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Marine Pollution as a Trigger of Discoloration Phenomenon in The Hard Coral, *Pocillopora* Species at The Gulf of Aqaba, Red Sea, Egypt: Pathological and Molecular Evidences

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ABSTRACT

Hard corals are precious marine creatures that comprise a complex form of symbiosis between symbiont algae and coral holobiont. For decades, corals have been challenged by disastrous events of climatic and anthropogenic etiologies. Such complex interactions have resulted in devastating disease episodes among coral populations worldwide. There is a scarcity of information about diseases of hard corals in the Gulf of Aqaba, Red Sea, Egypt. Therefore, the current study aims to investigate various diseases of hard corals in this pristine habitat within the Red Sea. Whitening and dark green dots were the most noticeable morphopathology among hard coral samples collected from Gulf of Aqaba. Some human-based pathogens, such as *Rothia kristinae*, *Cupriavidus pauculus*, and *Delftia acidovorans*, were isolated from some of the examined coral tissues, while the Burkholderia *cepacia* group was isolated from the nearby sediment. The final identities of the above-mentioned bacterial isolates have been molecularly confirmed using 16S RNA sequence analysis. Pathologically, diseased corals have been observed with changes such as some forms of tissue losses, degenerative changes, and eosinophilic granular amoebocytes/agranular cells infiltration. The frequent detection of some microbial pathogens of human origin could suggest deleterious forms of environmental pollution of anthropogenic origin. Ultimately, the entire existence of hard coral populations is mostly threatened by swiftly erupting climatic changes as well as environmental aquatic pollution. Thus, the current study concludes the real need for extensive ecological, biological, pathological, and immunological studies to determine the eminent threats and propose possible control means for better/sustainable hard coral populations.

Keywords: Hard corals, Gulf of Aqaba, Red Sea, Pollution, Coral diseases, coral discoloration, Molecular screening.

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INTRODUCTION

Coral reefs are the globe's most diverse symbiotic ecosystem. Reefs are referred to as "rainforests of the sea" due to the richness of life found in the ecosystems provided by corals. According to **NOAA (2019)**, healthy coral reefs support around 25% of the ocean's fish population. Moreover, it has a vital role in human life, such as economically in providing job opportunities for local populations and giving recreational possibilities, protecting coasts from hurricanes and erosions, and having biomedical importance in the production of pharmaceutical products. Stony corals (common name) are 232 families that originated from (Phylum: Cnidaria, Class: Anthozoa, Subclass: Hexacorallia, Order: Scleractinia) **(World Registry of Marine Species, 2023)**, the fundamental coral reef structure builders, consisting of a mutualistic relationship between the coral and a dinoflagellate of the species *Symbiodinium*, as well as different populations of microorganisms (Bacteria, Archaea, viruses, and Eucarya) **(Bourne & Munn, 2005)**. This has given rise to the notion that the host (coral animal) and its accompanying microbes are regarded as holobionts **(Rosenberg *et al.*, 2007)**.

The incidence and severity of reef deterioration have significantly risen. Coral diseases are believed to be a key contributor to this deterioration **(Porter *et al.*, 2001)**. Coral disease outbreaks are expected to worsen in the future because of ongoing issues caused by human misuse and global climate change **(Ziegler *et al.*, 2016)**.

Climate change has too many variables such as rising water temperature, increased CO_2 levels, increased Nitrogen levels that lead to ocean acidification, diminished dissolved oxygen levels with consequent decrease in water pH. The ongoing decrease in pH has deleterious impacts on the coral-associated bacterial population (coral microbiome) **(Meron *et al.*, 2011; Eissa and Zaki, 2011)**. The coral microbiome, with its patterns of geographical and host-specificity has significance for the host's acclimatization and adaptability to climate change **(Van Oppen & Blackall, 2019).** The first coral disease was described in 1965, and only four additional diseases were recorded during the next three decades. In the mid-1990s, reports of new coral illnesses grew globally **(Sutherland *et al.*, 2004)**. Between 1996 and 2000, the number of diseased coral species grew from 11 to 36 **(Porter *et al*., 2001)**.

Six coral diseases appear to be Indo-Pacific endemics **(Bruckner, 2015)**; these diseases are Black Band Disease (BBD)**,** Skeleton Eroding Band (SEB), White Syndromes (WS), Growth Anomalies (GAs), Ulcerative White Spots (UWS), and Brown-Band Disease (BrB). In 1998, a worldwide bleaching event had an insignificant impact on Coral reef damage in the Red Sea has accelerated through the previous three decades because of rising manmade disruptions and their combination with environmental influences, notably across the Egyptian Red Sea coast ***(Ali et al., 2011)***. One critical reason for difficulties in identifying the diseased coral colony is defining if the etiology was due to tissue loss/injury caused by predation or an infectious agent. Also, a variety of coral anomalies have been classified as coral illnesses in the literature, while they are caused by predation rather than disease **(Sutherland *et al.*, 2004).**

Research is urgently needed to overcome information gaps about disease patterns, predominance, and causation in Red Sea reefs **(Neave *et al.*, 2019)**. Thus, the current study aimed to perform a preliminary diagnostic investigation panel (microbiological, histopathological, and molecular examinations) to unveil the hidden etiologies behind some morphopathological changes of hard corals in the Gulf of Aqaba, Red Sea, Egypt.

