STUDIES ON SHELL DISEASES OF CAPTIVE TURTLES

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By

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CERTIFICATE

This is to certify that the thesis entitled **"Studies on shell diseases of captive turtles"** submitted by **Ms. PREETI GOSWAMI I.D. No. MVHK 1456** in partial fulfillment of the requirements for the award of degree of **MASTER OF VETERINARY SCIENCE** in **WILDLIFE** of the Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, is a record of bonafide research work carried out by her during the period of her study in this University under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

Bangalore
August, 2016

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AFFECTIONATELY DEDICATED

TO

MY MOM & DAD

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LIST OF ABBREVIATIONS

-	Negative
%	Per cent
+	Positive
°C	Degree Celsius
AD	Anno Domini
CCL	Curved carapace length
CCW	Curved carapace width
cfu	Colony-forming unit
CIRUM	Circumference
Cm	Centimeters
Co.	Company
CPL	Curved carapace length
CPW	Curved plastron width
CZG	Sri Chamarajendra Zoological Gardens, Mysuru
g	Gram
hrs	Hours
IBT	Indian black turtle
IFT	Indian flapshell turtle
IUCN	International Union for Conservation of Nature
kg	Kilo gram
lbs	pound

Ltd	Limited
max	Maximum
mcg	Micro gram
mg	Milli gram
min	Minutes
min	Minimum
ml	Milli liter
no.	Number
P	Pink
PBT	preferred body temperature
pH	Power of hydrogen
POTZ	Preferred Optimal Temperature Zone
Pvt.	Private
RES	Red eared slider turtle
SCL	Straight carapace length
SCUD	Septicemic cutaneous ulcerative disease
SCW	Straight carapace width
SPL	Straight plastron length
spp.	Species
V	Variable
Y	Yellow

Introduction

I. INTRODUCTION

Turtles and tortoises have existed for nearly 300 million years, since the Triassic Era, long before many dinosaurs walked the earth. Turtles and tortoises have evolved remarkable body armour that has remained relatively unchanged through evolution, and while other vertebrate species have arisen and subsequently gone extinct, the basic body form of the turtle shell has remained an obvious testament to the success of turtles and their ability to survive millions of years of natural selection pressures. However, the previously successful survival adaptations of turtles, including delayed sexual maturity, high juvenile mortality, and a long adult life-span with low natural mortality, have left turtle populations vulnerable to new, potentially devastating threats posed by human exploitation and development related pressures.

Reptilia is a fundamental split that gives rise to two clades the Anapsida which include the Chelonia [Testidines] and the Diapsida (which includes all other reptiles). Anapsid reptiles are characterized by a primitive skull with no temporal openings. Turtles are the only living representation of this clade and belong to one order variously referred to as Testudines, Testudinata or Chelonia. Currently, approximately 12 families, 90 genera and 250 species are within this order.

The two suborders of Chelonians are the Cryptodira (Hidden neck turtles) and the Pleurodira (Side neck turtles). Cryptodira turtles are able to retract the neck straight back into the shell, thereby hiding the neck. Pleurodirans are not able to retract the neck and must fold it up sideways. Hence, the common name of side necks. Pleurodirans are the smaller suborder and are composed of aquatic to semi aquatic families. Turtles are found

in diverse habitats in all continents except the Arctic. Fifty three percent of the species are considered threatened, making them highly endangered among the major vertebrate groups. The environment of captivity has a great impact on the bacterial levels of water which should be considered for the management of sea turtles as well as other aquatic animals. Water quality and temperature is critical for aquatic animal health and welfare, particularly for the captive animals. However in captivity, inadequate care is more likely to result in mortality then due to the old age.

The environment of captivity has great impact on the bacterial levels in the water, which should be considered for the management of turtles as well as other aquatic animals. Water quality is critical for aquatic animal health and welfare, particularly for captive animals (Cheun-Im *et al*., 2010).

Turtles play a critically ecological role in the environment and they play a major role in nutrient cycling.Freshwater turtles help to control aquatic vegetation, serve as scavengers and help maintain rivers and lakes in a healthy condition. In addition, turtles play a significant role in the cultures of many people around the world.

Khan (1982) reported *Lissemys punctata*is as most commonly observed species of turtle in Bangladesh. Das (1991) stated that *Lissemys punctata* may be the most common species in the Indian sub-continent. However, Verma and Sahi (1998) reported that this species was rare in Jammu and Kashmir State of India. Choudhury *et al*. (2000) reported *Lissemys punctata* as the common and stable species in India.

With this background, the present study was undertaken with the following objectives.

1. To study the occurrence of shell diseases in turtles
2. Isolation and ABST pattern of bacteria associated with shell diseases in captive turtles
3. To study the influence of environment and managemental practice on prevalence of shell diseases in captive turtles

Review of Literature

II. REVIEW OF LITERATURE

2.1 Distribution of turtles

There are currently three hundred and fifteen recognized species of turtles and tortoises in the world, out of those that have been assessed on the International Union for Conservation of Nature (IUCN) Red List are 63 per cent which have been considered threatened whereas 10 per cent are critically endangered along with 42 per cent of all known turtle species threatened as reported by Buhlmann *et al.* (2009).

As reported by Van Dijk *et al.* (2012) the diversity of all turtles and tortoises (chelonians) in the world has existed since 1500 AD, and currently it has been recognised as distinct by specialists in turtle taxonomy and systematics, it consists of approximately three hundred and thirty one species, of which fifty six are polytypic, with one hundred twenty one additional recognised subspecies. A total of eleven taxa of tortoises and freshwater turtles have become extinct since 1500 AD, leaving us currently with three hundred and thirty two species and one hundred and nineteen additional subspecies. In the entire living turtle taxa, seven species are marine turtles, along with three hundred and fifteen species and four hundred and thirty four total taxa of modern living freshwater and terrestrial turtles and tortoises.

In IUCN Red list 2013, *Nilssonia nigricans* is 'Extinct in Wild' category but presence of wild populations has been reported in 2009 by Ahmed and Das from Kaziranga national Park. In India, chelonians are given protection through National as well as International legislation. Turtles are considered as endangered and categorized in schedules of the Indian Wildlife (Protection) Act, 1972. Since the declaration and strict

enforcement of Wildlife Protection Act, fishing in India has been brought down. Audithia turtle (*Pelochelys bibroni*), Terrapin (*Badagur baska*), Eastern hill terrapin (*Melanochelys tricarinata*), Ganges soft-shelled turtle (*Trionyx gangeticus*), Green sea turtle (*Chelonia mydas*), Hawksbill turtle (*Eretmochelys imbricate inlscata*), Indian soft-shelled turtle (*Lissemys punctate punctata*), Kerala forest terrapin (*Hoesemys sylratica*); Leathery turtle (*Dermochelys coriacea*), Logger head turtle (*Caretta caretta*), Olive back logger head turtle (*Lepidochely solivacea*) Peacock marked soft-shelled turtle (*Trionyx hurum*) and Three keeled turtle *(Geoemydas tricarinata*) in Schedule IV of the Indian wildlife protection act 1972.

As reported by Ramakrishna *et al.* (2014), turtles are much more at risk of approaching extinction than birds, mammals, amphibians and paralleled among the larger vertebrate groups. Northeast India has been recognized as major conservation area for tortoise and freshwater turtle. It is also regarded as one of the major centres of turtle diversity. Rise of awareness on Indian Wildlife Protection laws and also on the biological and also socio-economic impacts of turtle exploitation and trade should be brought to notice among local fishers, turtle collectors, to protect the highly exploited species like *Lissemys punctata punctata* in Punnamada and elsewhere in Kerala, *Badagur baska* and *Badagur kachuga, Chitra indica, Manouria emys, Nilssonia nigricans* and *Pelochelys cantonii* some among many which are at a very high risk of extinction.

2.2 Taxonomy

McArthur (2004) reported that turtles belong to the family Cheloniidae, class reptilia under the order Testudines. Indian flapshell turtle (*Lissemys punctata Anderson*)

has an olive-green carapace and soft body parts, with yellow blotches and hieroglyphs on the carapace and head and neck. The blotches tend to disappear in old individuals (Smith, 1931).

In Sri Lanka, populations of Indian black turtle are intact in the uplands and some of the lowland areas, where large numbers can be seen within protected areas, such as Pollanaruwa and Udawattakale. In the Maldives, where the species was probably introduced by sailors, it was reported to be common in the ponds of Hulule in the early 1930's (Deraniyagala, 1956).

The Indian black turtle species is distributed throughout India, except for a broad area of apparent absence from the arid plains of northern and north-western peninsular India, causing the species to be split into two major ranges. The distribution in India includes the hill ranges of the Western Ghats, South of Gujarat, and the Southeast Coast (Daniel and Shull, 1964).

It is believed that the Gahirmatha rookery in Orissa is the largest reported nesting ground for Olive Ridley sea turtles in the world after it was discovered by Bustard (1974).

In Indian flapshell turtles, femoral flaps and nasal septal ridges are present. Shell closure (femoral flaps and movable anterior plastral lobe) allows for complete concealment of head, neck and limbs thus providing protection from predators and desiccation (Auffenberg, 1981).

The carapace of Indian flapshell turtle is relatively jumped and smooth surfaced in juveniles and adults, but hatchlings have low, indistinct ridges of tubercles, most prominent along the lateral margins. The carapace shape is nearly circular in the hatchlings and more oval in adults. Adult females are larger than males (Auffenberg, 1981; Agarwal, 1987; Shrestha, 1997).

Outside India, the distribution of the Indian black turtles include Srilanka, Maldives (where it was probably introduced), Southern Nepal, Bangladesh, Myanmar and extreme Western Thailand from Tak Mae Hong Son Province (Dinerstein *et al.* 1987; Das, 1995; Mitchell and Rhodin, 1996; Rashid and Khan, 2000; Van Dijk and Thirakhupt, 2000; Nabhitabhata *et al.*, 2000).

The maximal curved carapace length (CCL) observed in Indian flapshell turtle was 350 mm and estimated plastron length was 334 mm in females (Bhupathy, 1989).

The current distribution of Red eared slider in most countries is poorly known, but new sites of occurrence are likely to be found following dedicated field surveys. Moreover, notwithstanding the European Union import suspension of *Trachemys scripta elegans*since 1997, every year new records are reported in most European countries (Ernest, 1990).

Frazier and Das (1994) reported a large population of Indian black turtle in Dhikala, Corbett National Park, and northern India within a moist deciduous forest. In the Madras area of south India, low population densities within apparently suitable areas are attributed to exploitation and pollution. In many other localities in southern India,

including the Western Ghats, this is often the most abundant species of turtle, frequently seen after the first rains.

As claimed by the wildlife wing of Government of Orissa (2000), Olive Ridleys visiting Gahirmatha represent about 50 per cent of the total world population and about 90 per cent of the Indian population of Olive Ridley sea turtles.

A medium to large (20-60 cm) freshwater Red slider turtle characterised by their prominent yellow to red patches on either sides of the head of *scripta elegans* are the most commonly traded subspecies. Carapace and skin are olive to brown with yellow stripes or spots. Males are usually smaller than females and have a long thick tail. Melanism is common occurrence in the older males of the species. These turtles lack the characteristics by which the species are identified. There are two other distinct morphs of the Red eared slider; pastel and albino. These morphs are common in nature, but the turtles are bred to specifically bear these unique colourings. It was reported Red eared slider turtles species native range is in Eastern USA and in the adjacent areas of north-eastern Mexico. Whereas, their known introduced range is Europe, South East and Far East Asia, the Caribbean, Israel, Bahrain, Mariana Islands, Guam and South Africa. There are 4 types of Red eared turtles namely observed Red eared slider, Melanistic Red eared slider, Pastel Redeared slider, and Albino Red eared slider (Burger, 2009).

Based on the studies of Van Dijk *et al.* (2012), reptilia is a fundamental split that gives rise to two clades, the Anapsida (which includes the chelonian 'Testudines') and the Diapsida (which includes all other reptiles). Anapsida reptiles are characterized by a primitive skull with no temporal openings. Turtles are the only living representatives of

this clade and belong to one order variously referred to as Testudines, Testudinata or Chelonia. Thus, chelonians refer to turtles, tortoises and terrapins as a group. The most commonly found species is the Indian black turtle (*Melanochelys trijuga*) and as observed in these species the carapace is elongated, which is relatively more elevated in adults and depressed in juveniles, with freely serrated marginals posteriorly. The nuchal scute is small and distinctly triangular. The plastron is about as long as the carapace, truncated anteriorly. The head is moderate in size, which is shorter than the diameter of the eye. The toes are fully webbed. The limbs can be gray, brown or blackish. The recognized subspecies are defined by differences in size and coloration. *Melanochelys trijuga trijuga* has a straight carapace length up to 25.0 cm and the head has pale yellow reticulations, especially on the temples. *Melanochelys trijuga coronate* has a carapace length up to 26.0 cm and a yellow head with a gray-black diamond-shaped mark on the forehead. The plastron is completely unpatterned and black in adults, which is not the same in juveniles. *Melanochelys trijuga edeniana*has a carapace length up to 28.0 cm and the head is uniform gray or brown, or with indistinct yellow reticulations that may be absent in older individuals. *Melanochelys trijuga indopeninsular* has a carapace length up to 34.2 cm, and the head is olive-brown with a long, spear-shaped mark on the forehead. In large individuals, the lighter plastral rim disappears. *Melanochelys trijuga parkeri* being the largest subspecies has a carapace length up to 38.3 cm, and the head is uniform olive-brown or finely spotted with orange. *Melanochelys trijuga thermalis* has a carapace length up to 22.9 cm and the head is gray-black, and spotted or blotched with red, orange or pink.

Indian flapshell turtle *(Lissemus punctata)* is restricted to the Indian sub region. The nominotypical subspecies occurs in southern peninsular India, in the states of Kerala and Tamil Nadu. The subspecies *Lissemus punctata andersoni* occurs in Pakistan, northern India and southern Nepal, Bangladesh in the east, Indus and Ganges-Bharmaputra drainage systems, and in north-western coastal Myanmar (Burma). The subspecies *Lissemus punctata vittata* occurs in peninsular India from Gujarat (west), Karnataka (south)and Andhra Pradesh in the east (Bhupathy *et al*., 2014).

Based on the studies of Ramakrishna *et al*. (2014) the presence of twenty nine species of tortoises and freshwater turtles and six species of marine turtles makes India one of the most diverse chelonian faunas in the world, and is considered to be one of the top five Asian countries in terms of its importance for turtle conservation because 40 per cent of its total chelonian fauna is threatened. There are seven marine turtle species in the world, but some consider there are a total of eight marine species including the Black turtle. The controversy on the taxonomy of the black turtle, which is considered as the eighth, is still not settled. Out of the reported seven sea turtle species, five are known to nest in the Indian coastal waters i.e, Olive Ridley's sea turtle (*Lepidochelys olivacea*), Hawksbill sea turtle (*Eretmochelys imbricata*), Green sea turtle (*Chelonia mydas*), Loggerhead sea turtle (*Caretta caretta*) and Leatherback sea turtle (*Dermochelys coriacea*) and the sixth sea turtle known to nest in Indian coastal water, is the Flat back sea turtle (*Natator depressus*).

Worldwide attention is naturally focused on the rookeries at Gahirmatha for conservation of Black turtle species. India's freshwater turtle's fauna was not known

clearly until a country wide survey was conducted during late 1980's. Occupancy of different species of freshwater turtles in various biogeographic zones and in different states of India has been reported. Turtle and tortoise diversity is highest in northeast region of India wherein 23 of 29 species are found in this region. Twenty three turtle species of three families from Northeast region of India, which include fifteen species of family Geoemydidae; six species of family Trionychidae and two species of family Testudinidae has been reported. The Ganges and Brahmaputra of North-eastern region of India have been identified as the areas where more than eleven turtle species are likely to co-occur. Few studies conducted on Indian fresh water turtles have mainly dealt with taxonomy and their broad distributional ranges. Indian star tortoise (*Geochelone elegans*) was reported to be the most common tortoise compared to other three species (*Indotestudo elongate, Indotestudo forestenii* and *Manouria emys*) in India (Ramakrishna *et al*., 2014).

2.3 Diseases/conditions affecting the shell in turtles

Disease/conditions in turtles that have been described are;

2.3.1 Retained scutes:

"Dysecdysis" is the term to describe the condition in which an old scute is retained and not shed properly. This condition is often associated with poor husbandry, and may occur if the turtle has not been able to dry off or bask sufficiently to lose its old scutes. Retained scutes often become infected as reported by Wilson *et al*. (2003).

2.3.2 Metabolic bone disease and pyramiding

Turtle with an inadequate calcium or Vitamin D intake, inadequate exposure to ultraviolet light, or disease of the liver, kidneys, or parathyroid glands may develop metabolic bone disease. This is the major cause for softening and malformation of bones. The shells of turtles with metabolic bone disease are often deformed, with the rear area of the carapace pulled downward, and the marginal scutes pulled upward. Tortoises with metabolic bone disease may develop pyramid-shaped scutes. Metabolic bone disease can be fatal. Husbandry and diet changes may be able to correct the calcium imbalance, but deformities are generally permanent as observed byMcArthur *et al.* (2004).

2.3.3 Pyramiding or pyramidal growth syndrome

This is a condition in which the scutes become conical shaped. This condition has been associated with feeding excessive protein, inadequate calcium, low fiber, and other dietary excesses or deficiencies. Renal failure in a turtle has been reported to cause a turtle to slough his scutes. Kidneys help to maintain the proper calcium and phosphorous levels in the blood. If the kidneys fail, the phosphorous level in the blood increases. The turtle's body attempts to compensate by moving calcium from the bones into the blood stream. The bones in the shell, then, can become soft as indicated by Wilson *et al.* (2003).

