were isolated from marine fishes. The most predominant species was *Vibrio alginolyticus* (31.8%), followed by *V. harveyi* (27.3%), *V. mimicus* (22.7%), *V. parahaemolyticus* (11.4) and *V. Cholera* (6.8%). Clinical examinations of fish in several cultured fish farms located in different provinces of Saudi Arabia with total of 370 naturally diseased live fishes including, Nile tilapia (*Oreochromis niloticus L*), marine tilapia (*Oreochromis spilurus L*), grey mullet (*Mugil cephalus L*), sea bass (*Dicentrachus labrax L*) rabbit fish (*Siganus rivulatus L*) and catfish (*Carus gariepinus L*), The bacteriological analysis of the diseased fishes resulted in recovery of 62 *Vibrio spp*. The isolates were identified as *Grimontia* (=Vibrio) *hollisae* (54.5%), *Vibrio. fluvialis* (20.5%), *Photobacterium* (=Vibrio) *damselae* (12.6%), *V. alginolyticus* (6.8%) and *V. vulnificus* (4.5%) [44]. [45] isolated five distinct Vibrio species. Cultural, morphological and biochemical characteristics of these isolates identified them as *V. Alginolyticus* (29.4%), *V. vulnificus* (26.8%), *V. anguillarum* (19.4%), *V. fluvialis* (15.3%) and *V. pelagius* (9.1%). The most predominant isolates were *V. Alginolyticus* and *V. Vulnificus*. In addition, [46] demonstrated the spread of these two bacterial diseases in Mediterranean fish farming. Strains of *Photobacterium damsela* ssp. *piscicida*, *Vibrio fluvialis*, *Vibrio alginolyticus, Vibrio parahaemolyticus, Vibrio metschnikovii*, isolated from Italian aquaculture (fish, shellfish and crustaceans). [47] examined A total number of 100 European sea bass fish through the course of determined episodes and found that Vibrio alginolyticus (*V. alginolyticus*) was the most prevalent bacterial pathogen 32.25 %, followed by Pseudomonas fluorescens (*P. fluorescens*) 24.19% , Tenacibacullum maritimum (*T. maritimum*) 17.74 % and Streptococcus agalactiae (*S. agalactiae*) 14.51 %. Vibrio vulnificus (*V. vulnificus*) infections recorded the lowest rate 11.29 %. The difference in prevalence of isolated bacteria in our study compared with previous study was due to many reasons such as, different localities of isolation, diverse type of investigated fish samples and variable magnitudes of environmental stressors.

The improper values of water quality parameters in both investigated fish farms demonstrated that the nitrogenous waste products may be significantly accused for predisposing for these bacterial infections, where suppression of immunity occur and so responsibility of fish to be highly susceptible for infection and this was in accordance with [47] who mentioned that deterioration in water quality stresses cultured fishes with consequent increase in the chance of opportunistic pathogens to invade fish causing disease condition. Virtually, there is a close relationship between environmental stress and emergence of outbreaks of fish diseases. Regarding to dissolved oxygen levels (DO), unfavourable levels lower than the optimal recommended values, relatively low DO levels as found in our investigation (4.5 to 5.4 mg/L) synergized with other unfavourable environmental aquatic conditions will lead to Impaired immune mechanisms triggered by theses hostile conditions are accused for the establishment of fish mortality [48]. Furthermore, the virulence of the causative pathogens is exacerbated by exposure to reduced dissolved oxygen levels [49]. The value of NH3, NO2 and No3 were 1.4, 0.96 and 1.7 mg/l respectively and these recorded values in both farms were far from the optimum recommended levels (the recommended marine high reliability trigger value.) and these values were nearly similar to that obtained by [47] who found that the value of (un ionized ammonia) NH3 were (0.9 and 1.2 mg/l), while the optimum level must not exceed 0.1 mg/l for salt water fish [50]. This high ammonia levels enhance microbial infections through suppressing the immune capacity of fish. Phagocytic and clearance efficiency are diminished. As an ultimate fate for the colossal immuno-suppression of fishes, bacterial invasion will be the most probable event [51].

Studying the epizootiology of bacterial infection in the randomly collected fishes from both farms revealed that susceptibility of fish to be infected with isolated bacteria in this study is various from one fish species to another. *Acanthopagrus bifasciatus* was the most susceptible species to be infected with different types of bacteria which infected with 4 types of bacteria, followed by *Sparidentex hasta* and *Sparus aurata* (infected with 3 types of bacteria), *Epinephelus coioides* and *Siganus canaliculatus* (infected with 2 species of bacteria), while the lowest susceptible species to be infected with different types of bacteria was *Diplodus noct* (infected with only one type of bacteria).