MATERIALS AND METHODS

Samples collection

In November 2021, at the Gulf of Aqaba, Dahab (South Sinai), we chose two busy sites with human activities, such as the daily high number of boats used for diving and snorkeling activities. The latitudes for the first site (Mashraba) and the second site (Eel Garden) were 28°29'44.8"N, 34°31'04.3"E, and 28˚30'05.3"N, 34˚31'19.8"E, respectively. In both sites **(Fig. 1A)**, hard coral *Pocillopora* spp. was carefully inspected for any possible gross lesions **(Fig.1 B and C).** Both diseased and apparently healthy fragments (those without any visible signs) from each colony were sampled. In the first site, three colonies of hard coral at depths of 3 m and 7 m with healthy and abnormal gross lesions were sampled **(Fig.1 D-G)**. At the second site, two colonies at a depth of 15 m were also sampled. The apparently healthy samples and the diseased *Pocillopora* spp. were photographed, and five tissue fragments (1-2 cm) from each with two replicates were collected using a sharp clipper. For histopathological examination, the samples were transferred in separate labeled plastic bags with a few surrounding seawaters for fixation and tissue processing, according to **Work (2012).** For microbiological examination, fragments from the same colonies were collected in labeled sterile falcon tubes, while sediment nearby the examined colonies was collected in sterile 2 ml cryovials, according to **Woodley *et al.* (2008).** The measured water temperature in both sites during sampling was 29 ºC. Then **the collected** samples were prepared for lab analysis, as all samples were kept in a container filled with ambient seawater until preserved according to **Price & Peters (2018)**.

Fig. 1: A: Sampling sites of hard coral, Pocillopora spp., in Dahab, Gulf of Aqaba, Red Sea, Egypt. **B:** The scientific divers of the study surveying the reef to detect any gross lesions showing an overview for the examined reef. **C:** Close-up for the examined hard coral colonies. **D:** Sampled colonies, hard coral, Pocillopora spp. showing tagging of diseased colony 1 for sample collection; the gross lesions appeared as abnormal white discoloration **E:** Tagging of diseased colony 2 for sample collection, the gross lesion appeared as abnormal white discoloration associated with dark green dots **F:** Tagging of diseased Colony 3 for sample collection showing same gross lesions of colony 2. **G:** Demonstration of the Size of fixed samples collected from different colonies of diseased Pocillopora spp. (samples 3, 4&5).

Microbiological Examination

Preparation of tissue samples

Each tissue sample was homogenized in a sterile mortar with a small amount of sterile artificial seawater and divided equally into two labeled 2ml cryovials. In one of these cryovials, glycerol 50% was added, frozen, and preserved as stock samples. The other vial was used for bacterial isolation through loopful spreading onto Tryptic Soya Agar (TSA) with 2% NaCl and Thiosulphate Citrate Bile salt Sucrose Agar (TCBS) plates (HIMEDIA)®, and then transferred in an ice box to the lab for further identification **(Woodley *et al.*, 2008; Aeby *et al.*, 2017)**.

Preparation of sediment samples

Sediment samples were divided into two subsets. Each subset was enclosed in a 2ml cryovial. One sediment subset was kept without any additives for bacterial isolation and the other received 1ml of glycerol 50 % solution until stored at -80 °C.

Preparation of samples for histopathology

According to **Price and Peters (2018),** the fixative, formalin seawater 10% was prepared by mixing 100 ml formalin 37%- and 900-ml artificial seawater. Firstly, samples were documented by photography, then transferred from the plastic bag to a pre- labeled Falcon tube and filled with the previously prepared fixative, at a ratio of 1:20 (tissue: fixative).

Laboratory examination

Microbiology

Loopfuls from the homogenized tissue & sediment samples were spread onto TSA with 2% NaCl, TCBS, and Strept agar plates incubated at 28^{o} C for 48 hrs. One single colony was picked up from different bacterial colonies from each plate and then re-streaked onto TSA with 2 % NaCl for further purification. Gram staining was implemented on single pure colony obtained from each plate. The same pure colonies were inoculated into cryovials containing nutrient broth 50% glycerol solution and stored at -80 °C. Representative samples from the diseased tissue and the nearby sediment were identified using VITEK 2 compact system **(Pincus, 2006)**.

Pathology

Gross Pathology

The lesion description, distribution, color changes and severity of the examined hard coral colonies were scored and tabulated. The lesion progression either acute, subacute, or chronic were categorized according to **Woodley *et al.*, (2008)** who scored and related the lesion severity of the hard coral to the affected surface area as mild (<10%), moderate (10-24%), severe (25-49%), and extreme (50-100%). Further, described the lesion progression into acute (recent tissue loss, no algal colonization), subacute (large area of exposed skeleton accompanied by filamentous algae), and chronic (diverse types and degrees of algal colonization with small area of exposed skeleton or without).

Histopathological Examination

After tissue samples were fixed in the special fixative solution, were cut into small pieces using an electric cutter (Dremel®) in accordance with **Price and Peters (2018).** They were further contained in plastic tissue cassettes for decalcification utilizing HCl, formic acid 10% and water. Decalcified tissue specimens were stained by H&E, Gram, Giemsa, Ziehl Neelsen and PAS stains **(Roberts *et al.*, 2012)**.