2.3.4 Ulcers (shell rot/scud)

Based on the studies of Wiles and Rand (1987), gross lesions were subcutaneous and creamy white areas and hemorrhagic ulcerations of the skin with tissue destruction, erythema and exudation. A mixed infection of *Acinetobacter calcoaceticus var Lwoffi*,

Acinetobacter-like, *Pasteurella*-llke, *Proteus*-like, and *Salmonella*-like strains, *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, was observed in these lesions.

Deep ulcers may need to be repaired through surgery and the application of acrylic or fiberglass material. In a disease called "septicemic cutaneous ulcerative disease" or SCUD, ulcers may be seen on both the shell and legs. This condition is often associated with the bacteria, *Citrobacter freundii* (Frye and Williams, 1995).

Ulcers of the shell may be superficial or deep, and may be termed "shell rot." Ulcers are generally a result of poor husbandry. Turtles with ulcerative shell lesions should be examined and treated by a veterinarian, as the ulcers may become infected and penetrate through the shell. The shell will need to be cleaned daily and dead tissue removed. Topical and/or injectable antibiotics are required in the case of bacterial infections. The shell of a turtle is an amazing adaptation which has allowed turtles to exist for millennia. Proper diet, husbandry, and care are vital to maintain a healthy shell as reported by McArthur *et al.* (2004).

2.4 Bacteria associated with shell disease in turtles

Samples taken from soft shell turtles having several fresh lesions and the healing lesion on shell were plated on agar and incubated at 37°C were overgrown with *Proteus* spp. and the sample incubated at 24°C produced pure cultures of gram positive *Bacillus* spp. based on biochemistry and morphology (Rosen, 1970 and Campbell, 1974).

Beneckea chitinovora, another Gram-negative bacteria, is implicated as the cause of chronic ulcerative shell disease in captive turtles especially soft shell turtles Trionyx

species. Prior injury to the shell is necessary for the bacteria to cause disease (Wallach, 1975).

The Gram-negative bacterium, *Baneckea chhitinovora,* causes a chronic ulcerative shell disease in captive turtles (Wallach, 1976).

Infectious shell and skin lesions in chelonians have been diagnosed with parasitic, bacterial, fungal and viral causes as reported by Rhodin and Anver (1977), Garner *et al.* (1997), Boyer (1996), Rossi (1996), Lackovich *et al.* (1999), Harkewicz (2001), Rose *et al.* (2001) and Oros *et al.* (2003). The causative organisms isolated were *Baneckea chhitinovora, Bacillus* spp., *Proteus* spp. and *Corynebacterium* spp.

Very little is known about the diseases of shell of wild turtles, although there are several accounts in the literature of the infectious shell disease in captive turtles. Shell rot due *Mucorales* spp, *Geotrichum* spp., *Trichosporon* spp., and *Coniothyrium* spp. has been reported by Austwick and Keymer (1981).

Bacteria appear to play a very important role in marine turtle diseases, both as primary pathogens and as secondary invaders when host's immune system has been compromised. Those species of bacteria that have been found in turtles are commonly isolated in localized infections but are also involved in epizootics characterized by bacteraemia and septicaemia (Cooper, 1983).

Among the organisms involved in the presentation of SCUD, the most common are Gram-negative bacteria *Aeromonas hydrophila*, *Citrobacter freundii* and *Serratia* spp. (Frye and Williams, 1995; Rossi, 1996; Jacobson, 2007).

Poor husbandry, including suboptimal environment temperature and malnutrition, can predispose reptiles to *Pseudomonas* spp., with frequent isolation from lesions associated with ulcerative stomatitis, pneumonia, dermatitis and septicemia (Boyer, 1996).

The most frequently cultured organisms were *Aeromonas* spp., *Vibrio alginolyticus, Pseudomonas* spp., *Proteus* spp. and *Citrobacter* spp.The same genus or suite of microorganisms may be cultured from more than one of these diseases. It is not understood why a single bacterial species, such as *Aeromonas hydrophila*, may cause a variety of different disease syndromes (Lutz, 1996).

In the study conducted by Garner *et al.* (1997) three hundred ten (76%) of four hundred ten turtles captured had evidence of shell lesions. Two adult male river Cooters and two adult male Yellow-Bellied turtles captured from Lake Blacksheare.

A slow growing photochromogenic mycobacterium was isolated from carapace and lungs of a captured turtle and grown in pure culture (Oros *et al.*, 2003)

A wide range of Gram-negative and Gram-positive bacteria, including *Bacillus* sp., *E. Coli.*, *Pasteurella* spp., *Proteus* spp., *Staphylococcus* spp. and *Vibrio alginolyticus*, were isolated from the lesions of marine turtles (Oros*et al.*, 2004).

Venezuela has reported a case of mortality in a neonatal turtle (*Podocnemis expansa*) held in captivity due to septicemia apparently associated with *Citrobacter amalonaticus* and *Klebsiella pneumoniae* , while in Brazil the reported case of septicemia

in the same species of turtle, has been associated with *Salmonella* serotype Carrau (Boede and Hernandez, 2004).

Septicemic cutaneous ulcerative disease (SCUD), is a chronic or secondary problem which is a common presentation in immunocompromised turtles, mostly associated with aquatic environments of poor quality and hygiene, environmental temperature below the proper range and the presence of wound untreated skin (McArthur, 2004a; Jacobson, 2007).

Gram-negative bacteria are the most common bacterial pathogens because they are the common isolates found in healthy reptiles. Most Gram-positive bacteria are not considered pathogenic in reptiles; they are actually common inhabitants, especially of the skin. However, some Gram-positive bacteria can cause disease. A report identified *Corynebacterium* spp. as a cause of abscess in Desert Tortoise (Mader, 2006).

Serratia spp. are part of normal flora of the oral cavity in reptiles. They are commonly isolated from cutaneous lesions and appear to be introduced by traumatic events, such as bite wounds. Cutaneous infection with *Serratia* spp. typically causes caseated abscesses that require surgical curettage and antibiotic therapy for resolution (Mader, *loc. cit*).

Culture of *E. Coli* from the faeces of reptiles is not unusual. These organisms are a normal component of bacterial flora of the reptiles (Paterson, 2005).

Approximately 90 per cent of all reptiles carry and shed *Salmonella* with their faeces into the environment, where they may readily survive and multiply. Aquatic

environment represents favourable condition for maintaining these bacteria. Ingestion of faeces or contaminated water is considered as a probable way of infection with *Salmonella*, since turtles feed mainly into water. Although the bacteria have already been isolated from healthy reptiles of immune deficient animals, with repeated cases of *Salmonella* can effectively cause diseases in reptiles (Sousa *et al*., 2011).

Skin lesions were the most prominent macroscopic findings in the turtles examined for skin lesions of different form and origin observed in seventy three (48.67 %) of the turtles examined. The most common lesions found on the skin were abscesses, dermatitis, regenerative changes, cutaneous ulcerative disease, keratin layer abruption, blepharoconjuctivitis and pododermatitis (Sanja *et al*., 2013).

As observed by Sanja *et al*. (*loc. cit*), microbiological examination of 115 turtles showed the presence of a variety of bacteria such as *Aeromonas hydrophila* (74%), *Citrobacter freundi* (41.33%), *Serraita* (65.33%), *Escherichia coli* (65.33%), *Proteus* spp. (76.67%) and *Pseudomonas chitinovora* (38.67%) which were either primary pathogens or potential polluters, which invaded the skin and shell. Bacterial infection was diagnosed in 76.66 per cent of the turtles.

2.4.1Isolation of aerobic bacteria from soil samples

Janssen *et al*. (2002) in their studies observed that the genetic diversity of soil bacteria is high and that soil contains many bacterial species.

Jasuja *et al*. (2013) reported that the common bacteria such as *Staphylococcus, E. Coli, Pseudomonas aeroginosa, Klebsiella pneumonia, Enterobacter aerogenes, Shigella*

spp., *Proteus mirabilis, Bacillus anthracis, Bacillus subtilis* and *Staphylococcus epidermidis* are the dominating species in the soil sample.

Nandhini and Josephine (2013) reported that the common bacterial isolates included *Bacillus, Actinomycetes, Staphylococcus, Streptcoccus, Pseudomonas, Microccous, Proteus* and *Mycobacterium* from soil samples.

2.4.2 Isolation of aerobic bacteria from water samples

The nutritional properties of *Acinetobacter* and their ubiquitous occurrence in soil and water suggest that these organisms may be very important agents in the aerobic mineralization of organic matter in nature. *Acinetobacter* overgrows *Pseudomonas* either because of its greater growth rate or because of its greater numbers in water and soil (Baumann, 1968).

Bacterial infections are common in captive sea turtles. Large populations of bacteria may accumulate in recirculating water systems or conduct water system or crowded open flow-through systems, provide a ready source of pathogens. Turtles confined together often traumatize each other by aggressive biting or damage their integument on tank/pond sides/barriers. Such dermal injuries allow the pathogenic bacteria to enter tissues and possibly the bloodstream often resulting in clinical diseases. Turtles kept in close proximity to healthy turtles can "seed" the water with bacteria therefore increasing the likelihood of spread of diseases. Any infectious agent, be it bacterial, viral, fungal or parasitic, can be easily transmitted in the closed water system. The most commonly cultured pathogens were *Aeromonas hydrophila, Vibrio alginolyticus, E. Coli, Citrobacter* spp., *Enterobacter* spp., *Proteus* spp., *Pseudomonas*

spp., *Salmonella* spp., *Mycobacterium* spp., *Edwardsiella* spp., *Arizona* spp., and *Flavobacterrium* spp. quite often, several species have been cultured from a single lesion (Lutz and Musick 1996).

Shell infections were most common consequences of unclean water; unclean areas are a potential source of infectious bacteria such as *Salmonella* (Boyer and Boyer, 2006).

High frequency of shell lesions and other clinical signs related to a harmful aquatic environment were found. Bacteriological results showed potentially pathogenic microorganisms in lesions due to contaminated water, including *Staphylococcus aureus, Klebsiella* spp., *E. Coli*, *Proteus vulgaris*, *Serratia* spp., and *Candida* spp. (Rangel-Mendoza *et al.*, 2013).

2.5 Effect of environment on occurrence of shell disease

Turtles require specific habitat characteristics to achieve their basic needs for reproduction and survival, both in the wild and in captivity. If these requirements are not available, these reptiles generally do not do well in captivity (McKeown, 1996).

Captive management demands knowledge of several aspects of the biology of species to ensure that artificial conditions reach their biological requirements. Some basic aspects, such as housing, water quality, temperature, sunning area, diet and care of hatchlings should be considered for captive maintenance of aquatic turtles (Boyer and Boyer, 2006).

The environment of captivity has a great impact on the bacterial levels in water, which should be considered for the management of turtles as well as other aquatic animals. Water quality is critical for aquatic animal health and welfare, particularly for the captive animals. However in captivity, inadequate care is more likely to result in bacterial infections/mortality than old age. The inadequate environmental parameters can cause skin/shell lesions in turtles (Chuen-Im *et al.*, 2010).

2.5.1 Water quality

One of the most important husbandry requirements for captive aquatic reptiles is the water quality. Under-gravel filtration systems and power filters to circulate the water through the filter, must be of the highest quality and perform equal to those used for tropical fish. Even when using the best equipment in the tank, it is difficult at best to maintain good water quality. It is recommended that water be changed a minimum of once weekly to prevent the build-up of "dirty water," which can change the pH and nitrogen content in the water. Substrates such as gravel can be used; however, turtles are prone to dig up the gravel and therefore create more stress for the filtration system by adding sediment (Ramakrishna *et al.*, 2014).

Ideally turtles tank water should be kept at a pH ranging from 7.0 to 8.4which is considered neutral to slightly alkaline (Vella, 2013). Park (2007) reported that the permitted level for total viable count of portable water should be less than 100 cfu/ml of water.

2.5.2 Water temperature

Reptiles are ectothermic by nature- that is they rely on the environment to provide sufficient heat to warm them to their preferred body temperature (PBT). To do this the reptile must be provided with a preferred optimum temperature zone (POTZ) within which it may position itself in order to maintain its preferred body temperature (PBT). This necessitates the provision of some form of artificial heating within the tank, or vivarium, as it is more commonly known turtles should be maintained at temperature between 20-35°C and humidity between 50-70 per cent as reported by Girling (2003).

2.5.3 Haul out area

Aquatic reptiles need a haul out area, in other words, a place to get out of the water. These areas can be made of rocks or other non-organic materials and should be placed under a basking light. Basking lights are usually incandescent spot lights focused on the basking platform. Timers should be used to maintain a basking time of at least twelve hours. By increasing or decreasing the distance from the spotlight to the basking platform or by changing the wattage of the spotlight bulb, one can change the temperature of the basking area. The basking site should be at least 10 to 15 degrees warmer than the water temperature to provide a thermal gradient for the turtle (Madar, 2006).

2.5.4 Ultraviolet lights

Full-spectrum ultraviolet bulbs should be used to provide the proper Ultra voilet light for calcium metabolism. These bulbs should be suspended no greater than two feet above the aquarium and be put on an automatic timer to provide at least twelve hours of

exposure. Those animals kept outside in larger ponds will get natural exposure from filtered sunlight (Mader and Divers, 2014).

2.6 Antibacterial sensitivity

Although some earlier generation cephalosporins, such as cefalexin and cefazolin, have a place as general broad-spectrum antibiotics, effective against anaerobes, *Pseudomonas* are often resistant. Cephalosporins are generally safe, even in dehydrated patients. Later generation drugs, especially the third generations ceftazidime, have reduced activity against Gram-positive organisms but a greatly enhanced effectiveness against Gram-negatives, particularly *Pseudomonas* (Lawrence *et. al.*, 1984).

Liptak (1997) opined that important considerations in the selection of a topical drugs include the antimicrobial spectrum, dose, pharmacokinetics, tissue and systemic toxicity timing, route of administration and type of preparation (lavage, ointment, cream, or powder).Topical and systemic antibiotics have less benefit if infection has become established as the presence of wound coagulum prevents antibiotics from reaching effective levels in deep tissues and systemic antibiotics from reaching superficial bacteria.

A successful topical regime consisting of equal volumes of nystatin, oxytetracycline is beneficial. The animals left out of water for an hour after application of tropical treatment worked really well on the shell infection (Ladyman *et. al.*, 1998).

Opportunistic bacterial pathogens such as *Pseudomonas*, *Proteus* and *Klebsiella*, *Morganella* are the common reptile pathogens which are often resistant to antibiotics.

Antibiotics such as fluroquinolones, ceftazidine, amikacin, ticarcillin, gentamicin and amikacin have been widely used in reptile medicine because of their broad spectrum of activity, especially against Gram-negative organisms such as *Pseudomonas* (Barrows *et al.*, 2004).

Fluroquinolones such as enrofloxacin, ciprofloxacin and ofloxacin have a broad spectrum of activity but are particularly effective against Gram-negative organisms such as *Pseudomonas, Proteus* and *Staphylococcus* spp., which are frequently isolated from reptiles. Fluroquinolones also show good activity against many beta-lactamase producing bacteria, but poorly effective against anaerobes (Davies and Klingenberg, 2004)

Isolation and identification of *E.coli* was done and subjected to antimicrobial susceptibility testing by disc diffusion method using antibiotic discs such as neomycin, gentamicin, streptomycin, chloramphenicol, ofloxacin, trimethoprim-sulfamethoxazole, tetracycline, amphicillin, nalidixic acid, nitrofurantoin, cephalothin, and sulfisoxazole (Sayah*et al.*, 2004).

Carpenter (2005) treated turtle suffering from erythema and multifocal plastron and skin ulceration with enrofloxacin effectively.

Materials and Methods

III. MATERIALS AND METHODS

3.1 Materials

The present study was taken with the objective to identify the shell diseases in captive turtles housed at Sri Chamarajendra Zoological Gardens (CZG), Mysuru, to study the epidemiological aspects of shell diseases in captive turtles and the bacterial load of water in the tank and isolation and identification of aerobic bacteria/fungi from the water samples collected from the enclosure. A study on antibacterial sensitivity of aerobic bacterial isolates associated with shell diseases in captive turtles at CZG, Mysuru, was also conducted. The materials and methods adopted in this present study are presented in this chapter.

3.1.1 Glass-ware

The glass wares used in this study were of neutral glass of Corning, Borosil India Ltd., Mumbai.

3.1.2 Chemicals

The buffers and reagents were procured from M/s. **Hi-Media**, Mumbai.

3.1.3 Equipments

Following equipments were used for this study,

a. **Laminar flow** from Thermo Fisher Scientific Co., Marietta, OH, USA

b. **Incubator** from Lawrence and Mayo Co., Kolkata, West Bengal, India

c. **Autoclave** from National Steel Equipment Co., Mumbai, India

d. **Double distillation unit** from Borosil India Pvt Ltd., Mumbai, India

e. **Vortex mixer** from Vertex Genie Scientific Industries Inc., NY, USA

f. **Digital weighing balance** from Shimdzu Corporation, Japan

g. **Vernier calliper** procured from SBS Scientific, Bengaluru

h. **Measuring tape** procured from SBS Scientific, Bengaluru

i. **Electro-India weighing machine** from Electro-India Pvt Ltd, Madhavnagar, Kolhapur

j. **Temperature and Humidity loggers** procured from SBS Scientifics, Bengaluru

k. **pH test strip** procured from SBS Scientifics, Bengaluru

3.1.4 Culture media

Following culture media and biochemical reagents were used in this study. Their detailed compositions are given in Appendix I.

Brain Heart Infusion (BHI) broth

Peptone buffered water

Nutrient agar

Brain Heart Infusion (BHI) agar

MacConkey's agar

Mannitol salt agar

Eosin Methylene Blue (EMB) agar

Mueller Hinton agar

Xylose Lysine Desoxycholate (XLD) agar

Agar agar, Type I

Kovac's indole reagent

Ferric chloride

Methyl red

Hydrogen peroxide (5%)

Oxidase discs

3.1.5 Plastic-ware

Plastic-ware including micro centrifuge tubes, micropipette, micropipette tips and autoclave bags were procured from M/s. Tarson Products Pvt. Ltd., Kolkata. Sterile cotton swabs were procured from M/s. Hi-Media, Mumbai, and were used in this study.

