ORIGINAL ARTICLE Mirror of Research in Veterinary Sciences and Animals MRVSA/ Open Access DOAJ

Incidence of *Mycobacteria spp*. in shrimp in Iraq

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ARTICLE INFO
Received: 25.10.2014
Revised: 22. 11.2014
Accepted: 25.11.2014
Publish online: 29. 11.2014

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Abstract
This study was designed to determine the incidence of mycobacteria in shrimp. Totally, 162 Shrimp samples (every sample was batch of 3 shrimp) were collected from Basra Governorate (24 fresh samples) and (30 frozen samples) from Baghdad. The samples were cultured on special media for *mycobacteria* (Lowenstein – jensen medium) and incubated at 25 ºC for 8 weeks. Diagnosis of Mycobacteria species was based on rate of growth, colonies morphology, direct microscopic examination stained by acid-fast stain. The results revealed growth of bacterial isolates during 2-6 weeks that morphologically resemble mycobacterial colonies. Microscopically, acid-fast Ziehl-Neelsen staining bacteria showed red bacilli. Also, the results revealed 11 (20.3%) isolates out of 54 samples. The isolates were 9 (16.6%) of fresh samples and 2 (3.7%) from frozen samples. This is the first record of the occurrence of acid-fast bacterial infection in species of shrimp in Iraq.

To cite this article: Waffa, A. Ahmed; Nagham. M. AL-Gbouri; Maysoon, S. Abbas (2014). Incidence of Mycobacteria spp. in shrimp in Iraq. MRVSA. 3 (3), 24-32.
DOI: 10.22428/mrvsa. 2307-8073.2014. 00333.x

Keywords: Diagnosis, food, Iraq, *Mycobacteria*, shrimp

Introduction

Nontuberculous mycobacteria (NTM) are transmitted to humans from the environment, through ingestion of food. They have been isolated from beef, pork, lamb (Tison *et al.*, 1966), milk and other dairy products (Dunn and Hodgson, 1982; Tacquet *et al.*, 1966; Thomas and McDurmont, 1975; Wolinsky, 1979, Wolinsky, 1992; Yajko *et al.*, 1995), water (Andern *et al.*, 1983, Du moulin and Stottmeier, 1986, von Reyn *et al.*, 1993), vegetables (broccoli, spinach, and lettuce), fruits (cherries, pomegranates, and apples), herbs (basil and parsley) (Yoder *et al.*, 1999), preserves and brine (Tison *et al.*, 1966) and oysters (Thomas and McDurmont, 1975). In addition, NTM has been isolated from fish such as Pacific salmon

(Arakawa *et al.,* 1986) and Channastriatus (Chinabut *et al.,* 1990). So these food samples were considered as sources of human infection or colonization.

A genus Mycobacteria contains species that cause human leprosy, and tuberculosis. They are ubiquitous in the aquatic environment. Seafood-associated mycobacteria include *M. marinum, M. fortuitum, M. chelonae, M. shottsi*, and others. *M. marinum* is perhaps the most well-known of these, the organism causes disease in both fish and shrimp. It can result in devastating losses in aquaculture facilities. The human, illness might be occurred during exposure of the skin (wound) to infected shrimp, contaminated water and sometimes after cleaning aquariums. The fish infection is sometimes known as fish TB, and in shrimp as shrimp TB. Whereas, infection in human has been called fish tank granuloma, fish handler's disease, and swimming pool granuloma. This terminology can be confusing and overlaps with several distinct illnesses (Daniel, 2005). Other species of atypical (or non-tuberculous) mycobacteria are saprophytes in the aquatic environments, such as *M. fortuitum* and *M. chelonae,* (Biondi, 1982). These species have been reported as etiological agents of human skin infection over the world (Wolinsky, 1979; Rosenmeier, 1991; Inglis, 1993; Hautmann and Lotti, 1994; Campo *et al.,* 2003). Cutaneous mycobacterial infections associated with shrimp have been previously reported, with both *Mycobacterium kansasii* and *M marinum* (Owens, 1969; Miller, 1973). In 1972 and 1986, researcher mentioned that aquatic animals such as fresh or saltwater fish, snails, shellfish, dolphins, shrimps and water fleas can be considered as vectors of human skin infection (Huminer, 1986; Jolly and Seabury, 1972). An increasing number of cases have been reported from most countries with temperate climates (Falkinham., 1996). Predisposing occupations and activities include fishery worker, seafood handler, fish-tank owner, fisherman, pet shop worker, and water-related recreational exposure (Dobos *et al.,* 1999).