Molecular screening

16S rRNA sequencing

Genomic DNA was extracted from purified bacterial isolates using the QIAamp DNA Mini Kit (Qiagen, Germany) following the manufacturer's instructions. The eluted DNA was utilized as a template for PCR detection of the universal 16S rRNA gene. The 16S rRNA was amplified using the universal primers fD1 (5′AGAGTTTGATCCTGGCTCAG3′) and rP2 (5′ ACGGCTACCTTGTTACGACTT3′) described by **Weisburg *et al.* (1991)**. The PCR amplification was prepared in a total volume of 25 µl contained 5 µL of dNTP at 1 mM, 2.5 µL 1 U Taq DNA polymerase, 5 µl genomic DNA, 1 µl of each primer (10 pmol), and the volume was completed to 25 µL by adding nuclease-free water. The PCR was performed using a thermal cycler (Biometra, Germany) with the following conditions: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The PCR amplicons were purified using the QIAquick PCR purification kit. Sequencing of PCR products was performed by utilizing a 3500xL Genetic Analyzer (Applied Biosystems) at Faculty of Agriculture Research Park, Faculty Agriculture, Cairo University.

Phylogenetic analysis

The nucleotide sequences of bacterial isolates were checked and assembled using Bio-Edit version 7.0 **(Hall, 1999)**. The assembled nucleotide sequences were submitted to the database of GenBank and compared to the related sequences by BLASTN search. Multiple sequence alignments were performed using CLUSTAL W. Phylogenetic trees were constructed from the resulting alignments using the neighbor-joining method in MEGA X 11, with 1000 bootstrap replicates **(Kumar *et al.*, 2018)**.

RESULTS

Microbiological results

Presumptive bacterial identification using VITEK 2 system revealed that the bacterial isolates retrieved from diseased tissue of coral colony 4 were presumptively identified as *Rothia kristinae* with 95% probability and very good identification. *Cupriavidus pauculus* and *Delftia acidovorans* were presumptively isolated and identified from diseased tissue of coral colony 1 and *Burkholderia cepacia* group (*Burkholderia cepacia, Burkholderia vietnamiensis, Burkholderia multivorans,* and *Burkholderia stabilis)* were isolated and identified from the nearby sediment of coral colony 3 with 95 % probability and good identification.

Pathological findings

Gross pathology

At site 1 (Mashraba) and in comparison with the normal *Pocillopora* spp., the gross pathological findings of examined *Pocillopora* from coral colony 1, at 3m depth, exhibited a 1cm, mild, acute, white colored with smooth margins, and apical diffused lesion **(Fig.1 D)**. At the same depth, coral colony 2 showed a 2cm, severe, acute, white colored with dark green dots, irregular margins, and apical diffused lesion **(Fig.1 E)**. At 7m depth, coral colony 3 showed a 2cm, extreme, chronic, white colored with dark green dots, irregular margins, and medial coalescing lesion **(Fig.1 F)**.

At site 2 (Eel Garden), at a depth of 15m, coral colony 4 showed a 1cm, moderate, subacute, white colored with dark green dots, irregular margins, and apical diffuse distribution lesion. Coral colony 5 exhibited a 2cm, extreme, chronic, white color with dark green dots, irregular margins, and apical/medial diffused lesion **(Fig.1 G)**. A morphometric scoring system describing the reported hard corals' lesions was presented in **Table (1)**.

Table 1: Gross pathological findings of the corals in the examined two sites

Site	Depth	Coral Spp.	Coral Colony No.	Lesion size	Lesion color	Lesion Distrib-ution	Lesion Location	Lesion Margin	Lesion Severity	Duration
Mashraba (Site 1)	3m	Pocillopora	1	1 cm	White	Diffuse	Apical	Smooth	Mild	Acute
	3m		2	2 cm	White with dark green dots	Diffuse	Apical	Irregular	Severe	Acute
	7m		3	2 cm	White with dark green dots	Coalesc	Medial	Irregular	Extreme	Chronic
Eel Garden (Site 2)	15m		4	1 cm	White with dark green dots	Diffuse	Apical	Irregular	Moderate	Subacute
	15m		5	2 cm	White with dark green dots	Diffuse	Apical/Medial	Irregular	Extreme	Chronic

*****Lesion severity scoring: Mild (<10%), Moderate (10-24%), Severe (25-49%), Extreme (50-100%)

*The lesion severity scoring of our study was performed according to Woodley et al., 2008.

Histopathology

The histopathological examination of the apparently healthy patient showed normal histological structure. Prominently normal four layers were noticed in such samples: epidermis, gastrodermis, mesoglea, and calecodermis. The epidermis contained mucocytes, nematocysts, ciliated supporting cells, and pigment cells **(Fig.2 A–B)**, while the gastrodermis contained the symbiotic algae *zooxanthellae* in vacuoles, an eosinophilic organelle with basophilic nuclei, and granular gland cells with few mucocytes **(Fig.2 C)**.