3.1.6 Gram stain

Gram's staining kit, which contained crystal violet, Gram's iodine, decolourizer and safranin was procured from M/s. Hi-Media, Mumbai.

3.1.7 Antimicrobial discs

Antimicrobial discs procured from M/s. Hi-Media, Mumbai, and were used in this study.

List of antimicrobial discs used in the study (Table No. 11 and 12)

Sl. No.	Antimicrobial discs	Code	Concentration
1	Amikacin	AK	30mcg per disc
2	Cefuroxime	CXM	30 mcg per disc
3	Ceftriaxone	CTR	30 mcg per disc
4	Ceftriaxone + Sulbactum	CIS	30+15 mcg per disc
5	Chloramphenicol	C	30 mcg per disc
6	Penicillin-G	P	10 units
7	Enrofloxacin	E	10 mcg per disc
8	Tetracycline	T	30 mcg per disc
9	Kanamycin	K	30 mcg per disc
10	Neomycin	N	30 mcg per disc

3.1.8 Study animals

A total of 52 captive turtles maintained at Sri Chamarajendra Zoological Gardens (CZG), Mysuru, were included in this study.

Sl. No.	Location/particulars	No. of turtles
1.	Sri Chamrajendra Zoological Gardens (CZG), Mysuru	52
2.	No. of infected animals	19
3.	No. of enclosures	2
4.	No. of water samples collected from the enclosure for the period of four months	8
5.	No. of soil samples collected from the enclosure for the period of four months	8

3.1.9 Weight and body measurements of the study animals (Appendix XIII)

The weight and body measurements of fifty two animals were taken using digital weighing machine (kg) and vernier calliper/measuring tape was used to take the body measurements. Straight carapace length (SCL) , Curved carapace length (CCL), Straight carapace width (SCW), Curved carapace width (CCW), Straight plastron length (SPL), Curved plastron length (CPL), Straight plastron width (SPW), Curved plastron width (CPW), and Circumference (CIRUM) were the measurements taken to identify different types of turtles.

3.2 Methods

3.2.1 Preparation of glass-ware

The glasswares used in the study were prepared by soaking them in detergent (Teepol) solution overnight. The following day, they were washed thoroughly in running tap water and rinsed in distilled water. The oven dried glass wares were packed and sterilized in hot air oven for one hr at 160^{o} C.

3.2.2 Preparation of plastic-ware

The new plastic wares including sample containers and micropipette were placed in an autoclave bag and sterilized by autoclaving at 121^{o} C for 15 minutes at 15lb/sq.in pressure.

3.2.3 Preparation of growth media (Appendix I-XII)

The media used in the study were prepared using double glass distilled water and prepared as per the instructions recommended by manufacturer. The culture media for biochemical tests were prepared according to Collee *et al.* (1996).

i) Mac Conkey's agar (MCA)

MCA	5.1g
Distilled water	100 ml

ii) Muller Hinton agar (MHA)

MHA	7.6 g
Distilled water	100 ml

iii) Xylose Lysine Deoxycholate agar (XLD)

XLD agar	5.6 g
Distilled water	100 ml

iv) Mannitol Salt agar(MSA)

MSA agar	11.10 g
Distilled water	100 ml

v) Eosin Methylene Blue agar (EMB)

EMB agar	3.59 g
Distilled water	100 ml

vi) Brain Heart Infusion agar (BHI)

BHI agar	5.2 g
Distilled water	100ml

vii) Nutrient agar (NA)

NA	2.8 g
Distilled water	100ml

The media in Section 3.2.3 was reconstituted separately and autoclaved at 121°C at 15 lb for 15 minutes. When media had cooled to 45-50°C, it was poured aseptically into sterilized Petri plates and allowed to solidify. The plates were stored at 4°C, after sterility check-up at 37°C for 24 hours, until further use.

viii) Buffer Peptone water

Buffer Peptone	2.07 g
Distilled water	100 ml

ix) Brain Heart Infusion broth

BHI	9.7 gram
Distilled water	100 ml

All the broth in Section 3.2.3 was reconstituted separately in double distilled water and autoclaved at 121°C at 15 lbs for 15 minutes. On cooling, ten ml aliquots were prepared, sterility check-up was done at 37°C for 24 hrs and were stored at 4°C until further use.

x) Nutrient-glycerol broth

Nutrient broth	85 ml (sterile)
Sterile glycerol	15 ml

Glycerol was sterilized in hot air oven at 160°C for one hour and added into sterilized nutrient broth, mixed well and aliquot into one ml sterile tubes before storing at 4 °C.

xi) Nutrient agar slants (NA)

Nutrient agar	2.8 g
Distilled water	100 ml

The medium was reconstituted in double glass distilled water and autoclaved at 121°C at 15 lb for 15 minutes. When media had cooled to 40-50°C, it was poured aseptically into sterilized test tubes and placed in a slant position to obtain butt. After solidifying, tubes were kept for sterility check-up at 37°C for 24 hours and were stored at 4°C until further use.

3.2.4 Preparation of biochemical test media and reagents

i) Christensen's Urea agar base

Christensen's Urea agar	2.40 g
Distilled water	95 ml
Urea (40%)	5 ml

The reconstituted medium was autoclaved at 121°C at 15 lbs for 15 min. When media had cooled, it was poured aseptically into sterilized test tubes and placed in a slant

position to obtain one centimetre butt when cooled to 40-50° C. The slants were kept at 4°C after sterility check-up at 37°C for 24 hours until further use.

ii) Nitrate Reduction (NR)

Peptone	1 g
Potassium nitrate	0.02 g
Distilled water	100 ml

iii) Simmons Citrate agar

Simmons Citrate agar	2.40 g
Distilled water	100 ml

iv) Triple Sugar Iron agar (TSI)

TSI agar	6.4 g
Distilled water	100 ml

v) MR-VP broth

MR-VP	1.7 g
Distilled water	100 ml

All the biochemical media were reconstituted separately and autoclaved at 121°C at 15 lbs for 15 min. When media had cooled, it was poured aseptically into sterilized test tubes and placed in slant position to obtain one centimetre butt when cooled to 40-50° C. The slants were kept at 4°C after sterility check-up at 37°C for 24 hours until further use.

3.2.5 Gram's staining

Gram's staining kit, which contained crystal violet, Gram's iodine, decolourizer and safranin was procured from M/s. Hi-Media, Mumbai. The staining of all the isolates was carried out as per the kit protocol.

3.2.6 Restraint of the animal

Animals were restrained for examination using physical restraint methods. The turtles were made to lie on a clean surface and were eventually examined for lesions on the dorsal, ventral and lateral sides of the shell. Gender identification was done on the basis of tail length, cloacal opening and concave or convex plastron.

3.2.7 Collection of samples for Isolation of aerobic bacteria

A total of 52 captive turtles belonging to Sri Chamarajendra Zoological Garden (CZG), Mysore, were examined for the presence of shell (carapace and plastron) lesions and samples were collected using sterile cotton swabs and subjected for examination. Water (100 ml) and substrate (10 g) samples from two enclosures at Sri Chamarajendra Zoological Garden (CZG), Mysuru, were collected in sterile sample containers and transported to the laboratory for bacteriological investigation and water samples were also accounted for total water bacterial count.

The collected swabs were inoculated into BHI broth and incubated at 37° C for 12 hrs. A loop full of broth cultures was streaked on to different enriched and selective media and incubated at 37°C for 24 hrs. Different isolated colonies were picked up and

streaked on to BHI agar slants to get pure cultures for further identification as per Collee *et al.* (1996).

To analyse the bacterial load (Total viable count) of water from each enclosure, the water samples were collected 10 cm depth from the surface using a 25ml sterile container and kept under cold condition until examination. Enumeration of aerobic bacterial load in water samples was carried out using spread plate technique. The water sample was subjected to a series of 10-fold dilutions and the diluted sample was poured on the nutrient agar. All plates were incubated at 25°C for 48 hours and estimation total viable count of bacteria per ml of water was according to the colony-forming unit (cfu) method (Park, 2007).

Confirmation of bacterial identification was performed on randomly selected bacterial taxonomy procedures according to the 8^{th} edition of Bergey's Manual of Determinative Bacteriology (2009).

Isolation of organisms from soil were done by serial dilution technique on BHI agar plates. In this technique, a sample suspension was prepared by adding 1.0 g sample to 10 ml distilled water and mixed well for 15 min and vortexed. Each suspension was serially diluted 10^{-1} to 10^{-6}. 0.1 ml was pipette onto plates with BHI media, spread with the glass spreader and incubated at 37°C for bacterial observation (Waksman, 1927).

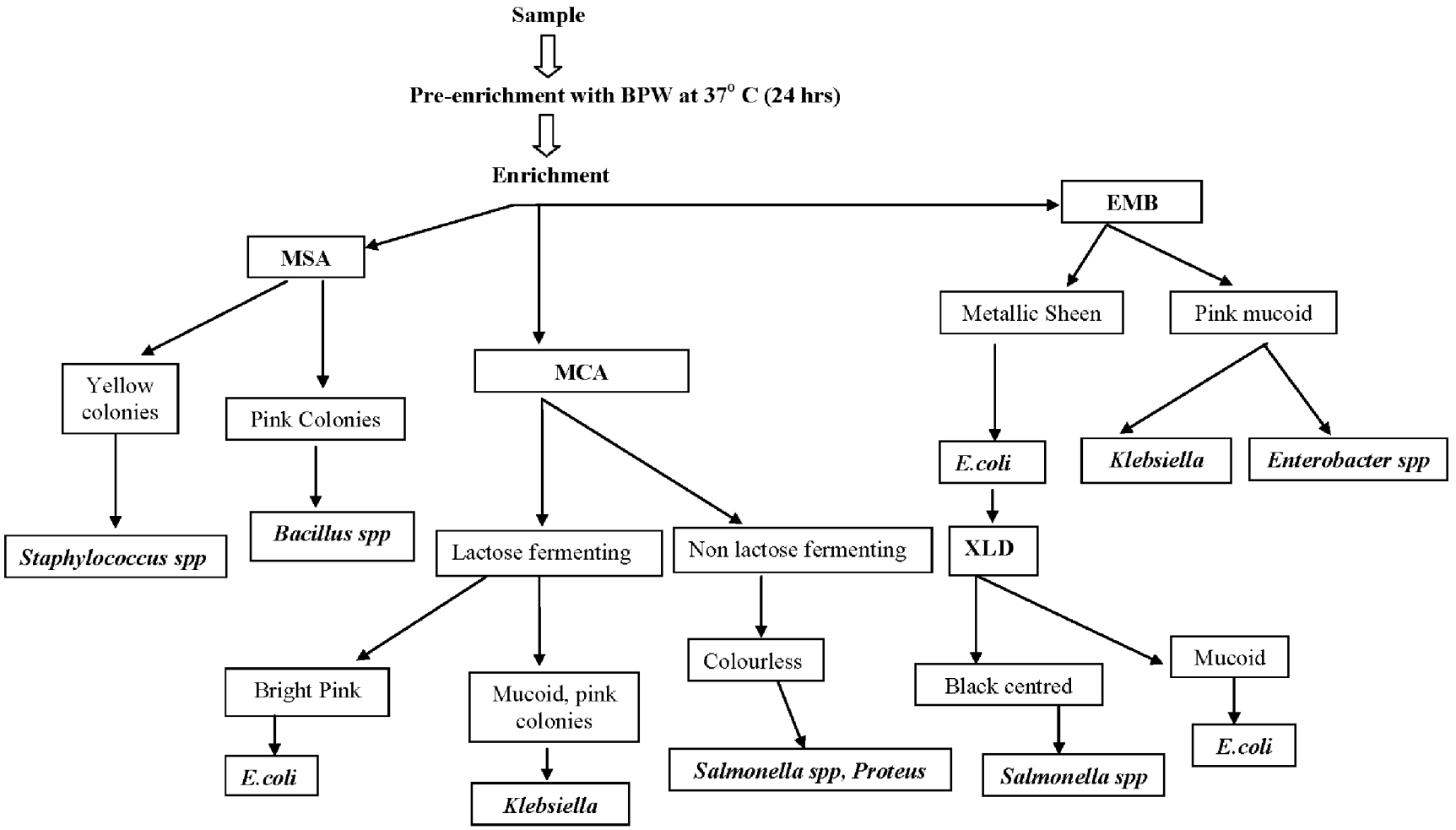
3.2.8 Identification of bacteria isolates

Preliminary characters of the pure cultures were identified by Gram staining, growth on selective media and biochemical tests. The isolates were further identified as per the Bergey's Manual of systemic bacteriology, 8th*Edn.* (2009).

The flow chart suggested by Subramanian *et al.* (2010) was employed for characterization of isolates.

- The collected swabs were inoculated into BHI broth and incubated at 37^{o}C for 24 hours.
- A loopful of culture was streaked on to different enriched and selective media like MSA plates, MCA plates, EMB agar plates and XLD agar plates and incubated at 37^{o}C for 24 hours.
- On plate reading suggested colonies were again streaked onto MSA, MCA, EMB and XLD agar plates to obtain presumptive colonies.
- Different pure cultures were picked up and streaked on to BHI agar slants for further identification procedures.

Schematic representation of isolation of aerobic bacterial isolates from clinical samples



(Subramanian *et al*. 2010)

3.3 Gram staining of the pure isolated cultures

1. A thin culture smear was prepared on a clean glass slide and air dried and heat fixed by passing over the flame.
2. Crystal violet solution was applied over the smear and allowed for two minutes.
3. Slide was washed with tap water.
4. Gram's iodine was applied and allowed for one minute.
5. The slide was washed and flooded with absolute alcohol, followed by washing the slide with running tap water.
6. Safranin was applied and allowed for 30 seconds
7. Slide was washed, air dried and observed under microscope.

3.3.1 Biochemical tests

The main objective of the biochemical tests was to determine the nutritional and metabolic capabilities of a bacterial isolates to identify bacteria. Methods described by Collee *et al.* (1996) were employed. Pure cultures were subjected for following biochemical tests:

a) **Indole test:** Development of red or pink colour in the alcohol layer after addition of Kovac's reagent to the 48 hrs old Tryptone broth with culture was considered positive for indole production.

b) **Methyl Red (MR) test:** The methyl red test was performed to find out the ability of the organism to utilize glucose with the production of a stable acid as the end product.

Development of red color on adding of 3-4 drops of alcoholic MR reagent to the 24-48 hrs old MR-VP broth medium with culture was considered positive for MR test.

c) **Voges - Proskauer (VP) test:** The VP test was conducted to detect ability of the bacteria to utilize glucose with the production of acetyl methyl carbinol (acetoin) as neutral end product. Development of rose pink color on addition of 0.2 ml of VP solution I (α-Naphthol), followed by addition of 0.6 ml of VP solution II (40% KOH) to 24-48 hrs incubated MR-VP broth medium inoculated culture was considered positive.

d) **Citrate utilization test:** The pure culture was streaked on to the Simmons citrate slants and incubated for 24 hours at 37°C. Changing of colour from green to blue indicated positivity and no change in colour was declared negative when incubated for 96 hours.

e) **Urease test:** The urease production test was performed to find out whether the organisms possesed urease activity. The cultures were transferred to Christensen's urea agar slants with 40% urea. After incubation at 37°C, the slants were examined at 6 and 12 hrs and thereafter every 24 hrs up to four days. The cultures showing alkaline reaction (pink) indicated urease activity and were discarded. The cultures showing unchanged position of the slant at the end of 96 hours incubation were declared negative for urease activity.

f) **Catalase test:** Effervescence of gas bubbles after addition of 24 hrs culture to three per cent hydrogen peroxide indicated positive reaction. Absence of effervescence of gas bubbles indicated negative reaction.

g) **Oxidase test (disc method):** Oxidase discs procured from M/s. Hi-media, Mumbai were used for this test. A loopful of pure culture was streaked on to the oxidase discs moistened with distilled water and observed for a change in colour from white to bluish purple. Culture which did not change colour was considered as oxidase negative.

3.3.2 Preservation of pure culture

Pure cultures obtained were sub cultured onto nutrient agar slants. These pure culture slants were preserved at 4°C for further identification of the organisms. A loopful of the isolated organism was added to the sterile nutrient glycerol broth vials and mixed well using vortex mixture. The vials were then labelled and stored at -20°C.

3.3.3 Antibacterial sensitivity test

Sterile cotton swabs were used to collect sample from lesions (turtle shell) and were subjected for antibacterial sensitivity as per Kirby - Bauer method of antibiotic sensitivity test (Bauer *et al.*, 1966).

All the samples to be tested were incubated in one ml BHI broth for 6-12 hrs at 37°C. After incubation, 0.5 ml of the bacterial suspension was taken in sterile serological tubes separately. The growth of all the cultures was adjusted to McFarland 0.5 turbidity standard using sterile peptone buffered saline. Then the bacterial suspension was placed

on Mueller Hinton agar, with the help of a sterile cotton swab. The antibiotic test discs were laid on the dried inoculums with the help of a dispenser.

The plates were incubated at 37°C for 24 hrs. The zone of inhibition was measured using antibiotic clearance zone measuring scale and expressed in millimetre. Based on diameter of zone of inhibition, antibiotics were classified as sensitive and resistant using interpretative chart provided by M/s. Hi-Media, Mumbai.

3.4 Statistical analysis

The data generated was analysed by using computer based statistical programme, Graph Pad Prism version 5.0 to arrive at the final conclusion.

Results

IV. RESULTS

The study was conducted for a period of four months at Sri Chamarajendra Zoological Garden, Mysuru, and Veterinary College, Bengaluru. The total numbers of animals were fifty two turtles housed in two enclosures. The Enclosure - I housed Red eared slider and Indian black turtle whereas the Enclosure – II housed Indian flapshell turtles. Out of the total population, thirty one were male turtles and twenty one were female turtles. A total of nineteen turtles had one or more shell problems. The environmental parameters (pH, temperature and humidity) were recorded for a period of four months.