Epinephelus coioides was the most susceptible species to be infected with *Photobacterium damselae* where the infection rate reached (72.72%), followed by *Sparidentex hasta* (42.85%), *Siganus canaliculatus (38.88%)*, *Acanthopagrus bifasciatus (16.66%),* while *Diplodus noct* and *Sparus aurata* not susceptible to be infected with the same bacteria. From sex fish species only two fish species, *Acanthopagrus bifasciatus* and

Sparus aurata were susceptible to be infected with *Vibrio vulnificus* and *Vibrio alginolyticus*. All sex fish species in this study were susceptible to be infected with *A. hydrophila*, the most susceptible was *Diplodus noct* (100%) followed by *Siganus canaliculatus* (61.11%), *Sparus aurata* (34.11%), *Acanthopagrus bifasciatus* (33.33%), *Sparidentex hasta* (32.14%) and *Epinephelus coioides* (27.27%). Only one fish species, *Sparidentex hasta* was susceptible to be infected with *Pseudomonas spp.* This variation on susceptibility may be attributed to many factors probably genetics, hereditary or the integumental defenses of the host [52; 53; 54].

Studying the seasonal prevalence of bacterial infection among the randomly collected fishes from both farms revealed that, the highest total prevalence of bacterial infections was recorded in the summer season (45%), followed by autumn (41.66%), then spring (38.33%) and was lowest in winter (31.66%). These results was agree with these findings reported by [28] who reported that, bacterial infection was prevalent all over the year with maximum prevalence during summer and autumn. The highest prevalence e of *Ph. damselae* was recorded during summer season (14.16%), followed by autumn (9.16%) then spring (6.66%), and was lowest in winter (1.66%). Results were in concordance with those reported by [55] who declared that *P. piscicida* causes high fish mortality only when the water is warm. On the other hand, [56] suggested that temperature has no strong influence on the course of Pasteurellosis. The highest prevalence of *V. vulnificus* and *V. alginolyticus* infection was recorded during the summer (11.66% & 9.16%), followed by spring (9.16% & 7.5%), autumn (8.33% & 5.83%), and only (1.66% & 0.83%) were recorded in winter. The results of the seasonal prevalence of *Vibrio spp* were in concordance with those reported by [29] who demonstrated that in wild, Vibriosis normally occurs in fish in late summer when the temperatures are high. On the other hand, [57] reported that *V. alginolyticus* were not associated with a particular season. The highest prevalence of *A. hydrophila* was recorded in winter season (23.33%) followed by autumn (16.66%), spring (15%) and only (10%) in summer. These results were supported by [58] who suggested that the highest isolation rates of *A. hydrophila* occurred during late winter followed by a progressive decline in density during summer and monsoon seasons. Moreover, [59] mentioned that there was clear seasonality in the prevalence of *A. hydrophila* as there were no isolates recovered in the summer months. On contrast, [60] reported that the most epizootics of motile aeromonads were generally reported in spring and early summer. The highest prevalence of *Pseudomonas spp* was recorded during the winter season (4.16%) followed by autumn (1.66%) and not recorded in summer and spring, this reveals that *Pseudomonas spp* has certain affinity to low temperature for propagation and wide spreading infection [61]. Results were supported by [57] who demonstrated that the Pseudomonads were isolated mainly in cold months of winter. On the contrary, results are not in accordance with those obtained by [62] who revealed that the prevalence of pseudomonads was lower in winter than summer. This may also be attributed to amplified activity of proteinases produced by pseudomonads at the low temperature (10-25°C) that play significant role in the pathogenesis of pseudomonas septicemia [63].

From pathological point of view, The histopathological examination was performed in severely infected fish species with higher incidence of *photobacterium damselae* infection where [15] mentioned that *photobacterium damselae* is belonging to *Vibrio spp*. On the other hand no histopathological demonstration was done on fish infected with *A*, *hydrophila* , due to slight or no clinical signs. In our study, the infection with *photobacterium damselae* induced chronic reaction characterized by granulomatous lesion and necrosis, the results were go parallel with that of [64] who mentioned that, fish infection with *photobacterium damselae* induced chronic stage with granuloma formation and focal necrosis in the internal organs.

The lesions in the hemopoietic tissue including kidneys and spleen indicated that, the *photobacterium damselae* infection may suppress the immune system and give chance to infection with other microorganisms. The lesions in the spleen and thrombi formation in the blood vessels may indicative for the effect of the bacterial toxins on the endothelial blood vessels. However, further studies are required to evaluate the pathogenicity of the isolated *photobacterium damselae* and types of bacterial toxins.