The tissue sample stained with Periodic Acid Schiff (PAS), showed clearly eosinophilic granular gland cells in the Gastrodermis **(Fig.2 D–E)**. Another fixed healthy tissue section exhibited the oral opening and actinopharynx downwards towards the aboral section, including the gastrovascular cavity, with the mesenteries showing the mesogleal pleats **(Fig.3 A-D)**, as well as the oocytes within the gonads at the mesentery **(Fig.3E)**, and the basal body wall and the skeleton have appeared histologically normal **(Fig. 3F)**.

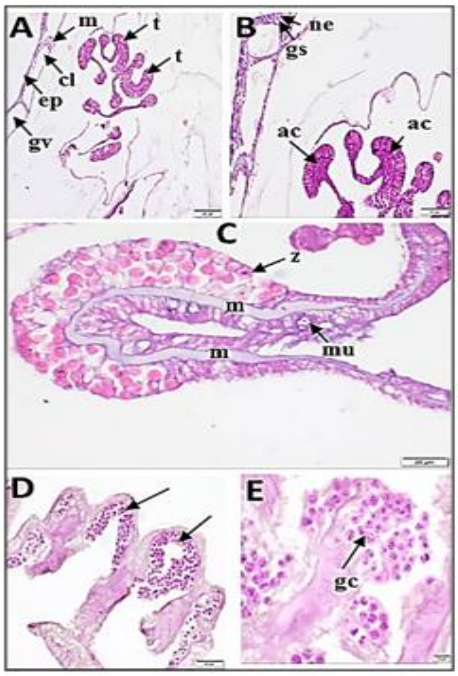

Fig. 2: Photomicrograph of a healthy tissue sample of colony 1, stained with H&E, showing A: tissue layers of the surface body wall that include the epidermis (ep), mesoglea (m), and gastrodermis; also, the gastrovascular canals (gv) connect the polyps of the colony. The basal body wall includes the thin layer of Calicodermis (cl); the tentacles (t) and the actinopharynx are noticed in the middle of the section. B: Higher magnification of the previous photo showing various structural cells of the polyp (Nematocyst (ne) and Mucocytes) of the epidermal layer and the symbiont (*Zooxanthellae*) in the Gastrodermis (gs). The actinopharynx (ac) appeared as a basophilic linear structure within the tentacles. C: Another section of the healthy tissue sample of the colony shows the surface body wall's tissue layers: the epidermis with its characteristic mucocytes (mu), the mesoglea (m), and the gastrodermis, which includes the Zooxanthellae (z) (a vacuole that surrounds the eosinophilic cytoplasm and basophilic nucleus). D: Healthy tissue sample of colony 1, stained with PAS, showing, the Gastrodermis includes eosinophilic granular gland cells and *Zooxanthellae* (arrows). E: higher magnification of the Granular Gland cells (gc) stained with PAS.

The H&E-stained slides from gastrovascular cavity of coral colony 1 showed clumping of eosinophilic granules (yolk) within the ova, vacuolization of the surrounding wall, as well as the surrounding wall of the spermary and clumping of the spermatozoa, and hyalinization of the mesenteries **(Fig.4 A-B)**. The H&E-stained sections from disease tissues of coral colony 3 showed vacuolation, tissue necrosis and the absence of *zooxanthellae* **(Fig. 4 C–D)**, while Giemsa-stained sections exhibited many positively stained protozoa of different sizes and shapes **(Fig. 4 E)**.