The managemental practices recorded during the study period (4 months) included water, soil and feeding pattern in both the turtle enclosures. Soil was used as the substrate material for both the enclosures and the soil used as bedding material in enclosures was never changed or replaced during the period of this study. Both enclosures had a water body where the animals spent most of their time soaking. The water in the enclosure was changed on daily basis. Chicken was fed once in 24 hours on a regular basis.

The results are presented in Tables 1-12, Figures 1-6 and Plates 1-12.

4.1 Occurrence of shell diseases in captive turtles at Sri Chamarajendra Zoological Gardens, Mysuru

A total of fifty two captive turtles were examined in the present study and it was noticed that a total of nineteen had one or more shell problems which indicated that occurrence of shell problems in captive turtles was found to be 36.54 per cent.

4.1.1 Species-wise occurrence of shell diseases in captive turtles at Sri Chamarajendra Zoological Gardens, Mysuru

Out of the total 52 turtles housed at Sri Chamarajendra Zoological Gardens, Mysuru, the occurrence of shell disease was observed higher in Indian flapshell turtle (63.64%) followed by Red-eared slider turtle (50%) and Indian black turtle (25.71%) (Table 2 and Figure 1).

4.1.2 Gender-wise occurrence of shell diseases in captive turtles of Sri Chamarajendra Zoological Gardens, Mysuru

Out of nineteen turtles with shell diseases, ten (52.63%) were male turtles and nine (47.37%) were female turtles. This indicated that shell problems in captive turtles were comparatively more in males than female turtles (Table 3 and Figure 2).

4.2 Shell diseases encountered in captive turtles of Sri Chamarajendra Zoological Gardens, Mysuru

Out of nineteen affected turtles two turtles had shell deformities, three turtles had shell cracks and fourteen had shell lesions due to infection (Table 4 and Figure 3).

4.3 Isolation and identification of aerobic bacterial isolates from shell lesions in captive turtles of Sri Chamarajendra Zoological Gardens, Mysuru

Out of samples collected from the shell lesions of turtles, sixteen pure bacterial isolates were isolated, the Gram positive and Gram negative organisms constituted seven (43.75%) and nine (56.25%) isolates respectively. Out of these isolates, *Staphylococcus* spp. and *Salmonella* spp. were the most common (25%), followed by *Bacillus* spp., *Pseudomonas* spp. and *E. Coli* (12.5% each), *Proteus* spp. and *Corynebacterium* spp. (6.25%).

In addition to culture and colony characteristics, biochemical tests and staining morphology were used to confirm the identity of all the isolates (Table 6,7,8 and Figure 4).

4.3.1 Isolation and identification of aerobic bacterial isolates from soil sample from turtle enclosure in Sri Chamarajendra Zoological Gardens, Mysuru

From the soil samples collected from Enclosure I and II, 7 organisms were isolated. Gram positive and Gram negative isolates constituted five (71.42%) and two (28.57%) respectively, out of two Gram positive isolates. *Bacillus* spp. was most commonly isolated (42.85%) followed by *Staphylococcus* spp. (28.57%). Among Gram negative isolates, *E. Coli* (14.28%) and *Proteus* spp. (14.28%) were seen.

In addition to culture and colony characteristics, biochemical tests and staining morphology were used to confirm the identity of all the isolates (Table 6,7,10 and Figure 6)

4.3.2 Isolation and identification of aerobic bacterial isolates from water sample of turtle enclosures in Sri Chamarajendra Zoological Gardens, Mysuru

From water samples collected from Enclosure I and II, 9 organisms were isolated. Gram positive and Gram negative isolates constituted two (22.22%) and seven (77.77%) respectively. Out of the two Gram positive isolates, *Staphylococcus* spp. was only isolated (22.22%). Among Gram negative isolates, *E. Coli* was more common (44.44%), followed by *Salmonella* spp. (22.22%) and *Pseudomonas* spp. (11.11%).

In addition to culture and colony characteristics, biochemical tests and staining morphology were used to confirm the identity of all the isolates (Table 6, 7, 9 and Figure 5).

4.4Identification of bacterial isolates (Table 6 and Table 7)

4.4.1 Identification of *Staphylococcus* spp.

4.4.1.1 Colony morphology

On incubation for 24 hours at 37^{o}C, the colonies on MSA appeared yellow/white colonies surrounded by yellow zone with luxuriant growth and moderately sized white or golden colonies.

4.4.1.2 Staining and morphology

Gram staining of suspected colonies from 24 hour old broth culture revealed Gram positive cocci in clusters resembling bunches of grapes.

4.4.1.3 Biochemical characterization of *Staphylococcus* spp.

All the isolates suggestive of *Staphylococcus* were positive for Catalase test and reduced nitrates to nitrites, where as they were negative for Oxidase test. All the isolates were positive for MR reaction and variable for VP reaction and negative for Indole production, Urease activity, and motility. Based on the biochemical characterization, twenty seven out of one hundred and eight isolates were identified as *Staphylococcus* spp. (Plate 13).

4.4.2Identification of *Bacillus* spp.

4.4.2.1Colony morphology

On incubation for 24 hours at 37°C, the colonies on BHI agar plates were up to 5 mm in diameter, flat dry with greenish tinge, with characteristic hair like outgrowths were seen.

4.4.2.2 Staining and morphology

Gram staining of suspected colonies from 24 hour old broth culture revealed Gram positive rods occurring in single and in short chains. The ends of the cells were rounded.

4.4.2.3Biochemical characterization of *Bacillus* spp.

All the isolates suggestive of *Bacillus* were positive for Catalase test and reduced Nitrates to Nitrites, where as they were negative for Oxidase test. All the isolates were positive for motility and variable for VP reaction and negative for Indole production,

MR, and Urease activity. Based on the biochemical characterization twenty six out of one hundred eight isolates were identified as *Bacillus* spp.

4.4.3 Identification of *Corynebacterium* spp.

4.4.3.1 Colony morphology

The colonies did not grow on Mac Conkey agar. On BHI agar they appeared as small grey/black shiny surface with smooth surfaces. On blood agar, colonies appeared as small gray, dull opaque colonies which are non-hemolytic.

4.4.3.2 Staining and morphology

Gram staining of suspected colonies from 24 hr old broth culture revealed Gram positive rod shaped bacteria that were straight or slightly curved. Metachromatic granules were present, the stored phosphate regions. The bacteria grouped together in a characteristic way which has been described as the form of a "V" palisades or "Chinese letters".

4.4.3.3 Biochemical characterization of *Corynebacte*rium spp.

All the isolates suggestive of *Corynebacterium* spp. were positive for catalase test and reduced nitrates to nitrites where as they were negative for oxidase test.

4.4.4 Identification of *E. Coli*

4.4.4.1 Colony morphology

The colonies on Mac Conkey agar plates appeared as small, circular, convex, and pink in colour indicating lactose fermentation. On EMB agar, after 24 hours of incubation

the colonies appeared as large, blue black colonies with characteristic appearance of metallic sheen which is unique identification for *E. Coli* (Plate 10).

4.4.4.2 Staining and morphology

Gram staining of suspected colonies from 24 hour old broth cultures revealed Gram negative rods arranged in single.

4.4.4.3Biochemical characterization of *E. Coli*

All the isolates were positive for Indole reaction, MR reaction and for motility. They were found negative for VP reaction, Urease activity and Citrate utilization. In carbohydrate fermentation, they fermented the sugars and turned the slant and butt into yellow colour indicating acidic pH with production of gas in TSI (Triple sugar iron) agar test.

4.4.5 Identification of *Proteus* spp.

4.4.5.1Colony morphology

The colonies on MacConkey agar appeared glistening, colourless to grey colonies indicating non-lactose fermenters, some species showed the swarming growth. On Xylose lysine deoxycholate (XLD) agar, the colonies appeared yellow to grey colour with variable reaction to H_2S production. On Eosin methylene blue (EMB) agar, colonies appeared pink colour with no fermentation of lactose or acid production.

4.4.5.2 Staining and morphology

Gram staining of suspected colonies from 24 hour old broth culture revealed Gram negative rods/bacilli arranged in singles or short chains.

4.4.5.3 Biochemical characterization of *Proteus* spp.

All the isolates suggestive of *Proteus* spp. were Catalase positive and reduced the Nitrates to Nitrites, where as they were negative for Oxidase test. All the isolates were positive for Indole, MR reaction, Urease activity and motility. They were negative for VP and variable for Citrate utilization and H_2S production.

4.4.6 Identification of *Pseudomonas* spp.

4.4.6.1 Colony morphology

On incubation for 24 hours at 37°C, the colonies on nutrient agar plates were surrounded by bluish green coloration. On MCA agar after 24 hours of incubation the colonies appeared as irregular pale yellow colonies indicating non-lactose fermenters with a characteristic fruity, grape like odour.

4.4.6.2 Staining and morphology

Gram staining of suspected colonies from 24 hours old broth cultures revealed Gram negative medium sized rods.

4.4.6.3 Biochemical Characterization of *Pseudomonas* spp.

All the isolates suggestive of *Pseudomonas* were positive for Oxidase, Catalase activity and they reduced Nitrate to Nitrite and they were Negative for Urease activity.

All the isolates were positive for Citrate utilization and negative for Indole production, MR reaction and VP reaction.

4.4.7 Identification of *Salmonella* spp.

4.4.7.1 Colony morphology

The colonies on MacConkey agar appeared smooth, circular, translucent, glistening, convex and colourless indicating non-lactose fermenters. On XLD agar, the colonies appeared pinkish to red in colour with change in colour of medium to pink. Two samples showed black centered colonies indicating H_2S production. On EMB agar colonies appeared grey mucoid with no fermentation of lactose or acid production.

4.4.7.2 Staining and morphology

Gram staining of suspected colonies from 24 hr old broth culture revealed Gram negative slender rods arranged in single or in short chains.

4.4.7.3 Biochemical characterization of *Salmonella* spp.

All the isolates suggestive of *Salmonella* were positive for catalase test and reduced nitrates to nitrites where as they were negative for oxidase test. Two isolates were positive for MR, citrate utilization and motility test and negative for VP, indole and urease activity. In carbohydrate fermentation they were non-lactose fermenters and turned the slant alkaline by changing to red color and butt acidic by showing yellow color with production of gas and variable with production of H_2S.

4.5 Bacterial load (Total viable count) of water samples collected from water bodies of turtle enclosures at Sri Chamarajendra Zoological Gardens, Mysuru

Total viable counts from the water sample of Enclosure -I was found to be 530 cfu/ml and that of Enclosure -II water sample was 410 cfu/ml.

4.6 Observation on environmental parameters (pH, temperature and humidity) from Enclosures –I and II at Sri Chamarajendra Zoological Gardens, Mysuru

The pH of the water bodies from both the enclosures showed 7 which was under the recommended pH for turtles.

The maximum and minimum temperature recorded in the present study of Enclosure -I at Sri Chamarajendra Zoological Gardens, Mysuru, was 35.67±1.39 and 20.94±0.89, whereas the recorded value in Enclosure –II at Sri Chamarajendra Zoological Gardens, Mysuru, was 34.81±1.057 and 22.21±0.844 (Table 5).

The maximum and minimum humidity recorded in the present study of Enclosure –I at Sri Chamarajendra Zoological Gardens, Mysuru, was 79.15±1.96 and 28.43±2.92, whereas the recorded value in Enclosure -II at Sri Chamarajendra Zoological Gardens, Mysuru, was 72.39±7.28 and 28.28±3.32 (Table 5).

4.7 Antibacterial sensitivity pattern of isolated bacteria

The results of the present study conducted on samples collected from the shell lesions of captive turtles indicated that the mixed bacteria colonies were sensitive to amikacin and choramphenicol (92.85% each) followed by kanamycin and neomycin (71.42%) and cefuroxime (50%) whereas, resistant to teracycline and erythomycin

(35.71%) followed by ceftriaxone+sulbactum (28.57%), ceftriaxone (21.45%) and Penicillin-G (14.28%) (Table 11).

In the present study, amikacin, cefuroxime, chloramphenicol, ceftriaxone, erythromycin, kanamycin, penicillin-G, tetracycline, neomycin, and ceftriaxone+sulbactum antibiotics were used for assessing the sensitivity pattern of the isolated bacteria namely *Staphylococci, Bacillus, Corynebacterium, E. Coli, Proteus, Pseudomonas* and *Salmonella* spp.

The isolates of *Staphylococcus, Bacillus, Corynebacterium, Proteus, Pseudomonas* and *Salmonella* spp. were sensitive to the entire (10) range of antibiotic disc used in the study, whereas *E. Coli* was found to be resistant to all the antibiotic discs used in the study (Table 12).

Table 1: Species and gender of captive turtles included in the present study at Sri Chamarajendra Zoological Gardens, Mysuru (n=52)

Species	No. of turtles	No. of male turtles	No. of female turtles
Red eared slider	6	4	2
Indian flapshell turtle	11	8	3
Indian black turtle	35	19	16
Total	**52**	**31**	**21**

Table 2: Species-wise occurrence of shell diseases in captive turtles at Sri Chamarajendra Zoological Gardens, Mysuru (n=52)

Sl. No.	Turtle species	Total no. of turtles	Turtles with shell diseases	Percentage
1	Red eared slider	6	3	50 %
2	Indian flapshell turtle	11	7	63.64 %
3	Indian black turtle	35	9	25.71 %

Table 3: Gender-wise occurrence of shell diseases in captive turtles at Sri Chamarajendra Zoological Gardens, Mysuru (n=19)

Gender	Number affected	Percentage
Male	10	52.63 %
Female	9	47.37 %
Total	**19**	**100**

Table 4: Types of shell diseases in captive turtles at Sri Chamarajendra Zoological Gardens, Mysuru (n=19)

Shell problem	No. of affected animals	Percentage
Shell deformity	02	10.53 %
Shell crack	03	15.79 %
Septicemic ulcerative cutaneous disease (SUCD)	14	73.68 %
Total	**19**	**100**

Table 5: Mean temperature and humidity for the present study period (4 months) at Sri Chamarajendra Zoological Gardens, Mysuru

Enclosure name	Temperature observed		Humidity observed	
	Maximum (°C)	Minimum(°C)	Maximum(%)	Minimum(%)
Enclosure–I	35.67±1.05	20.94±0.844	79.15±1.96	28.43±2.92
Enclosure–II	34.81±1.05	22.21±0.844	72.39±7.28	28.28±3.32

Table 6: Biochemical tests results of Gram-positive aerobic isolates (n=13)

Isolates	Gram stain	C	O	NR	I	MR	VP	Citrate	Urease	TSI		
										Slant/Butt	Gas	H_2S
Staphylococcus spp. (n=07)	+Cocci	+	-	+	-	+	-	-	-	Y/Y	-	-
Bacillus spp. (n=05)	+ Bacilli	+	-	+	-	-	V	-	-	Y/Y	-	-
Corynebacterium spp.(n=01)	+ Bacilli	-	-	-	-	-	-	+	+	Y/Y	-	-

+: Positive- **:** Negative **V:** Variable **Y:** Yellow

C- Catalase test**; O-** Oxidase test; **NR=** Nitrate reduction test; **I-** Indole test**; MR-** Methyl Red test**; VP-** Voges – Proskauer test

Table 7: **Biochemical tests results of Gram-negative aerobic bacterial isolates (n=19)**

Bacterial isolates	Gram stain	Motility	C	O	NR	I	MR	VP	Citrate	Urease	TSI		
											Slant/Butt	Gas	H_2S
E. Coli (n=08)	-Rod	Motile	+	-	+	+	-	-	-	-	Y/Y	+	-
Pseudomonas spp. (n=03)	-Rod	Motile	+	+	+	-	-	-	+	+	R/R	-	-
Proteus spp.(n=02)	-Rod	Motile	+	-	+	V	+	V	V	+	Y/R	-	V
Salmonella spp. (n=06)	-Rod	Motile	+	-	+	-	+	-	+	-	R/Y	+	+

+: Positive;**- : Negative**;**V: Variable**;**Y:** Yellow; **R:** Red

C- Catalase test**; I**- Indole test**; O**- Oxidase test**; NR=** Nitrate reduction test**; MR**- Methyl Red test**; VP- Voges –** Proskauer test**;**

O- Oxidase test

Table 8: Aerobic bacterial isolates from the shell lesions of captive turtles at Sri Chamarajendra Zoological Gardens, Mysuru (n=16)

	ORGANISMS	RES1	RES2	RES3	IFT4	IFT5	IFT6	IFT7	IFT8	IFT9	IBT10	IBT11	IBT12	IBT13	IBT14	TOTAL
G -ve	*E. Coli*	-	-	-	-	-	+	-	-	-	+	-	-	-	-	2
	Pseudomonas spp.	-	-	+	-	-	-	-	-	+	-	-	-	-	-	2
	Proteus spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	+	1
	Salmonella spp.	+	-	-	+	-	-	-	-	-	+	-	+	-	-	4
G +ve	*Staphylococcus* spp.	-	+	-	-	+	-	-	-	-	+	-	-	-	+	4
	Bacillus spp.	-	-	-	-	-	+	-	-	-	-	+	-	-	-	2
	Corynebacterium spp.	-	-	-	-	-	-	-	-	-	-	-	+	-	-	1

RES: Red eared slider turtle; **IFT:** Indian flapshell turtle; **IBT:** Indian black turtle

+: Positive - : Negative

Table 9: Aerobic bacterial isolates from water samples from enclosure of captive turtles at Sri Chamarajendra Zoological Gardens, Mysuru (n=9)