So far as we are aware, no articles have been published regarding the isolation and identification of *Mycobacteria spp.* from shrimp in Iraq. So this study was designed to determine the incidence of mycobacteria in shrimp.

Material and methods

A total 162 (every sample was a batch of 3 shrimp) (24 sample from fresh shrimp from Basra governorate and 30 samples from frozen shrimp from Baghdad governorate), were transported in closed plastic box to the laboratory. Subsequently, samples were subjected to a careful external and internal examination to detect the presence of any obvious disease signs. Each sample was homogenized with sterile Phosphate Buffer Saline (PBS). Then, the suspension was treated with 4% sodium hydroxide solution, and the mixture was incubated at 37 ^{o}C for 15 min with occasional shaking. The suspension was centrifuged and the supernatant removed. Then, the sediment suspended in 4 ml sterile distilled water and centrifuged at 3000 x g for 10 mins. The sediment was mixed with 1 ml of sterile distilled water, and 0.1 ml of this solution was used as an inoculum on the Lowenstein –jensen medium and cultured at 25 ^{o}C for 8 weeks. The samples were observed weekly (de Kantor and Laszlo, 1998; Laidlaw, 1989; Watt *et al.,* 1996). The presence of mycobacteria in the positive cultures was confirmed by; the colony morphology on Lowenstein-

Jensen medium, Ziehl-Neelsen staining method and time of growth through the weeks (Quinn *et al.,* 2006).

Results

Microscopic examination

Stained smears prepared from the sediment of the homogenized samples and from growth colonies, revealed red staining rods with different length. The isolation percentages were 11 (20.3%) out of 54 samples and 9 (16.6%) of fresh samples. While, it was 2 (3.7%) of frozen samples in different growth time from 2-6 week (Table.1).

Colony morphology

The isolated colonies appeared as whitish, sticky small colonies and broken up easily. Some of the colonies were smooth however, others were rough or intermediate. The colonies were appeared as pale yellow, buff or tan pigment colonies on LJ medium.

Table 1. Mycobacterium spp. incidence in fresh and frozen shrimp by culturing between 2-6 weeks

Positive samples	Types	Growth Time/ week	Percentage %
1	Fresh	6 weeks	16.6
2	Fresh	6 weeks	
3	Fresh	2 weeks	
4	Fresh	4 weeks	
5	Fresh	2 weeks	
8	Fresh	6 weeks	
9	Fresh	6 weeks	
11	Fresh	4 weeks	
12	Fresh	4 weeks	
46	Frozen	6 weeks	3.7
47	Frozen	4 weeks	
Total 11	9/2	Between 2-6 weeks	20.3

Discussion

The demand in the consuming the natural and fresh food has increased recently between populations. There is a growing concern about the nutritional food losses upon processing and possible health risk of chemical preservatives. The number of foodborne illness remains a public concern, despite the improvement in the manufacturing standard and effective legislative control on processing procedures (Tharmaraj and Shah, 2009). The characteristics features of colony morphology,

including the appearance of different lengths red staining rods by microscopic examination of the acid-fast stain smear from the centrifuged sediment were in agreement with (Quinn *et al.,* 2006). Colony morphology of MA complex, exist in 3 forms; smooth transparent, smooth opaque and rough opaque (Ryan and Ray, 2004). *M.avium*, *M intracellular* and sometimes *M.scrofulaceum* are grouped together because there was no mean to distinguish one species from another (Thegerstrom, 2009).

The results of this study revealed 11 (20.3%) isolate out of 54 samples, 9 (16.6%) from fresh samples, and 2 (3.7%) from frozen samples.