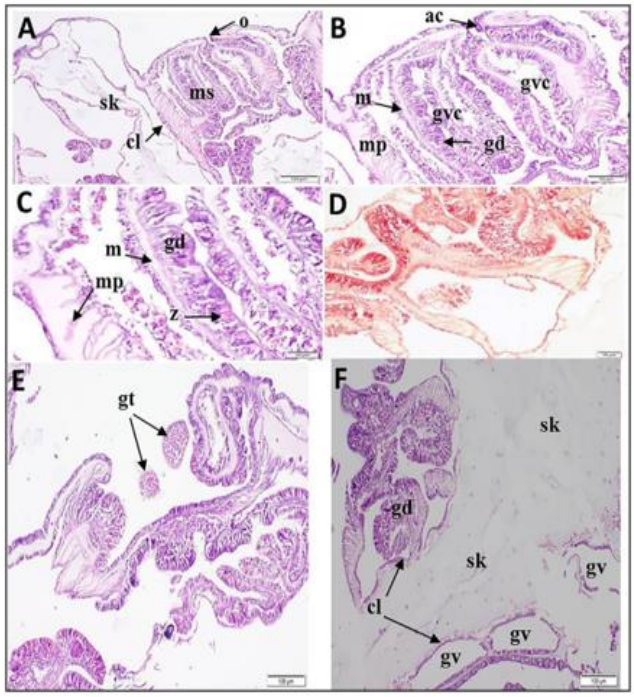

Fig. 3: Photomicrograph of healthy tissue sample of colony 3 (A-C) stained with H&E, (D) stained with Gram's stain; showing (A, B). A: gastrovascular cavity demonstrating the oral (o), aboral parts and mesentery (ms) attached to the skeleton (sk) bounded by the calicodermis (cl) of the polyp. B: higher magnification of the oral, aboral, and mesentery, showing the actinopharynx (ac) in the oral part covered with the epidermal layer, followed by the aboral part that begins from the end of the actinopharynx toward the gastrovascular cavity (gvc) and mesentery. The Gastrodermis (gd), Mesoglea (m), and Mesogleal Pleats (mp) are observed. C: Higher magnification of the mesentery shows the Gastrodermal layer (gd) covering the mesentery and including *Zooxanthellae* (z) and Mucocytes; the basal mesogleal layer (m) is observed underneath the gastrodermis. The characteristic shape of the mesogleal pleats (mp) is noticed. D: A healthy tissue sample of colony 3 stained negative with Gram's stain, showing no evidence of bacterial infection. E&F: Photomicrograph of healthy tissue samples of colony 5 stained with H&E showing E: Gonadal tissue (gt) in the mesenteries shows multiple stages of developing oocytes containing the eosinophilic yolk. F: skeleton of the polyp (sk) formed of the basal body wall layers, Gastrodermis (gd) and Calicodermis (cl). The gastrovascular canals (gv) run along the superfacial layer of the skeleton, connecting the polyps of the colony.

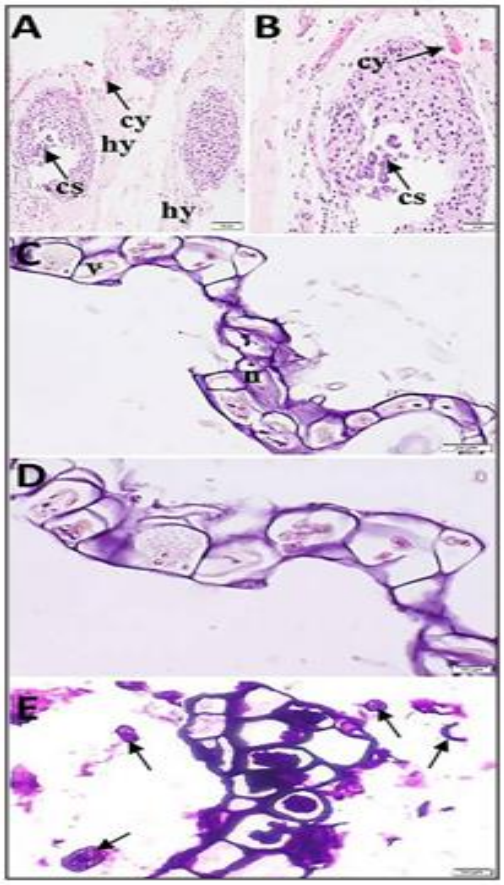

Fig. 4: Photomicrograph of diseased tissue sample of colony 1 stained with H&E (gonads within the mesentery) showing; **A:** clumping of yolk (cy); the yolk appeared as homogenous and more eosinophilic within the ova together with vacuolation of surrounding cell wall. The spermary showing central area of clumping of the spermatozoa (cs). Hyalinization and dissociation (hy) of the surrounding Gastrodermis is observed. **B:** Higher magnification of the previous photo demonstrating the clumped yolk (cy) and spermatozoa (cs) of the gonads. **C-E:** Photomicrograph of diseased tissue sample of colony 3 showing **C:** Diseased tissue stained with H&E showing degenerative changes; vacuolation (v), necrosis and fragmentation (n) of the gastrodermal cells. **D:** Higher magnification of the previous photo showing the degenerative changes of the cells. **E:** Diseased tissue sample stained with Giemsa stain, showing many positively stained protozoa of different sizes and shapes (arrows).

Additionally, the Gram stained apparently healthy tissue sample of colony 3 showed aggregations of Gram-positive coccobacilli bacteria. The H&E stained sections of Colony 5 showed eosinophilic granular cell infiltration **(Fig.5 A-C)**. Further, the Gram stained tissues showed the presence of Gram-positive protozoal spores and Gram-positive bacterial aggregation **(Fig.5 D & Fig.6 A-D)**.

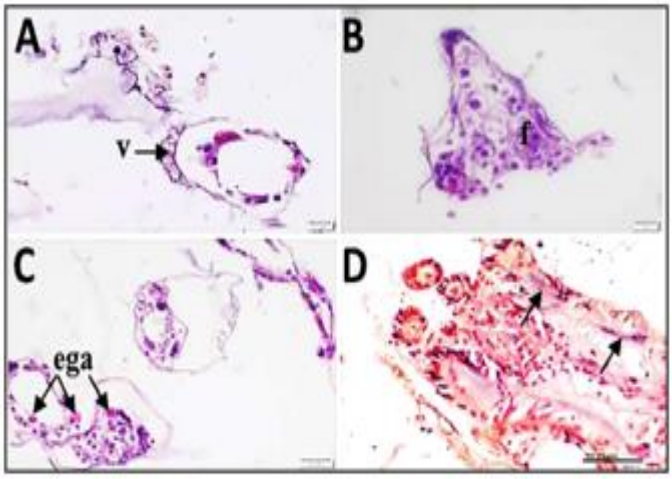

Fig. 5: Photomicrograph of diseased tissue sample of colony 5 showing **A-C:** Degenerative changes such as vacuolation (v), fragmentation (f) of the gastrodermis and aggregation of eosinophilic granular amebocytes (ega) with absence of *Zooxanthellae* (H&E stain). **D:** Gram-positive coccobacilli bacteria (arrows) aggregated on the degenerative tissue.