	Organisms	Water sample 1	Water sample 2	Water sample 3	Water sample 4	Water sample 5	Water sample 6	Water sample 7	Water sample 8	TOTAL
G -ve	*E. Coli*	-	+	+	-	-	+	-	+	4
	Pseudomonas spp.	-	-	-	-	+	-	-	-	1
	Salmonella spp.	+	-	-	-	+	-	-	-	2
G +ve	*Staphylococcus* spp.	+	-	-	-	-	-	+	-	2

+: Positive - : Negative

Table 10: Aerobic bacterial isolates from soil samples from enclosure of captive turtles at Sri Chamarajendra Zoological Gardens, Mysuru (n=7)

	Organisms	Soil sample 1	Soil sample 2	Soil sample 3	Soil sample 4	Soil sample 5	Soil sample 6	Soil sample 7	Soil sample 8	TOTAL
G -ve	*E. Coli*	+	-	-	-	-	-	-	-	1
	Proteus spp.	-	+	-	-	-	-	-	-	1
G +ve	*Staphylococcus* spp.	-	+	-	+	-	-	-	-	2
	Bacillus spp.	+	-	+	-	-	-	-	+	3

+: Positive - : Negative

Table 11: Antibacterial sensitivity of samples collected from shell lesions in captive turtles at Sri Chamarajendra Zoological Gardens, Mysuru

Antibiotic Sensitivity Test																															
Sl. No.	Name of the antibacterial	Samples collected																													
		RES1		RES2		RES3		IFT4		IFT5		IFT6		IFT7		IFT8		IFT9		IBT10		IBT11		IBT12		IBT13		IBT14			
		S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R		
1	Amikacin	0	1	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0		
2	Cefuroxime	0	1	0	1	1	0	1	0	0	1	1	0	1	0	1	0	1	0	0	1	0	1	0	1	1	0	0	1		
3	Chloramphenicol	1	0	1	0	1	0	1	0	0	1	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0		
4	Ceftriaxone	1	0	0	1	0	1	0	1	0	1	0	1	0	1	0	1	1	0	0	1	0	1	1	0	0	1	0	1		
5	Erythromycin	0	1	1	0	1	0	0	1	0	1	0	1	0	1	0	1	1	0	1	0	1	0	0	1	0	1	0	1		
6	Kanamycin	0	1	1	0	1	0	1	0	0	1	1	0	0	1	1	0	1	0	1	0	1	0	0	1	1	0	1	0		
7	Penicillin-G	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	1	0	0	1	0	1	1	0	0	1	0	1		
8	Tetracycline	0	1	1	0	1	0	1	0	0	1	0	1	0	1	0	1	1	0	1	0	1	0	1	0	1	0	0	1		
9	Neomycin	0	1	1	0	1	0	1	0	1	0	1	0	0	1	0	1	1	0	1	0	1	0	1	0	0	1	1	0		
10	Ceftriaxone +Sulbactum	0	1	0	1	0	1	0	1	0	1	0	1	0	1	1	0	1	0	0	1	0	1	1	0	0	1	1	0		

S: Sensitive; **R**: Resistant; **RES**: Red eared slider turtle; **IFT**: Indian flapshell turtle; **IBT**: Indian black turtle

Table 12: Antibacterial sensitivity test of pure bacterial isolates from the shell lesions of captive turtles at Sri Chamarajendra Zoological Gardens, Mysuru (n=16)

Antibacterial Sensitivity Test																						
Sl. No.	Name of the bacteria	No. of isolates	No. of pure isolates																			
			AK		CXM		C		CTR		E		K		P		T		N		CIS	
			S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
1	*Staphylococcus* spp.	03	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0
2	*Bacillic* spp.	02	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0
3	*Corynebacterim* spp.	01	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0
4	*E.Coli*	03	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3
5	*Proteus* spp.	01	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0
6	*Pseudomonas* spp.	02	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0
7	*Salmonella* spp.	02	2	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0

S: Sensitive; **R:** Resistant

AK: Amikacin; **CXM**: Cefuroxime; **C**: Chloramphenicol; **CTR**: Ceftriaxone; **E**: Erythromycin; **K**: Kanamycin; **P**: Penicillin-G; **T**: Tetracycline; **N**: Neomycin; **CIS**: Ceftriaxone + Salbactum.

Fig 1: Occurrence of shell diseases species-wise in captive turtles at Sri Chamarajendra Zoological Gardens, Mysuru (n=52)

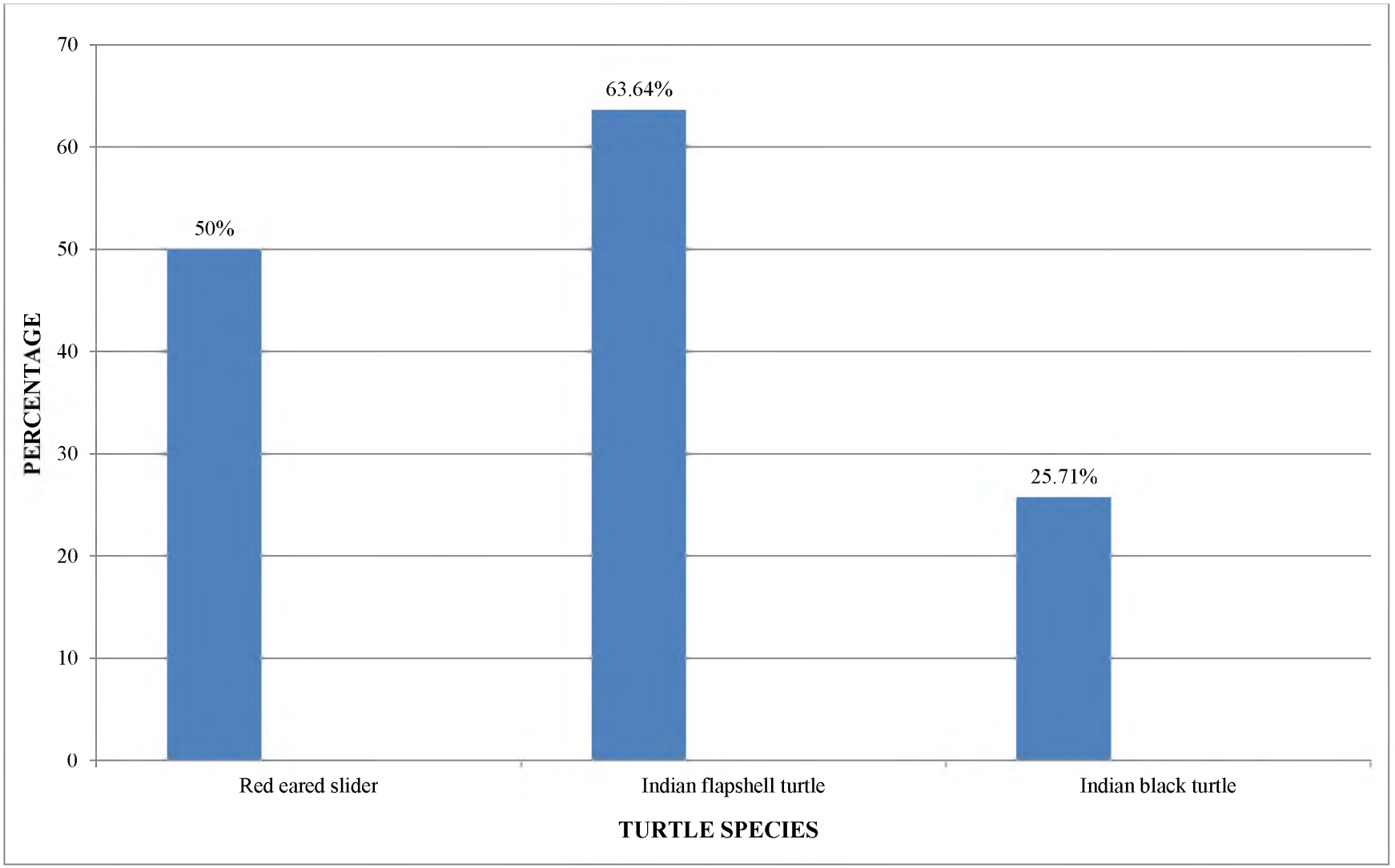


Fig. 2: Gender-wise occurrence of shell diseases in captive turtles at Sri Chamarajendra Zoological Gardens, Mysuru (n=52)

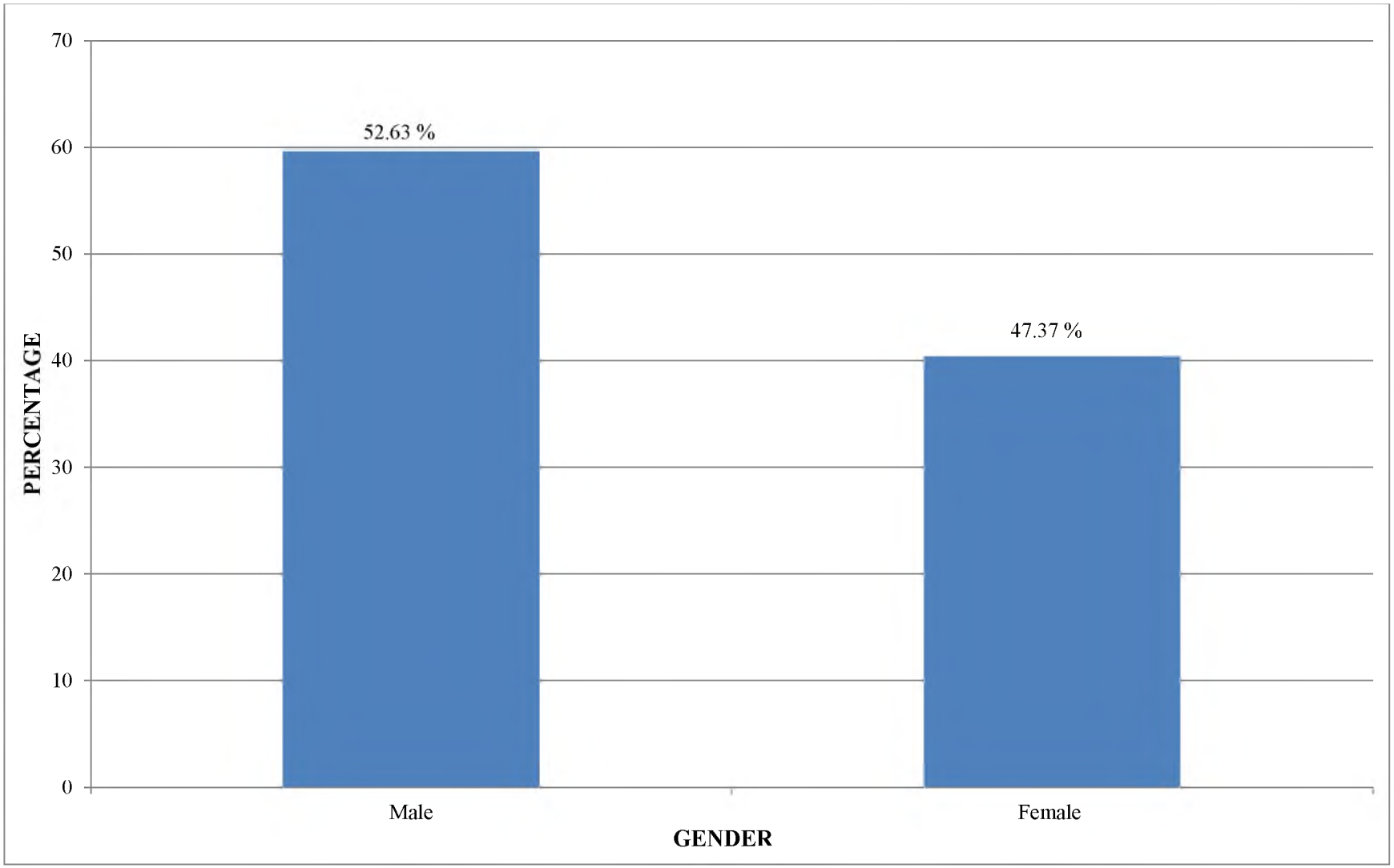


Fig. 3: Types of shell diseases in captive turtles at Sri Chamarajendra Zoological Gardens, Mysuru (n=19)

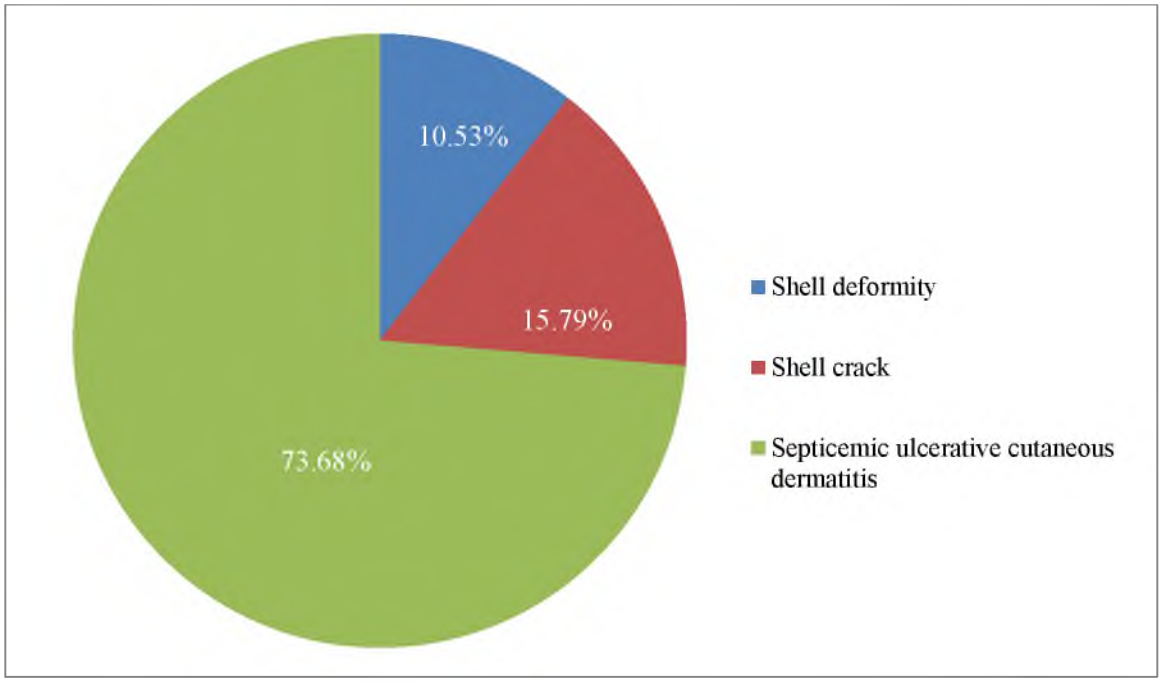


Fig. 4: Aerobic bacterial organisms isolated from shell diseases of captive turtles at Sri Chamarajendra Zoological Gardens, Mysuru (n=16)

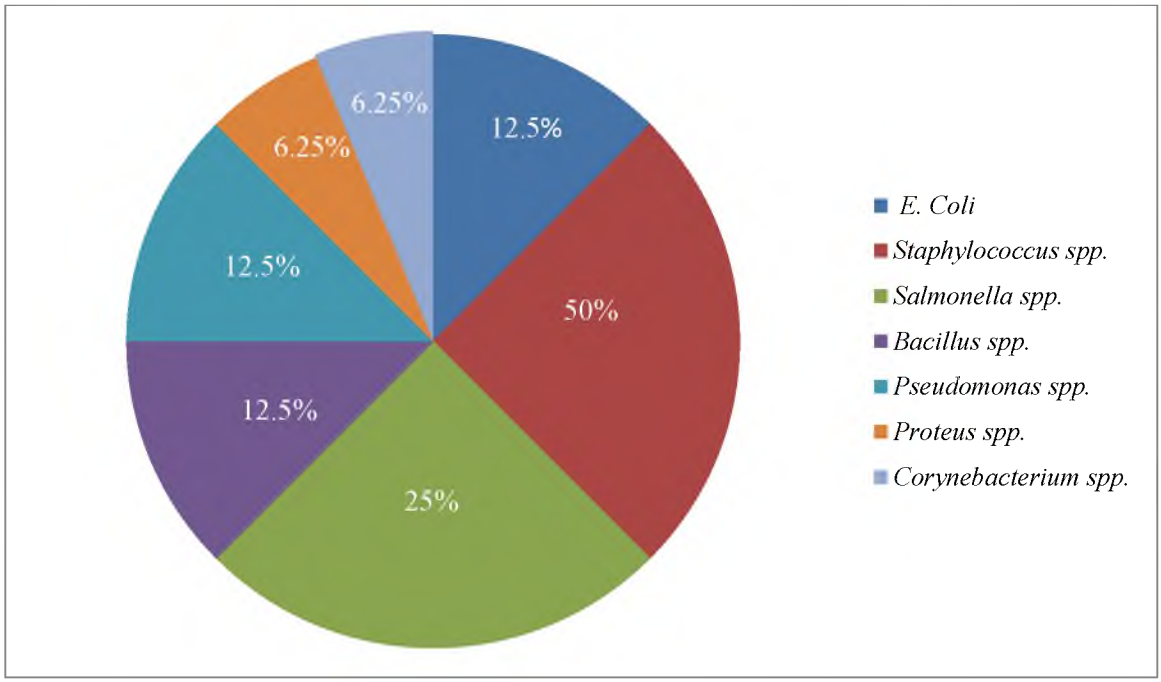


Fig.5: Aerobic bacterial organisms isolated from water samples of captive turtles enclosures at Sri Chamarajendra Zoological Gardens, Mysuru (n=9)