The percentages of isolation of Mycobacterium in this study from the fresh samples was higher than from the frozen samples. This results may be attributed to the cleaning and shelling of the frozen samples. Also, the number of microorganisms were killed by freezing and thawing (Sokatch and Ferreti, 1979).

Mycobacteria can survive under environmental conditions. They are intolerable for most other bacterial genera, including temperatures below 0°C. These strains are known to have remained viable in nutrient broth at -70°C for years (Iivanainen *et al*., 1995; Kim and Kubica, 1973). This may be due to the specific properties of their cell walls, such as high lipid content and therefore hydrophobicity, which renders them resistant to changes in environmental conditions (Ratledge, 1982). Due to the association between mycobacteria and a variety of different aqueous environments, it seems reasonable to believe that these organisms may occur in frozen foods, including shrimp widely consumed by humans (Carson, 1978; Falkinham, 1996; Slosa´rek *et al.,* 1993). Mycobacterial colonies appeared within 2-6 weeks on *L.J* medium this indicate that these mycobacteria were of the slow grower that may be *M. marinium* ,*M. kensasii, M. simiae, M. scrofulaceum, M. avium intracellular, Mulcerance*or *M. xenopi* (Quinn *et al.,* 2006). The contamination and growth of psychotropic and pathogenic spoilage microorganisms in refrigerated foods is a major risk in the food industry (Quinn and Markey 2003). Mycobacteria are widely distributed in both fresh and marine waters. It includes pathogenic species to marine animals and humans (Collins *et al.,* 1984; Falkinham, 1996; Dailloux *et al.,* 1999). Isolation of *Mycobacterium* from shrimp indicated that the shrimp carry or infected with this microorganism. However, many studies reported *Mycobacterium* infection from crustaceans such as, white shrimp, *Penaeus vannamei* (Lightner and Redman, 1986; Mohney *et al.,* 1998), *Macrobrachium rosenbergi* (Brock *et al.,* 1986; Lightner, 1996). LeBlanc *et al.,* (2012) also isolated *Mycobacterium marinum* infection from sea monkeys (type of shrimp). This infection originated from the direct inoculation of bacteria into skin and wounds, or ingestion of contaminated shrimp. Shrimp-vectored human infections can be grouped into two types according, to the route of entry and subsequent site of infection those originating from direct inoculation of skin and wounds, and those from ingestion of contaminated shrimp. Wound infections can be caused by bacteria that also cause disease in shrimp or those that are incident a line the marine environment from which the shrimp are harvested and processed. Cleaning and shelling shrimp can result in small lacerations or puncture wounds, especially on the hands, which provide sites of entry for the bacteria. Shrimp consumers may also be at a slight risk when preparing shrimp for the table (Daniel, 2005). *M. marinum* cutaneous infections have been reported from

exposure to contaminated water in aquariums or unchlorinated swimming pools, this has led to the nickname of 'swimming pool granuloma' or 'fish tank granuloma' (Bhambri *et al.,* 2009; Griffith *et al.,* 2007). Identified vectors include dolphins, snails, water fleas, saltwater and freshwater fish, oysters and shrimp (Bhambri *et al.,* 2009; Bhatty *et al.,* 2000; Jolly and Seabury 1972).Cutaneous mycobacterial infections associated with shrimp have been previously reported, with both *Mycobacterium kansasii* and *M marinum* (Owens and McBride, 1969; Miller, 1973). A review of the literature by Jernigan and Farr, (2000) identified nine cases of *M marinum* infection secondary to injury associated with shrimp. Many species of nontuberculous mycobacterium can cause extra pulmonary infections such lymphadenitis and osteoarticular infections in immunocompetent persons (Claudio and Claudio, 2009).

In conclusion, the results of this study revealed the incidence of *Mycobacteria* in the fresh and frozen shrimp, for the first time in Iraq. However, other studies need to be done and further microbiological and molecular investigation need to confirm and identifi the *Mycobacteria spp*. in shrimp.

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