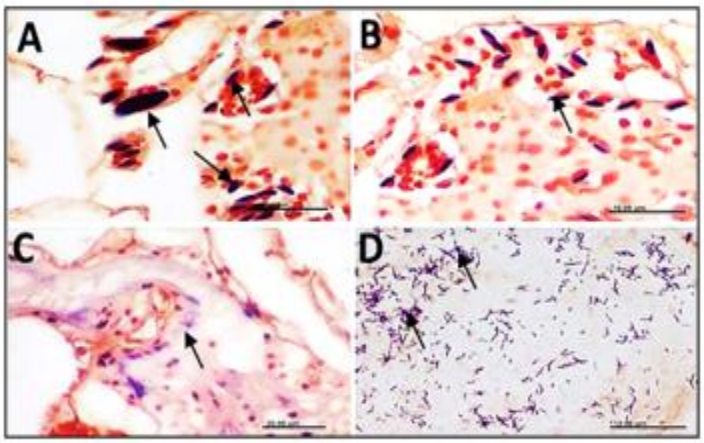

Fig. 6: Photomicrograph of diseased tissue sample of colony 5, stained with Gram's stain showing **A:** aggregation of Gram-positive bacterial and protozoal aggregates (arrows). **B-C:** Higher magnification of the previous photo. **D:** Gram-positive bacterial aggregation (arrows).

Phylogenetic analysis

The nucleotide sequences of the bacterial isolates in this study were submitted to GenBank database and each was assigned an accession number **(Table 2)**.

Table 2: The accession numbers of 16S rRNA sequences of bacterial isolates.

Isolate	Accession number	Length (bp)
Rothia Kristinae	PP709030	1412
Delftia acidovorans	PP715431	1520
Cupriavidus Pauculus	PP709032	1492
Burkholderia cepacia	PP709062	1480
Burkholderia vietnamiensis	PP709065	1490
Burkholderia multivorans	PP709069	1510
Burkholderia stabilis	PP709070	1478

The neighbor-joining phylogenetic tree was constructed based on the sequenced 16S rRNA gene of *Rothia kristinae*. *R. kristinae was* grouped with their relevant *R. kristinae* sequences, which were genetically apart from other related species *K. kristinae* (99%) and *K. massiliensis* (97%) depending on their degree of similarity **(Fig. 7)**. While the BLAST alignment of *Delftia acidovorans* showed 100–99% identity to the accession numbers of *Delftia* species (AF149849.1, AF538930.1, OP986396.1, NR024711.1, KC572558.1, JN644603.1, KC292489.1, NR116495.1, OM764634.1, and OR623221.1) **(Fig. 8).**

Figure 7

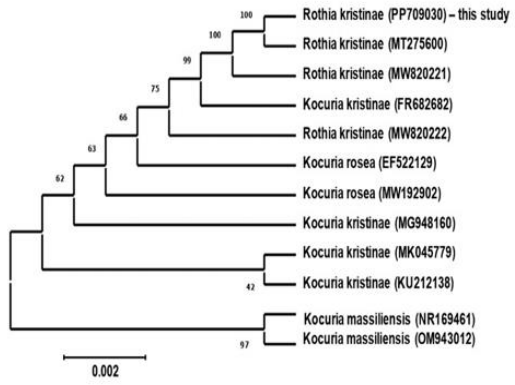

Fig. 7: The neighbor-joining phylogenetic tree is based on the comparative sequence of 16S rRNA gene of Rothia Kristinae isolated from coral tissues.

Figure 8

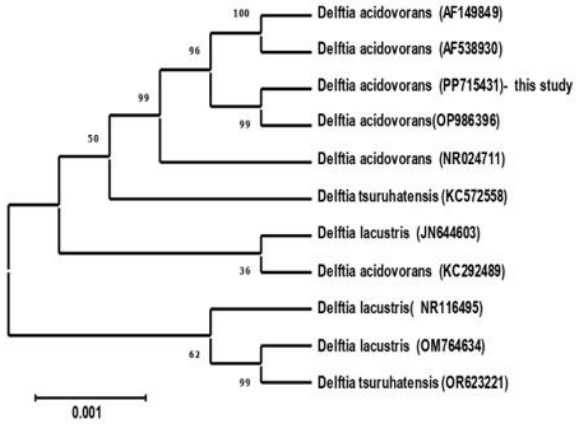

Fig. 8: The phylogenetic tree of Delftia acidovorans isolated from coral tissues based on 16S rRNA gene sequences.

The phylogenetic analysis of *Burkholderia* cepacia group and *Cupriavidus pauculus* showed two main clades. The first linage included *Burkholderia* cepacia group (*Burkholderia cepacia*, *Burkholderia vietnamiensis*, *Burkholderia multivorans*, and *Burkholderia stabilis)*, which were embedded with other related bacterial species. The second clade showed that *Cupriavidus pauculus,* which was grouped with their relevant *Cupriavidus* spp. sequences, showed 100% identity to the accession number of *C. pauculus* (AM418462.1) and 99.86% identity to *C. pauculus* (JQ811852.1 and AB109753.1) and *C. metallidurans* (JQ046372.1).