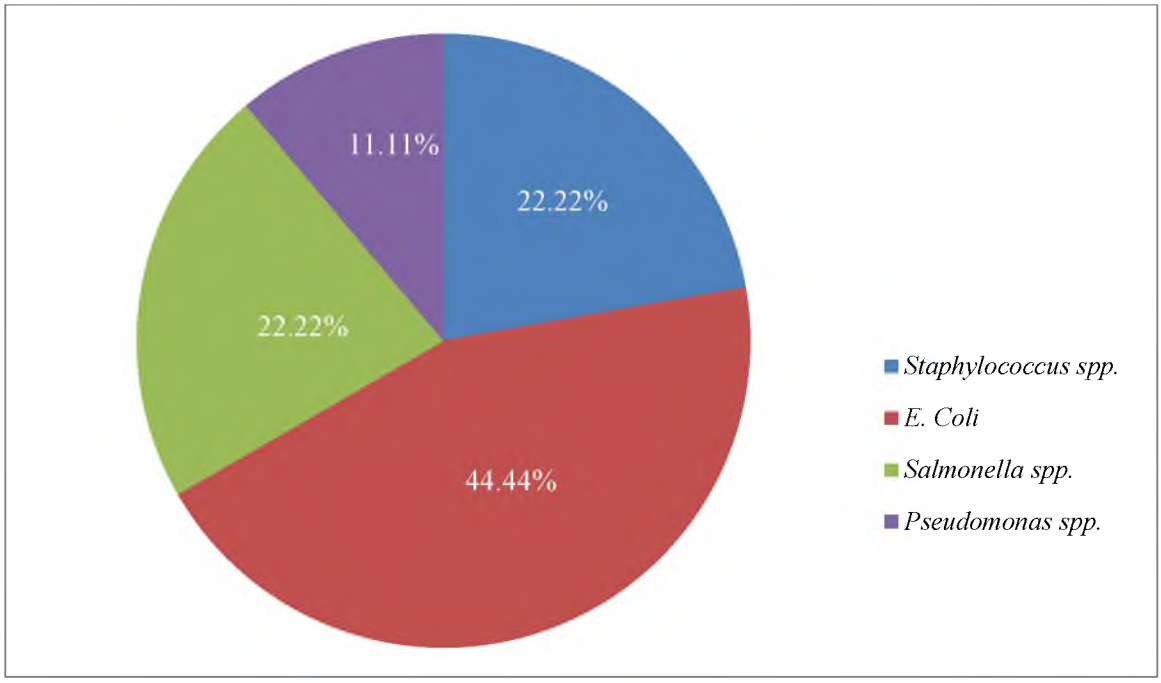


Fig.6: Aerobic bacterial organisms isolated from soil samples of captive turtles enclosures at Sri Chamarajendra Zoological Gardens, Mysuru (n=7)

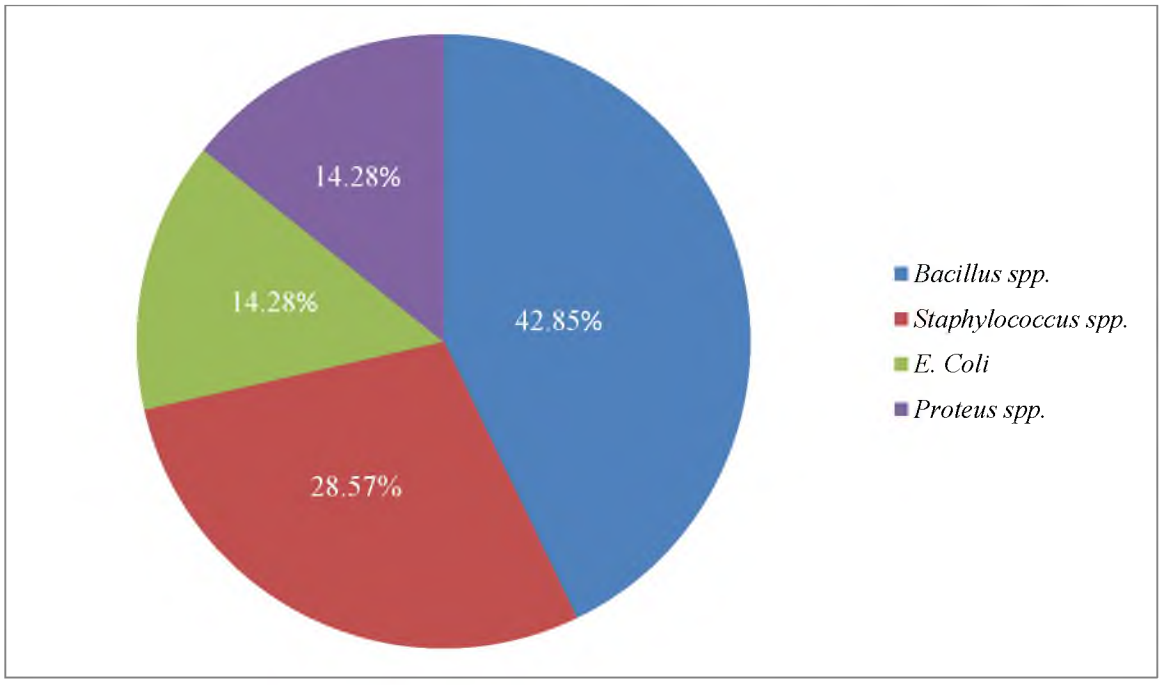


Plate 1: Turtle Enclosure - I at Sri Chamarajendra Zoological Gardens, Mysuru

Enclosure -I

Plate 2: Turtle Enclosure - II at Sri Chamarajendra Zoological Gardens, Mysuru

Enclosure -II

Plate 3: Shell deformity in captive turtles at Sri Chamarajendra Zoological Gardens, Mysuru

Shell deformity

Plate 4: Shell crack in captive turtles at Sri Chamarajendra Zoological Gardens, Mysuru

Shell crack

Plate 5: Septicemic ulcerative cutaneous disease (Indian blank turtle) at Sri Chamarajendra Zoological Gardens, Mysuru

Septicemic cutaneous ulcerative disease

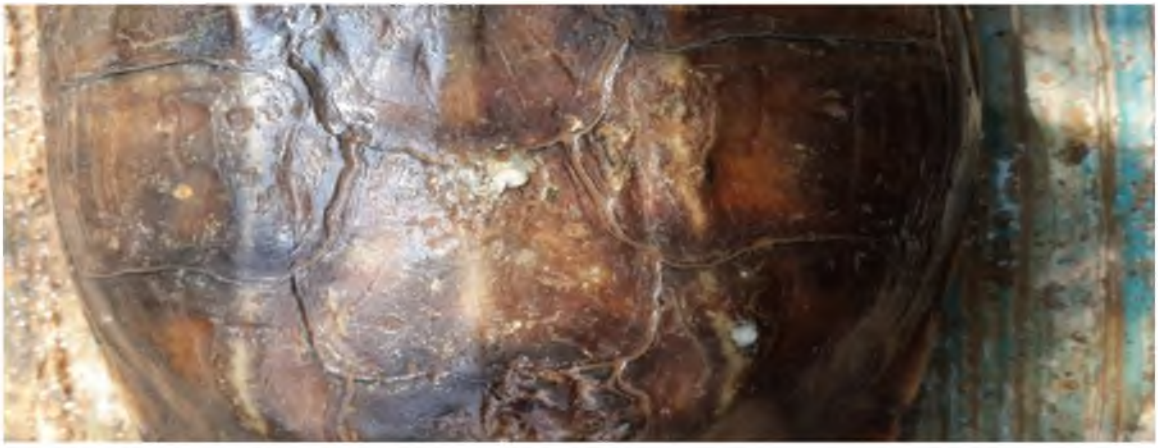


Plate 6: Septicemic ulcerative cutaneous dermatitis (Indian flapshell turtle) at Sri Chamarajendra Zoological Gardens, Mysuru

Plate 7: Septicemic ulcerative cutaneous dermatitis (Red eared slider turtle) at Sri Chamarajendra Zoological Gardens, Mysuru

Septicemic cutaneous ulcerative disease

Plate 8: Body weight of captive turtles housed in Sri Chamarajendra Zoological Gardens, Mysuru

Digital weighing machine

Plate 9: Body measurements (Carapace and Plastron) of captive turtles at Sri Chamarajendra Zoological Gardens, Mysuru

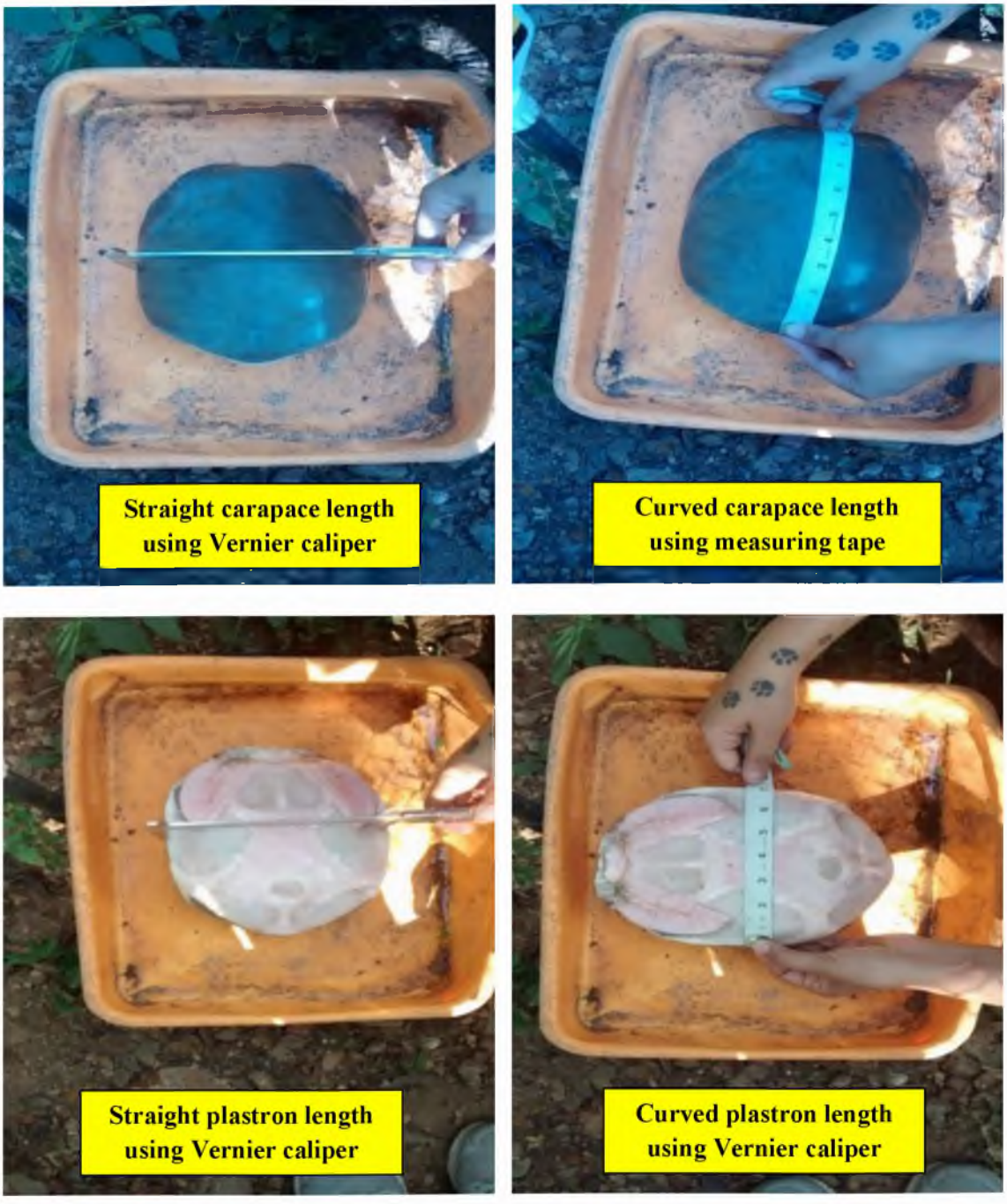
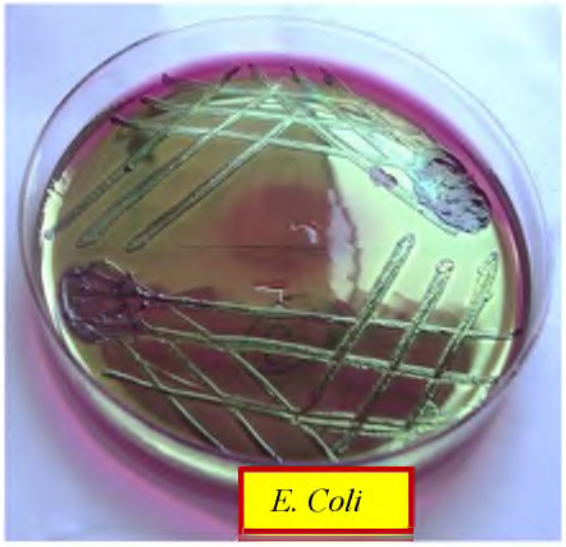