Regarding the degree of susceptibility of the most prevalent bacterial isolates (*Ph. damselae* and *V. vulnificus*) towards 8 different types of antibiotics in this study isolates of Photobacteria were susceptible to chloramphenicol, Nalidixic acid, sulfamethoxazole with trimethoprim and Nitrofurantoin while it was resistant to Ampicillin, Neomycin and Erythromycin. On the other hand *V. vulnificus* isolates were sensitive to chloramphenicol, sulfamethoxazole with trimethoprim and Nalidixic acid and resistant to other antibiotics. and this was nearly in a accordance with [34] who described the resistance of *P. damselae* ssp. *Piscicida* to

ampicillin, amoxicillin, oxytetracycline and tetracycline. Moreover, all isolates were susceptible to trimethoprim-sulfamethoxazole, oxolinic acid and flumequine.

The same results were obtained by [65] who illustrated the antimicrobial susceptibility patterns of isolates of *Photobacterium damselae* ssp. *damselae* and *P. damselae* ssp. *piscicida* from red banded seabream and found that they were susceptible to chloramphenicol, sulfamethoxazole with trimethoprim and Nitrofurantoin and resistant to ampicillin, erythromycin and kanamycin. Most Vibrio spp. isolated from marine fish farms located in different regions of Saudi Arabia were relatively highly sensitive to chloramphenicol, trimthoprime, colistin and tetracycline and resistant to nitrofurantoin, ampicillin, lincomycin, sulphonamides, penicillin and amoxicillin [44]. This resistance of *Vibrio* spp. to nitrofurantoin and sulphonamides was disagree with our results. In contrary antibiotic resistance pattern of *Vibrio spp*. isolated from shrimp rearing ponds in Bangladesh showed the highest resistance to ampicillin (100%), followed by amoxicillin (78%), nalidixic acid (40%), vancomycin (13.33%), neomycin (6.66%) and chloramphenicol (6.66%). All the Isolates were observed sensitive to gentamycin, erythromycin, ciprofloxacin and doxycyclin [66]. [46] studied the antibiotic resistance patterns of aetiological agents responsible for vibriosis and pasteurellosis to contribute to control the spread of these two bacterial diseases in Mediterranean fish farming. Strains of *Photobacterium damsela* ssp. *piscicida*, *Vibrio fluvialis*, *Vibrio alginolyticus, Vibrio parahaemolyticus, Vibrio metschnikovii*, isolated from Italian aquaculture (fish, shellfish and crustaceans) sites. The bacterial strains showed resistance to ampicillin, carbenicillin, kanamycin, cefalothin, while they were sensitive to chloramphenicol, nitrofurantoin and tobramycin; the sulfadiazine-trimethoprim association was completely ineffective and this was in accordance with recorded results in our research. Lastly, [67] demonstrated that *Photobacterium damselae* isolated from marine ornamental yellow tail surgeon was sensitive to sulfamethoxazole gentamycin, and streptomycin and this was in accordance with results of applying sulfamethoxazole in our work and in contrary with results obtained from applying gentamycin and streptomycin against photobacterium strains isolated in our work.

CONCLUSION

Fish samples randomly collected from two private marine fish farms located at Arabian Gulf, Eastern Province, Saudi Arabia during a period of October 2013 until September 2014 showed that *A. hydrophila* were the most prevalent isolated bacteria by which fish exhibit no apparent external clinical signs except rare individuals that display slight hemorrhagic areas around the head and gills, followed by *Ph. damselae and V. vulnificus* by which diseased fish have a prominent external clinical signs. Fish mortalities have been observed in some cages of Sparidentex hasta infected with *Ph. damselae* or *V. vulnificus* in summer months only. The improper values of water quality parameters in both investigated fish farms demonstrated that the nitrogenous waste products may be significantly accused for predisposing for these bacterial infections. *Epinephelus coioides* was the most susceptible species to be infected with *Photobacterium damselae*, while *Acanthopagrus bifasciatus* and *Sparus aurata* were susceptible to be infected with *Vibrio vulnificus* and *Vibrio alginolyticus*. The highest total prevalence of bacterial infections was recorded in the summer season, followed by autumn then spring, and the minimal prevalence of infection was recorded in winter. The degree of susceptibility of the most prevalent isolates causing mortality (*Ph. damselae, and V. vulnificus*) towards 8 different types of antibiotics demonstrated that they were susceptible to chloramphenicol, Nalidixic acid and Sulphamethoxazole with trimethoprim while they were resistant to Ampicillin, Neomycin and Erythromycin.

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