DISCUSSION

The current research is considered an initial step in a long trail of hard coral disease investigations from the perspective of aquatic veterinary medicine. Moreover, the supervisory professional veterinary collaboration with governmental field marine biologists/ecologists and NGO marine conservationists has resulted in the development of a pile of comprehensive data about the rarely investigated diseases of hard corals on the Egyptian side of the Red Sea.

In the current study, *K. kristinae* was isolated from the diseased hard coral tissue and identified by VITEK 2 with a 95% probability. *K. kristinae* is a Gram-positive coccus of the *Micrococcaceae* family. Recent taxonomical studies have changed the genus name of Kocuria species into genus *Rothia*, where the bacterial species name was changed into *Rothia kristinae* **(Nouioui *et al.*, 2018)**. It is often thought to be a member of the natural flora of humans and animals.

However, it is rarely isolated from clinical samples. Yet, several studies have reported its implication in the progression of pathogenic diseases in a variety of systems and organs, such as catheter-related bacteremia, infection of an immunocompromised patient **(Dudeja *et al.*, 2013)**, and isolation from a bovine vaginal sample **(Styková *et al.*, 2016)**. Locally, the first reported Egyptian case was a catheter-related bacteremia referenced to *K. kristinae* **(Hassan *et al.*, 2016)**. Such implications could lead to a conclusive notion that the major source of these bacteria in hard coral tissues is of anthropogenic pollution's origin. On the other hand, a new *Kocuria* species, *Kocuria coralli*, was isolated from the coral reef seawater at a depth of 4.2 meters. The organism's phylogenetic and morphological characteristics supported the notion that it belonged to the genus *Kocuria* **(Li and Zhang, 2020)**.

Cupriavidus pauculus is another bacterium that was successfully isolated from the examined hard corals. It is a Gram-negative bacillus that belongs to the family *Burkholderia*. It is a commonly existing environmental bacterium that is rarely reported as a human pathogen. In the past decade, *C. pauculus* has been identified as a water pollutant that has resulted in many human pathological cases **(Balada-Llasat *et al.*, 2010)**. In our research, we hypothesized that the isolation and identification of *C. pauculus* from diseased coral tissue (1 D-T) may have been linked to heavy metal pollution, as they commonly exist in heavy metal-polluted soil and water. Thus, they are considered a bioindicator for such types of chemical pollution, particularly copper. Several studies have supported this hypothesis, in which we attribute the revealed lesions and bacteria to heavy metal pollution, particularly copper **(Dar *et al.*, 2018).**

Copper is a major component of the antifouling paints used in ship and boat manufacture, with continuous release into aquatic environments. The photosynthetic efficiency of coral symbionts *(Zooxanthellae)* decreases significantly as the quantity of heavy metal pollutants increases **(Bielmyer *et al.*, 2010)**. **Zeng *et al.*, 2020**, studied the heavy metals (copper, cobalt, and Nikel) concentration range that promotes *C. pauculus* growth and concluded that the concentration range was within 400 mg L^{-1}. In these regards, **Ammar *et al.*, (2013)** analyzed water and sediment samples from the same locality of our study (Eel Garden) at the Gulf of Aqaba, Red Sea, Egypt, and noticed that the copper concentration in the water samples at this site was 0.13 ppb and in the sediment was 3.63 ppb. Although these findings did not go in parallel with our results, the cumulative effect of this heavy metal has increased over time.

Delftia acidovorans is also considered an unusual pathogen and has been reported in many human clinical cases **(Bilgin *et al.*, 2015)**. It is used as a pollution indicator **(Simpson, 2020)** and in removing microplastics **(Pan *et al.*, 2023)** because of its ability to attach to these particles. The presence of this bacteria in hard coral tissues could support the assumption that the local study area in Gulf of Aqaba is intensively polluted by oil and sewage from diving and snorkeling boats, as well as other anthropogenic activities.

At the same sampling site, Burkholderia cepacia complex (Bcc) bacteria (B. cepacia, B. vietnamiensis, B. multivorans, and B. stabilis) were isolated and identified from the sediment in the vicinity of some examined hard coral colonies. Burkholderia cepacia complex, a group of bacteria that are known to be widely spread in the natural as well as polluted environment **(Vial *et al.*, 2011)**. Even though Bcc has an ecologically significant role in environmental biodegradation and bioremediation, it is implicated in the pathogenesis of fatal pathologies in immunocompromised patients. **(Mahenthiralingam *et al.*, 2008; Cauduro *et al.*, 2021)**.

The above-mentioned bacterial etiologies were biochemically identified utilizing VITEK 2 technology, according to **Pincus (2006)**. Recent molecular tools, including PCR, sequence analysis and phylogenetic tree assembly, are accredited confirmative diagnostic tools that can be relied upon in declaring the final identities of bacterial pathogens **(Fairfax & Salimnia, 2013)**. The molecular screening of the presumptively identified bacterial isolates has confirmed their final identities as Delftia acidovorans and Burkholderia cepacia complexes based on the 16SrRNA sequence analysis and consequent phylogram **(Weisburg *et al.*, 1991; Kumar *et al.*, 2018)**.