Plate 10: Aerobic bacteria isolated

***Bacillus* on BHI**

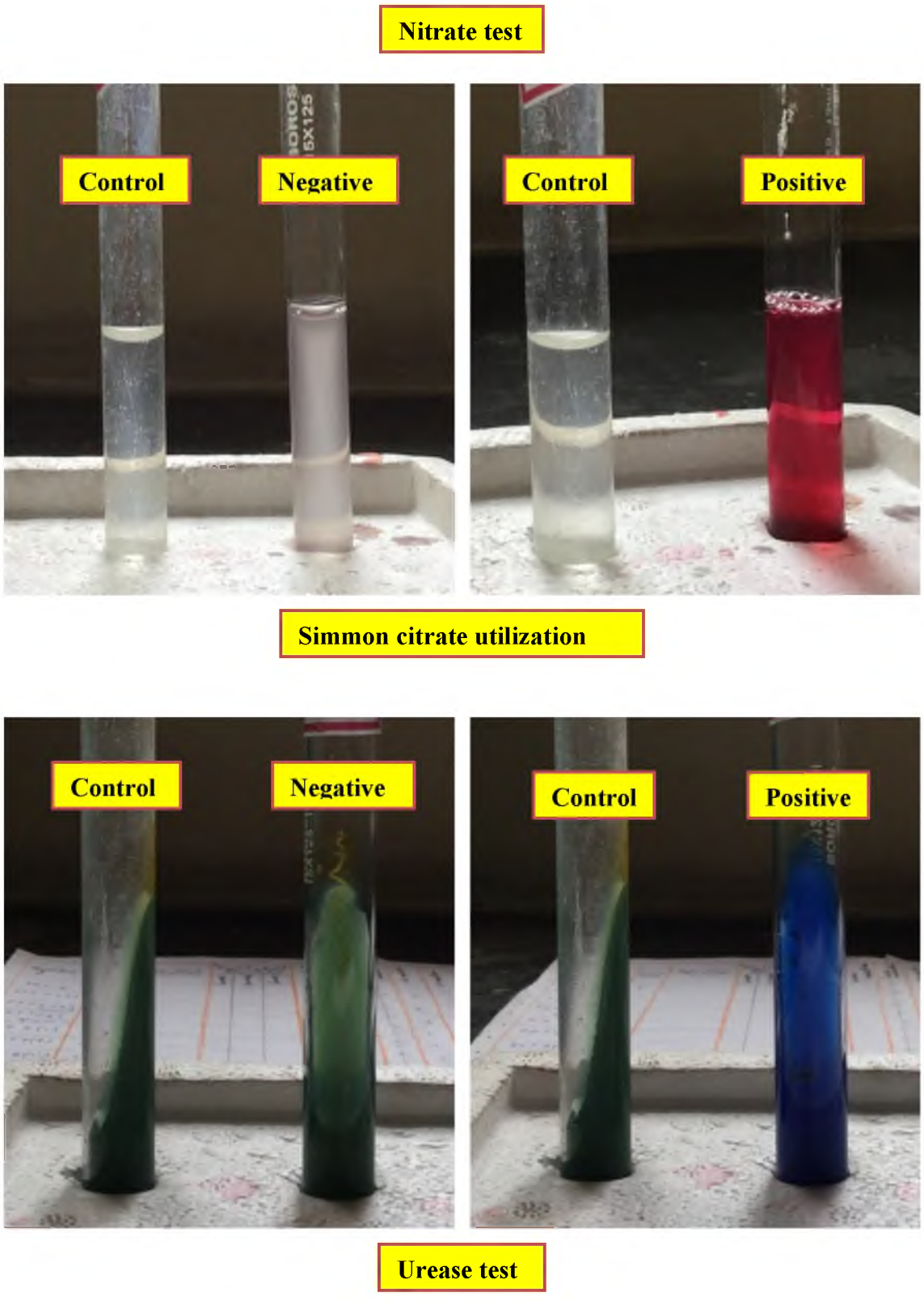
***Pseudomonas* on BHI**

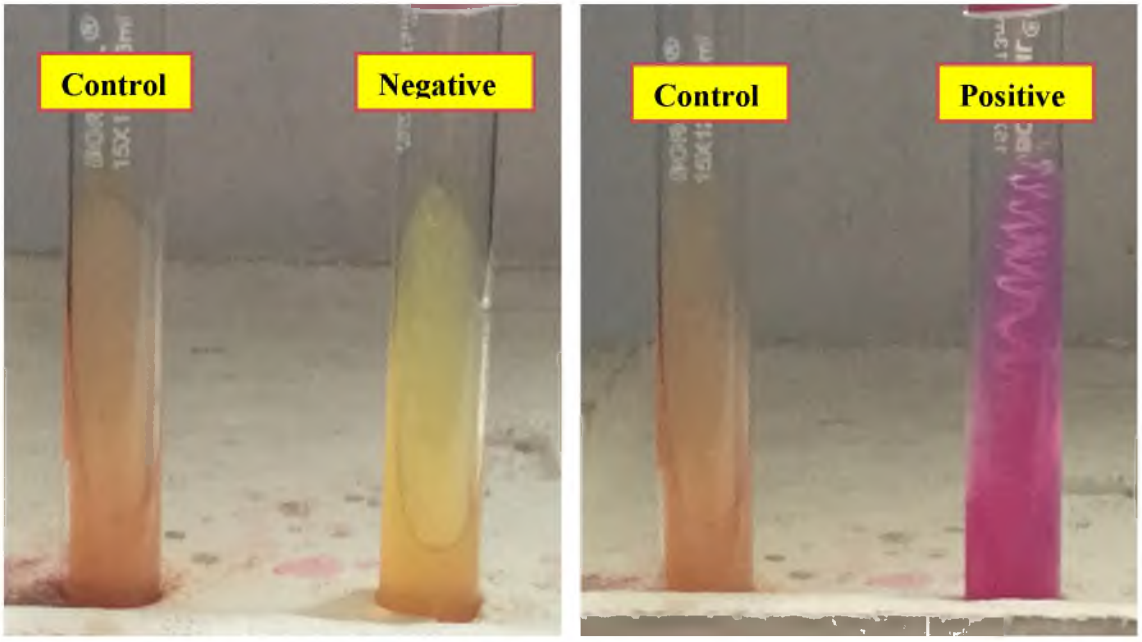
***Salmonella* on XLD**



***E. Coli* on EMB**

Plate 11: Biochemical test results of the isolated aerobic bacteria





TSI fermentation Test

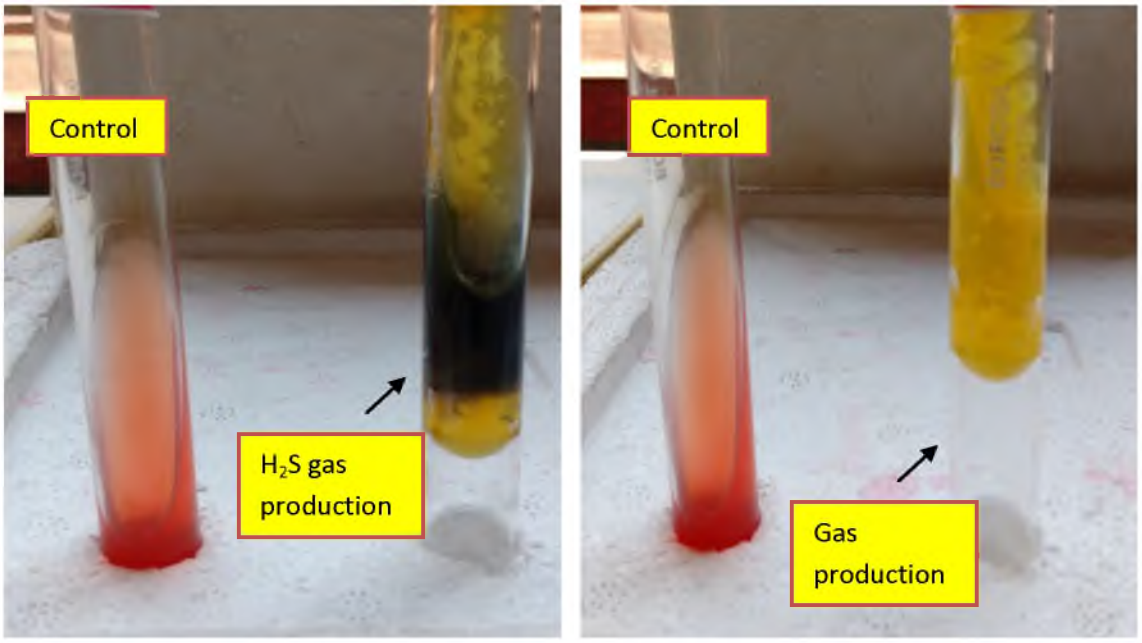
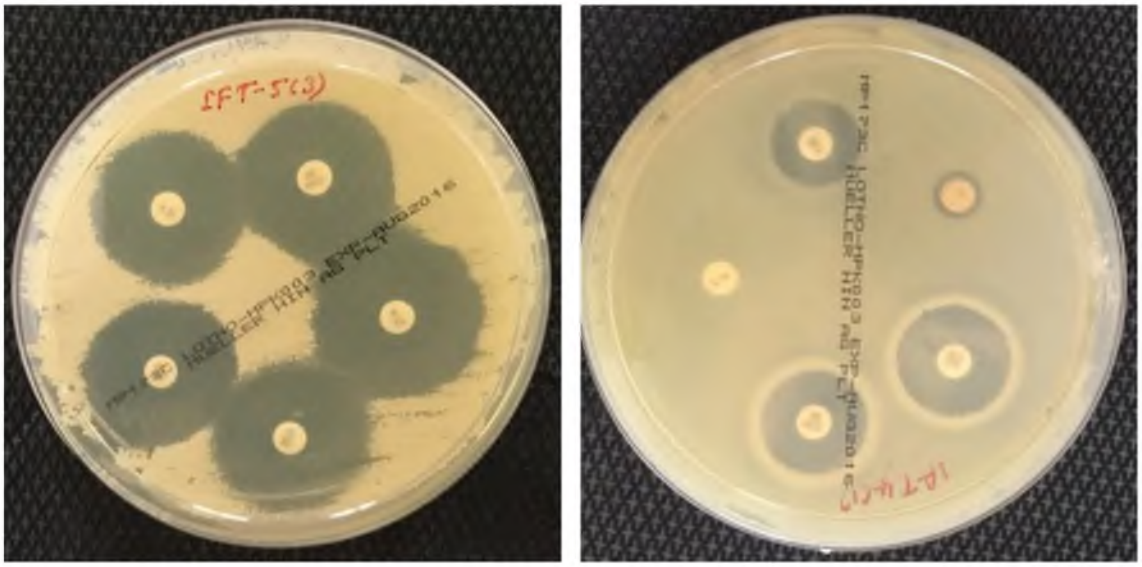


Plate 12: Antibiotic sensitivity pattern of isolated aerobic bacteria

Antibiotic sensitivity test on Mueller Hinton agar



Discussion

V. DISCUSSION

Turtles play a critically important role in the environment via nutrient cycling and cultures of many people around the world, they also help control the aquatic vegetation by scavenging. Turtles reared in captivity have to be provided with appropriate environmental conditions which if not provided can lead to shell diseases in turtles, based on which the present work was carried out and its findings are discussed below.

5.1 Occurrence of shell diseases in captive turtles at Sri Chamarajendra Zoological Gardens, Mysuru

In the present study, the overall occurrence of shell diseases in turtles was found to be 36.54 per cent which is similar to the observation made earlier by Sanja *et al.* (2013) who stated the shell lesions to be around 38.67 per cent. However, Garner *et al.* (1997) in the studies reported the shell lesions to be 76 per cent in turtles in Georgia lake. However the variation in per cent may be due to type of enclosure, water management and environmental parameters as indicated by Sanja *et al.* (*loc. cit*)

There is a paucity of literature about the shell diseases of turtles, although there are records on infectious shell disease in the captive turtles (Austwick and Keymer, 1981).

As regards the occurrence of shell diseases in different species of turtles, the present study (Table 2) indicated that the highest (63.64%) shell diseases were observed in the soft shelled turtles (Indian flapshell turtles) followed by Red eared sliders

(50%)and the least in Indian black turtles (25.71%). The available literature failed to throw light on the occurrence of shell disease in different species of turtles.

Further it is also observed that the highest per cent of occurrence was in the enclosure where a particular species (Indian flapshell turtle) was kept as compared to other turtle (Indian black turtle and Red eared slider turtle) which were housed together.

5.1.1 Gender-wise occurrence of shell diseases in captive turtles of Sri Chamarajendra Zoological Gardens, Mysuru.

In the current study at Sri Chamarajendra Zoological Garden, Mysuru, the occurrence of shell diseases in male turtle was found to be 52.63 per cent and it was 47.37 per cent in females. It can be observed that apparently the shell diseases in males are slightly higher than females. Generally it has been regarded that the occurrence of shell diseases is more in males as they have an aggressive behaviour when housed together as opined by Vosjoli, (2000).

5.2Shell diseases in captive turtles

The result of the present study indicated that shell deformity (10.53%) was observed in captive turtles and was encountered in hard-shelled turtle. The result is supported by the work of McArthur *et al.* (2009) who reported that inadequate calcium or vitamin D intake, inadequate exposure to ultraviolet light, or disease of liver, kidney, or parathyroid glands may lead to metabolic bone disease or shell deformities causing malformation of the bones. Husbandry and diet changes may be able to correct the calcium imbalance, but deformities are generally permanent.

Shell crack was noticed in three turtles and it is assumed shell cracks can be due to inadequate exposure to ultra violet light, trauma, nutritional deficiencies, or improper husbandry and diet changes (Fleming, 2014).

The results of the present study indicated that septicemic ulcerative cutaneous disease (73.68%) was the most common shell disease in turtles and was more frequently encountered (Table 4 and Table 2) in Indian flapshell turtles (63.64%) followed by Red eared sliders (50%)and the least in Indian black turtles (25.71%).

This result is supported by the work of McArthur *et al.* (2009) who reported that the septicemic cutaneous ulcerative disease was one of the most common shell condition seen in turtles which may be the result of a deep ulcer and poor husbandry.

5.3 Isolation of aerobic bacteria from shell lesions in captive turtles of Sri Chamarajendra Zoological Gardens, Mysuru

In the present study a total of sixteen aerobic organisms were isolated from the shell lesions. Out of these, the Gram positive and Gram negative isolates constituted seven (43.75%) and nine (56.25%) bacterial isolates respectively suggesting that the Gram negative isolates were more commonly associated with shell lesions of turtles when compared to Gram positive isolates. This observation draws the support from the findings of Mader (2006) who stated that Gram negative bacteria were the most common bacterial isolates and that Gram-negative bacteria were common isolates in healthy reptiles. Most Gram-positive bacteria are not considered pathogenic in reptiles and they are actually common inhabitants, especially of the shell.

Out of the sixteen bacterial isolates from shell lesions in the present study, *Staphylococcus* spp. and *Salmonella* spp. were the most common (25%), followed by *Bacillus* spp., *Pseudomonas* spp., *E. Coli* (12.5% each), *Proteus* spp. and *Corynebacterium* spp. (6.25% each).

This indicated that *Staphylococcus* spp. and *Salmonella* spp. were isolated as the most common bacteria in the present study which is similar to the reports of Oros *et al.* (2003), Boede and Hernandez (2004), Sousa *et al.* (2011) who have reported in their study *Staphylococcus* and *Salmonella* spp. from the shell lesions of turtles belonging to different species.

Isolation of *Bacillus* spp. from turtles draws its support from the results of Rosen (1970) and Campbell (1974) who have reported *Bacillus* spp. from the fresh lesions as well as healing lesions of soft shell turtles.

In the present study, *E. Coli* was isolated from fourteen shell lesions which was the primary pathogen or potential pollutant or normal component of bacterial flora of the reptiles which invade the skin and shell as indicated by Oros *et al.* (*loc. cit*), Ayerdon (2006) and Sanja *et al.* (2013).

Pseudomonas spp. was isolated from the shell lesions of turtles. The observation draws its support from the finding of earlier workers like Boyer (1996), Lutz and Musick (1996) and Sanja *et al.* (*loc. cit*) who observed that poor husbandry, including suboptimal environment temperature and malnutrition can predispose reptiles to infection with *Pseudomonas* spp. and was associated with dermatitis in turtles.

In the present study, *Corynebacterium* spp. and *Proteus* spp. were observed similar to studies made by Rosen (1970), Campbell (1974), Lutz and Musick (1996), Mader (2006), Oros *et al.* (2003) and Sanja *et al.* (2013) who reported *Corynebacterium* spp. and *Proteus* spp. commonly being reported from shell lesions of marine turtles.

More than one species of bacteria was isolated from total of 14 samples collected from the shell lesions of turtles in Sri Chamarajendra Zoological Garden, Mysuru. Septicemic ulcerative cutaneous disease (SUCD) is a chronic or secondary bacterial infection of mixed flora which is commonly present in immunocompromised turtles, mostly associated with aquatic environment temperature below the proper range and presence of untreated skin wound as opioned by McArthur (2004a) and Jacobson (2007).

5.4.1 Isolation of aerobic bacteria from soil samples collected from two turtle enclosures in Sri Chamarajendra Zoological Gardens, Mysuru

In the present study, a total of eight soil samples were collected for a period of four months. Out of this, seven bacterial isolates belonging to the genus *Bacillus* spp. was found to be highest (42.85%), followed by *Staphylococcus* spp. (28.57%), *E. Coli* and *Proteus* spp. (14.28% each). These results indicated that *Bacillus* spp. is the most common isolate from the soil samples of turtle enclosures, followed by *Staphylococcus*, *Proteus* and *E. Coli*. This was similar to the report by earlier workers namely Jasuja*et al.* (2013) and Nandhini and Josephine (2013) who reported isolation of *E.coli*, *Staphylococcus, Proteus* and *Bacillus* spp. from the soil samples.

5.4.2 Isolation of aerobic bacteria from water samples collected from two turtle enclosures in Sri Chamarajendra Zoological Gardens, Mysuru

In the present study, from the two enclosures nine bacterial isolates were obtained belonging to the *E. Coli* (44.44%), *Salmonella* spp. (22.22%), *Staphylococcus* spp. (22.22%), and *Pseudomonas* spp. (11.11%).Thus, the results indicate that *E. Coli* was found to be the most common isolate from the water samples from the turtle enclosures, followed by *Salmonella*, *Staphylococcus* and *Pseudomonas* spp.

The most commonly cultured bacteria from water were *E. coli, Pseudomonas* species, *Salmonella* spp. as reported by Lutz and Musick (1996).

Shell infections are most common consequences of unclean water. Unclean areas are a potential source of infectious bacteria such as *Salmonella* (Boyer and Boyer, 2006).

The results of the present study draws the support of the earlier workers namely He *et al.* (2007) who reported that *E. Coli* is considered as one of the most common pathogens responsible for the contamination of water.

Bacteriological results showed potentially pathogenic microorganisms in lesions due to contaminated water, including *Staphylococcus aureus*, *Escherichia coli* and *Proteus* spp. (Rangel-Mendoza *et al.* 2013).

5.5 Bacterial load in water from turtle pond at Sri Chamarajendra Zoological Gardens, Mysuru

Total viable counts of bacteria from the water sample of Enclosure -I water body were found to be 530 cfu/ml and that of Enclosure -II water body was 410 cfu/ml. The present results indicated that the water bacterial load of both the water bodies from the enclosures was high compared to the previous studies of Park (2007) who reports that the

permitted level for total viable count of portable water should be less than 100 cfu/ml of water.

5.6 Observation of environmental parameters (pH, temperature and humidity) from Enclosure –I and II at Sri Chamarajendra Zoological Gardens, Mysuru.

Analysis of water samples collected from the turtle pond of both enclosures revealed a pH of 7 which was under the recommended pH for turtles. This observation draws its support from the findings of earlier worker namely Vella (2013).

The maximum and minimum temperature recorded in the present study of first enclosure at Sri Chamarajendra Zoological Garden, Mysuru, were 35.67±1.39 and 20.94±0.89, whereas the recorded value in second enclosure at Sri Chamarajendra Zoological Garden, Mysuru, was 34.81±1.05 and 22.09±0.84.

The recommended range of temperature for turtles is 20-35 ºC (Girling, 2003). This indicates that the observed temperature and humidity range for both enclosures was within the recommended range of temperature.

The maximum and minimum humidity recorded in the present study of Enclosure –I at Sri Chamarajendra Zoological Garden, Mysuru, were 79.15±1.96 and 28.43±2.92, whereas the recorded value in Enclosure -II at Sri Chamarajendra Zoological Garden, Mysuru, was 72.39±7.28 and 28.28±3.32.

The recommended range of humidity for turtles is 50-70 per cent (Girling, 2003). The minimum humidity noted during month of January, February, March and April in first enclosure was 36.28 per cent, 22.57 per cent, 26 per cent and 28.85 per cent and in

second enclosure was 37.42 per cent, 21.71 per cent, 25.85 per cent and 28.14 per cent respectively which indicates that the minimum humidity recorded in both the enclosures during four months period was lower than the recommended range.

Whereas, the maximum humidity noted during month of January, February, March, April in first enclosure was 79.71 per cent, 80.6 per cent, 73.57 per cent, 82.71 per cent and that in second enclosure was 83 per cent, 51 per cent, 74.85 per cent, and 80.14 per cent respectively, which indicates that the maximum humidity recorded in both the enclosures during the four months period was higher than the recommended range.

The environment of captivity has a great impact on the bacterial levels in water, which should be considered for the management of turtles as well as other aquatic animals. Water quality is critical for aquatic animal health and welfare, particularly for the captive animals. However in captivity, inadequate care is more likely to result in bacterial infections/mortality than old age. The inadequate environmental parameters can cause skin/shell lesions in turtles (Chuen-Im *et al.*, 2010). This may explain the reason for shell diseases in the captive turtles.

Hence, it may be concluded that inappropriate humidity in the enclosures may have precipitated the prevalence of shell diseases in the present study.

5.7 Antibacterial sensitivity pattern of isolated aerobic bacteria

The results of the present study indicated that all the bacterial isolates viz., *Staphylococcus, Bacillus, Corynebacterium, Proteus, Pseudomonas* and *Salmonella* were

sensitive to all the antibiotic discs used namely amikacin, cefuroxime, chloramphenicol, ceftriaxone, erythromycin, kanamycin, penicillin-G, tetracycline, neomycin, and ceftriaxone+sulbactum. Whereas, *E. Coli* was found resistant to all the antibiotic discs used in the study (Table No. 12).

The present findings draw support from the work of Anil and Shahid (2013) who reported that *Proteus, Pseudomonas* and *Staphylococcus* isolates isolated were found to be sensitive to chloramphenicol, neomycin, ceftriaxone and amikacin. Rasheed *et al.* (2014) reported that highest per cent of drug resistance was detected in *E. Coli*, stating antibiotic resistance in *E. Coli* was of particular concern because it was the most common Gram-negative pathogen. Sharvani *et al.* (2006) reported that *Salmonella* isolated in their studies was found sensitive to chloramphenicol, followed by third generation cephalosporins and tetracycline respectively.

Conclusions:

- The occurrence of shell disease in captive turtles at Sri Chamarajendra Zoological Gardens, Mysuru, was found to be 36.53 per cent with the highest occurrence rate in Indian flapshell turtles (63.64%).
- Indian black turtle, Red eared turtle and Indian flapshell turtle exhibited shell lesions during the period of study.
- The occurrence of shell diseases was seen more in male turtles (52.63%) than in female turtles (47.37%).

- The most common type of shell disease was found to be septicemic ulcerative cutaneous disease (73.68%), followed by shell crack (15.79%) and shell deformity (10.53%).

- The maximum temperature recorded in Enclosure -I was (35.67±1.39) and minimum was (20.94±0.89) and in Enclosure -II the maximum was(34.81±1.05) and minimum was (22.09±0.84) and it was found to be within the recommended range (20-35°C) in both the enclosures.

- The maximum humidity recorded in Enclosure –I was (79.15±1.96) and minimum was (28.43±2.92) and in Enclosure -II the maximum was (72.39±7.28) and minimum was (28.28±3.32) and it was found to be lower and higher than the recommended range (50-70%) in both the enclosures.

- The most common aerobic bacteria isolated form shell lesions of turtles were *Staphylococcus* spp. (25%), *Salmonella* spp. (25%), *Bacillus* spp. (12.5%), *E. Coli* (12.5%), *Pseudomonas* spp. (12.5%), *Proteus* spp. (6.25%) and *Corynebacterium* spp. (6.25%).

- Among the various aerobic bacteria isolated from the water samples of turtle enclosures at Sri Chamarajendra Zoological Garden, Mysuru, were *E. Coli* (44.44%), *Staphylococcus* spp. (22.22%), *Salmonella* spp. (22.22%) and *Pseudomonas* spp. (11.11%).

- Among the various aerobic bacteria isolated from the soil samples of turtle enclosures at Sri Chamarajendra Zoological Gardens, Mysuru, were *Staphylococcus* spp. (28.57%), *E. Coli* (14.28%), *Bacillus* spp.(42.85%),*Proteus* spp. (14.28%).

- The total viable count of water samples collected from the water in Enclosure -I (530 cfu/ml) and Enclosure –II (410 cfu/ml) of both the turtle enclosures was higher than the recommended range.
- Among the different antibiotic discs used, bacterial isolates were sensitive against amikacin (92.85%), choramphenicol (92.85%), kanamycin (71.42%), neomycin (71.42%) and cefuroxime (50%).

Summary

VI. SUMMARY

The present work was taken up with the objective to study the overall occurrence of shell diseases in turtles, to isolate and identify the aerobic bacteria associated with the shell disease, to study the antibiotic sensitivity test (ABST) pattern of aerobic bacteria, and study the possible relationship between the environment, management practice and shell diseases in turtles.

A total of 52 turtles belonging to 3 different species were examined for the presence of shell disease and it was noticed that 19 turtles among these exhibited shell problems which indicated the occurrence of shell diseases to be 36.54 per cent.

The occurrence of shell diseases in male turtles was 52.63 per cent and that in female turtles was 47.37 per cent indicating that shell disease in males was slightly higher than in female turtles.

Septicemic ulcerative cutaneous disease (SUCD) was found to be the most encountered shell disease (73.68%) with the highest occurrence in Indian flapshell turtles (63.64%), followed by Red eared slider turtle (50%) and Indian black turtles (25.71%).

Three cases of shell deformity and two cases of shell crack was seen in Red eared slider turtle and Indian black turtles respectively.

The temperature in the different turtle enclosures was found to be within the recommended temperature range. However, the humidity in both the enclosures was found to be lower than the minimum range and higher than the maximum range

recommended. This indicated that high humidity in the turtle enclosures had an effect on the occurrence of shell diseases in turtles.

Out of the fourteen shell swab samples collected, sixteen organisms were isolated. Out of these, *Staphylococcus* and *Salmonella* spp. were found to be the most common (25% each), followed by *Bacillus*, *Pseudomonas*, *E.Coli* spp (12.5% each), *Proteus* and *Corynebacterium* spp. (6.25% each). *Staphylococcus* and *Salmonella* spp. (25%) were isolated as the most common bacteria associated with shell diseases in turtles.

More than one species of bacteria was isolated from all fourteen samples in the present study and it ranged between 2-3 isolates.

The pH of water samples from both the enclosures was found to be within the normal range (7-8). However, bacterial load of the water samples from Enclosure - I was found to be 530cfu/ml and that of the Enclosure - II was 410cfu/ml. This indicated that the water bacterial load of water bodies of both the enclosures was is higher than the normal total viable count (100 cfu/ml of water) of portable water.

From the two turtle enclosures, water samples were collected and nine bacterial isolates were isolated which included *E. Coli* spp. (44.44%) followed by *Salmonella* and *Staphylococcus* spp. (22.22%), and *Pseudomonas* spp. (11.11%). This indicated that *E.coli* spp. was the most common isolate from the water sample.

From two turtle enclosures, soil samples were collected and seven bacterial isolates were identified which included *Bacillus* spp. (42.85%), *Staphylococcus* spp.

(28.57%), *E. Coli* and *Proteus* spp. (14.28% each). This indicated that *Bacillus* spp. was the most common isolate from the soil sample.

Among the different antibacterial agents used for ABST, the results of the present study indicated that most of the bacterial isolates viz., *Staphylococcus* spp., *Bacillus* spp,, *Corynebacterium* spp, *Proteus* spp., *Pseudomonas* spp. and *Salmonella* spp. were sensitive to all the antibacterial agents used namely amikacin, cefuroxime, chloramphenicol, ceftriaxone, erythromycin, kanamycin, penicillin-G, tetracycline, neomycin, and ceftriaxone+sulbactum. Only *E. Coli* was found to be resistant to all the antibacterial agents used in the study.

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Abstract

VIII. ABSTRACT

A study was taken up with the objectives to assess the occurrence of shell diseases in turtle, to isolate and identify the aerobic bacteria associated with the shell disease, to study the ABST pattern of aerobic bacteria, and study the possible relationship between the environment, managemental practices and shell diseases of turtle housed at Sri Chamarajendra Zoological Gardens, Mysuru. The occurrence of shell diseases was found to be 36.53 per cent with the highest occurrence in Indian flapshell turtle, followed by Red eared slider turtle and Indian black turtle. The occurrence of shell disease was seen higher in males (52.63%) than in females (47.37%). The common type of shell condition seen was septicemic ulcerative cutaneous disease (73.68%), followed by shell crack (15.79%) and shell deformity (10.53%). The water pH (7) from water bodies from both enclosures was within the recommended range. The total viable count of water samples from both enclosures revealed higher bacterial count. The temperature in the turtle enclosures were within the recommended range. However, the humidity in both turtle enclosures was below minimum and above maximum recommended range which might have acted as a precipitating factor for the occurrence of shell diseases. Among the various aerobic bacteria isolated, *Staphylococcus* and *Salmonella* organisms were the most common isolates from the shell lesions and water sample, and *Bacillus* and *Staphylococcus* were the most common isolates from soil samples. Among the different antibiotic agents used all the bacterial isolates showed higher sensitivity towards amikacin (92.85%) and choramphenicol (92.85%) except *E. Coli* being resistant to all antibiotics.

Appendices

IX. APPENDICES

The compositions of various media employed in the study are given below. All the media were sterilized by autoclaving at 15 lbs pressure (121°C) for 15 min unless otherwise specified.

Appendix I:Brain heart infusion broth

Ingredients	g/liter
Calf brain	200.00
Beef heart	250.00
Proteose peptone	10.00
Dextrose	2.00
Sodium chloride	5.00
Disodium phosphate	2.50

3.7 grams dissolved in 100 ml of DW and pH adjusted to 7.4 ± 0.2

Appendix II: Nutrient agar

Ingredients	g/liter
Peptic digest of animal tissue	5.00
Sodium chloride	5.00
Beef extract	1.50
Yeast extract	1.50
Agar	15.00

2.8 grams dissolved in 100 ml of DW and pH adjusted to 7.4 ± 0.2

Appendix III:Brain heart infusion agar

Ingredients	g/liter
Calf brain	200.00
Beef heart	250.00
Proteose peptone	10.00
Dextrose	2.00
Sodium chloride	5.00
Disodium phosphate	2.50
Agar	15.00

5.2 grams dissolved in 100 ml of DW and pH adjusted to 7.4 ± 0.2

Appendix IV: MacConkey's agar

Ingredients	g/liter
Peptic digest of animal tissue	1.50
Casein enzymic hydrolysate	1.50
Pancreatic digest of gelatin	17.00
Lactose	10.00
Bile salts	1.50
Sodium chloride	5.00
Crystal violet	0.001
Neutral red	0.03
Agar	15.00

5.15 grams dissolved in 100 ml of DW and pH adjusted to7.1 ± 0.2

Appendix V:Mannitol salt agar

Ingredients	g/liter
Peptic digest of animal tissue	5.00
Pancreatic digest of casein	5.00
Beef extract	1.00
Sodium chloride	75.00
D-Mannitol	10.00
Phenol red	0.025
Agar	15.0

11.10 grams dissolved in 100 ml of DW and pH adjusted to 7.4 ± 0.2

Appendix VI:Eosin methylene blue (EMB) agar

Ingredients	g/liter
Peptic digest of animal tissue	10.00
Dipotassium phosphate	2.00
Lactose	5.00
Sucrose	5.00
Eosin – Y	0.40
Methylene blue	0.065
Agar	13.50

3.8 grams dissolved in 100 ml of DW and pH adjusted to 7.2 ± 0.2

Appendix VII:Methyl red-Vogues Proskaur (MR-VP) medium

Ingredients	g/liter
Buffered HiVeg peptone	7.00
Dextrose	5.00
Dipotassium phosphate	5.00

1.7 grams dissolved in 100 ml DW and pH adjusted to 6.9 ± 0.2

Appendix VIII:Simmons citrate agar

Ingredients	g/liter
Magnesium sulphate	0.20
Ammonium dihydrogen phosphate	1.00
Dipotassium phosphate	1.00
Sodium citrate	2.00
Sodium chloride	5.00
Bromo thymol blue	0.08
Agar	15.00

2.42 grams dissolved in 100 ml of DW and pH adjusted to 6.8 ± 0.2

Appendix IX: Christensen's Urea agar base

Ingredients	g/liter
Peptic digest of animal tissue	1.00
Dextrose	1.00
Sodium chloride	5.00
Disodium phosphate	1.20

Monopotassium phosphate	0.80
Phenol red	0.012
Agar	15.00

2.40 grams dissolved in 100 ml of DW and pH adjusted to 6.8 ± 0.2

Appendix X: Mueller hinton agar

Ingredients	g/liter
Beef infusion	300.00
Casein acid hydrolysate	17.50
Starch	1.50
Agar	17.00

3.8 grams dissolved in 100 ml of DW and pH adjusted to7.3 ± 0.2

Appendix XI: Xylose Lysine Desoxycholate (XLD) agar

Ingredients	g/liter
Yeast extract	3.00
L-Lysine	5.00
Lactose	7.50
Sucrose	7.50
Xylose	3.75
Sodium chloride	5.00
Sodium deoxycholate	2.50
Sodium thiosulphate	6.80
Ferric ammonium citrate	0.80

Phenol red	0.08
Agar	15.00

5.693 grams dissolved in 100 ml DW and pH adjusted to 7.4 ± 0.2, boiled notautoclaved.

Appendix XII: Triple Sugar Iron (TSI) agar

Ingredients	g/liter
Peptic digest of animal tissue	10.00
Casein enzymic hydrolysate	10.00
Yeast extract	3.00
Beef extract	3.00
Lactose	10.00
Sucrose	10.00
Dextrose	1.00
Sodium chloride	5.00
Ferrous sulphate	0.20
Sodium thiosulphate	0.30
Phenol red	0.024
Agar	12.00

6.452 grams dissolved in 100 ml of DW and pH adjusted to 7.4 ± 0.2

Appendix XIII: Body measurements of the three species (Indian black turtle, Indian flapshell turtle, Red eared slider turtle) the present studies at Sri Chamarajendra Zoological Gardens, Mysuru.

(a) Indian flapshell turtle

Sl. No.	Body weight	Carapace (cm)				Plastron (cm)				Circumference (cm)
	kg	SCL	CCL	SCW	CCW	SPL	CPL	SPW	CPW	CIRUM
1	2.143	20.8	9.5	16.3	8.5	20.3	9.5	16.2	7.5	17
2	1.698	19.5	8.9	16.6	8.6	18.4	8.5	18.5	7.5	17
3	1.992	19.4	9	16	8.5	20.5	9.5	16	7.5	16.5
4	3.516	25.5	11.3	18.6	10.6	25	11.5	20	17.6	8.6
5	1.421	20.4	9.6	16.2	8.4	19.7	9	18.6	7.5	16.5
6	1.736	20.4	9.2	17.6	8.5	19.5	9.4	17.5	7.5	16.6
7	3.711	27.5	12.3	21.8	11.5	26	11.3	20	8.5	20.5
8	3.889	30	13	25.3	11.1	29	12.2	22.9	9.3	21.2
9	1.744	23.1	9.5	15.6	9	20.9	8.9	15.4	7	16
10	1.914	23.5	10.2	18.7	9	21	9.2	18.6	8	17.4
11	2.014	24	10.4	20.3	9.7	22.9	9.6	19.9	8	18.1

(b) Red-eared slider turtle

Sl. No.	Body weight	Carapace (cm)				Plastron (cm)				Circumference(cm)
	kg	SCL	CCL	SCW	CCW	SPL	CPL	SPW	CPW	CIRUM
1	0.583	15.5	7.1	12.2	6	14	5.6	12.5	5.4	11.5
2	1.308	19.1	8.5	14.4	8	17.5	7.4	14.5	6.1	14.4
3	0.932	15.9	7.4	13.8	7	15.4	6.4	13.5	6	13.2
4	0.418	13.6	5.9	10.8	5	12	5	10.5	4.8	10
5	0.792	12.2	5	12	5.6	15.2	6.5	10.6	3.5	12

(c) Indian black turtle

Sl. No.	Body weight	Carapace (cm)				Plastron (cm)				Circumference (cm)
	kg	SCL	CCL	SCW	CCW	SPL	CPL	SPW	CPW	CIRUM
1	1.892	23.5	10.2	16.7	8.4	21.8	9.1	16	7.4	16.1
2	2.070	25	10.6	16	8.5	20.6	8.6	15.6	7.2	16.5
3	1.642	21	9	14.1	7.6	19.7	7.7	14.3	6.5	14.9
4	1.092	19.9	8.5	13	6.6	20.1	6.9	13.7	6	13.2
5	1.466	20.5	9	14.2	7.2	17.6	7.5	14.4	6.6	14.2
6	1.987	23.6	10.7	16.5	9	20.8	8.3	16.0	7.1	16.5
7	2.896	25.7	11.9	17.2	9.5	21.1	9.2	18.6	7.9	18.2
8	1.844	22.9	10	15.5	8.1	20	8.2	15.3	7.2	15.8
9	2.481	24.6	11.5	16.9	9	19.8	8.5	16	7.5	16.8
10	2.012	26	11	16.7	9	22.6	9	16	7.6	16.8
11	2.7	19.5	12	18.7	9.5	22.4	9.4	16.8	8	17.8
12	2.105	18.9	10.5	16.6	8.3	21.5	8.9	15.6	7.6	16.2
13	4.401	31	13.5	21.3	12	25.9	10.5	19.7	9	21.1
14	1.991	21.1	9.7	15	8.5	19.4	8	15.2	7	16
15	0.355	12.6	5.8	9	4.8	12.2	4.9	9	4.1	9
16	1.308	19.1	8.5	14.4	8	17.5	7.4	14.5	6.1	14.4
17	0.588	11.9	7.0	12.8	6	14.4	6	12.1	5.5	11.5
18	2.465	25.2	11	17.7	9	21.7	9.1	16.9	7.9	17
19	2.652	26.2	12	20.4	8.9	26.6	9	16.9	8.4	17.2
20	1.131	17.6	8	13.5	6.6	16.2	7	15	6.7	13.4
21	1.610	21.7	9.2	15.8	8.2	19.5	8.5	15.1	6.7	15
22	0.383	13.2	6.2	9.7	5	12.9	5.2	9.5	4.5	9.5
23	2.014	27.2	11.5	17.4	9	23.3	9	18.6	7.5	17
24	0.701	15.5	7.5	11.9	6.5	15.3	6.4	12.1	5.2	12
25	1.926	24.4	10.5	16.2	8.5	19.7	8.1	15.3	7	15.7
26	2.047	23.7	10	19.1	8.7	22.1	8.7	18.4	7.9	17.9
27	2.182	24	11	15.9	8.2	19.7	8.2	15	6.8	16.4
28	1.440	21	9	14.4	7.6	20.5	8.2	16.1	7.4	14.9
29	2.207	22.9	10.5	16.2	8.6	19.1	8	15	6.8	16.5
30	1.836	22.9	9.8	14.8	7.5	19.1	7.6	15	7	15
31	2.016	23.8	10	17.2	8.9	21.7	8.6	15.4	7.4	16.4
32	1.918	22.3	10.6	16.1	8	19.9	8.2	15	6.7	15.2
33	1.353	21	9	15	7.6	18	7.8	15	6.9	14.5
34	1.883	21.4	10	14.5	7.4	20.5	8.1	14.5	6.4	15
35	1.927	22.3	9.4	14.8	8.3	21.4	8.4	14.2	6.6	15.7
36	2.001	24	10.2	16	8.4	19.6	8	15.4	7.2	16
37	1.573	22.5	10.2	17.2	8.4	19.6	8	16.2	7.1	15.8