Hard corals are naturally threatened by corallivores predation, such as fish, Crown of Thorns starfish, or gastropods. During the examination of the sampling sites, signs of fish predation on hard corals were observed. Such predation could have resulted in tissue erosion, which consequently predisposed the patient the patient to secondary microbial infection and increased disease susceptibility. **(Renzi *et al.*, 2022)**.

From a disease perspective, pathological examination is an important diagnostic tool for coral diseases since a single gross lesion might be linked with numerous putative causal agents **(Hawthorn *et al.*, 2023)**. In the current study, various pathological changes including both gross and histopathological lesions were detected on examination of sampled hard coral tissues. The detected gross lesions were variable in color, size, distribution, and severity. Our findings were consistent with **Work and Aeby (2006)**, who systematically described the various lesions of hard

corals based on morphometric measures. It is suggested that white discoloration in coral colonies could indicate initial bleaching or white disease **(Ainsworth *et al.*, 2007)**. Our hard coral colony 1 pathological findings consistently accord with this assumption; thus, the observed bleaching (white discoloration) could be attributed to the loss of *zooxanthellae* that was noticed in the histopathological sections. In this respect, **Ainsworth *et al.*, (2007)** have indicated that white disease is characterized by bacterial invasion of tissues, *zooxanthellae* loss, tissue structural loss, and necrosis, while white syndrome appears without bacterial invasion. The white discoloration with green dots in colonies 2, 3, 4, and 5 may indicate infection with ciliates such as *Halofolliculina coralliasia*, the causative agent of the Skeleton Eroding Band disease, in which the bare white coral skeleton is studded with several abandoned loricae of *H. coralliasia*. The histopathological findings revealed the presence of gram-positive stained protozoa deeply imbedded in the examined colony. These findings agreed with **Winkler *et al.*, (2004),** who confirmed that corals of the Red Sea at Gulf of Aqaba are vulnerable to the Skeletal Eroding Band (caused by *H. coralliasia)* when stressed by environmental stresses induced by pollution.

The histopathological examination showed degenerative changes in all layers (Epidermis, mesoglea, and gastrodermis) and fragmentation of the architecture, changes in *zooxanthellae* or eviction of *zooxanthellae*, vacuolation, tissue losses, immune cell infiltration (mainly eosinophilic granular amoebocytes and agranular cells), aggregation of Gram-positive bacteria, and Gram-stained protozoal spores **(Bruno *et al.*, 2006)**. With these pathological changes, it is difficult to detect only one causative agent, which might indicate that disease conditions are attributed to environmental pollution with toxic chemicals. The pollution and climatic changes could negatively affect corals' health status, leading to apoptosis and the ejection of symbiotic *zooxanthellae*, leaving the corals bleached **(Zhou *et al.*, 2018)**. It is worth mentioning that the bacteria were not identified in all diseased samples of the examined corals, which indicates the possibility of pollution-inducing lesions in such coral colonies. On the other hand, gram-positive bacilli and coccobacilli were detected in the apparently normal corals, indicating that the isolated bacteria could be considered a contaminant other than pathogenic for the corals. Attention could be paid to the possibility of adaptation and biodiversity of the isolated bacteria to become pathogenic for the corals. In this regard, bacteria normally inhabit hard corals and may adapt to infect and affect their host because of anthropogenic stressors **(Work and Aeby, 2014; Rodríguez-Gómez *et al.*, 2021)**.

CONCLUSIONS

The current research sheds light on the growing aquatic environmental pollution from a regional perspective. Despite the remarkable shortage in coral disease research, the current study is a novel initiative for an effective investigative approach for the diseased corals in the Gulf of Aqaba, Red Sea. The obtained results could present real answers to the dilemma of Red Sea coral degradation through a decade of environmental crises. Sewage and heavy metal pollution are the major environmental threats to such pristine creatures. The current study affirms that some human and terrestrial animal pathogens are frequently isolated from corals and their floor sediments. This has led to the critical assumption that municipal sewage, heavy metal pollution and its associated pathogenic load are the major environmental threats to such precious coral populations. Further, these assumptions mandate a swift move toward the establishment of reference coral disease laboratories across the region to disseminate coral disease knowledge, diagnostic knowledge and restorative skills among budding veterinarians and environmentalists. Ultimately, profound studies are required by multidisciplinary fields of veterinary medicine and marine biology to demonstrate the impacts of human activities and anthropogenic pollution on the health status and disease occurrence of hard corals in our sampling locality for reef-building corals' conservation in these areas. However, further parasitological identification of this protozoa is required as a complimentary study of our work.

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Ethical approval

The authors confirmed that sampling was approved by the Egyptian Environmental Affairs Agency (EEAA) through the South Sinai Protectorates Managers. Furthermore, all methods and procedures applied on the examined hard corals were conducted in compliance with the guidelines of the care and use of animals and have been approved by the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, Cairo University, Egypt (IACUC Protocol No.: CU II F Reg.1/21).

Availability of data and materials

All data are included in the manuscript.

Conflicts of interest

All authors declare that they have no conflict of interest.

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Author's Contributions

All authors contributed equally to the manuscript.

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