



Occurrence of Potentially Pathogenic *Vibrio* and related species in Seafoods obtained from the Eastern Province of Saudi Arabia

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Abstract

Seafoods are liable to bacterial contamination and could cause health risk to consumers. The present work is aimed to study the occurrence of potentially pathogenic and related species in seafoods obtained from the Eastern Province of Saudi Arabia. Studies were carried out to isolate, identify and characterized different bacterial species in 448 seafood samples comprising Fishes (353), Prawns (49) and Cuttlefishes (46), collected during the period from January 2015 until May 2016. TCBS agar was used for isolation and presumptive colonies both suspicious and non-suspicious for *Vibrios* were subcultured, characterized by biochemical tests and then identified at the genus and species level using API 20NE test kits. Species belongs to 8 bacterial families were isolated and the most predominant was *Vibrionaceae* 262 (58.4 %), followed by *Aeromonadaceae* 45 (10.4 %), *Shewanellaceae* 16 (3.57 %), *Pasteurellaceae* 13 (2.90 %), *Caulobacteriaceae* 9 (2.00 %), *Pseudomonadaceae* 7 (1.56 %), *Enterobacteriaceae* 7 (1.56 %) and *Burkholderiaceae* 6 (1.33 %). The presence of these organisms in the fresh seafood samples showed that the source of contamination may be from environment, catching, unhygienic handling and transportation. Thus it is strongly recommended that fresh seafoods must be properly stored at low temperature, good hygienic practices must be strictly followed and the seafoods must be adequately subjected to proper cooking before consumption.

Keywords: Seafoods, Potential pathogen, Contamination, API, Hygienic practices.

Introduction

Pathogenic bacteria in Seafoods may be classified in to two groups: the indigenous bacteria and non-indigenous bacteria. The indigenous bacteria consist of *Clostridium botulinum*, *Vibrio cholerae*, *Aeromonas hydrophila* and *Plesiomonas shigelloides*. These bacteria are widely distributed in the aquatic environment in various parts of the world (Falana, 2005).

Vibrios are among the most common surface organisms found in the surface waters of the world.

They occur in associations with aquatic animals of both marine and freshwater habitats. Some species are found to be potential pathogens of fishes, eels and frogs as well as other vertebrates and invertebrates (Todar, 2005)

Vibrios are gram negative facultative anaerobic organisms that are natural inhabitants of the marine environments. They are curved, motile by means of a single polar flagellum. Consumption of raw or undercooked shellfish are the main causes of transmission of *Vibrio* infection in the United States.

Patients with liver disease are at high risk with these infections which may result in the morbidity and mortality (Nicholas *et al.*, 2000).

Vibrios are naturally present in the marine environment and are particularly resistant to high salt concentrations. A number of species within the genus *Vibrios* are associated with food borne infections and food spoilage. However, only a few of the species are more specifically pathogens to humans, such as *Vibrio cholerae*, *Vibrio mimicus*, *Vibrio metschnikovii*, *Vibrio cincinnatiensis*, *Vibrio hollisae*, *Vibrio damsela*, *Vibrio fluvialis*, *Vibrio furnissi*, *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Vibrio carchariae* (Igbiosa, 2010; Adagbada *et al.*, 2012; Farmer *et al.*, 2003).

Italian anatomist Filippo Pacini in 1854 was first isolated *Vibrio cholerae* as the cause of cholera, but his discovery was not widely known. Thirty years later, Robert Koch brought the knowledge about this bacterium to the public notice by his works and the means of fighting the disease (Bentivoglio and Pacini, 1995). *Vibrio cholerae* is a waterborne pathogen that causes gastro intestinal disorders with a wide range of clinical manifestations, including vomiting and rice water like diarrhea (Madden *et al.*, 1989).

Vibrio parahaemolyticus is a halophilic bacterium which has been isolated from many species of fish, shellfish and crustaceans. It has a worldwide distribution in warm climate estuarine waters and found naturally in coastal environments (Oliver and Kaper, 1997). Regarding the public health hazard, *Vibrios* have been implicated in food poisoning and gastroenteritis (Okonko *et al.*, 2011). One of the leading causes of food borne gastroenteritis associated with the ingestion of undercooked shellfish throughout the world including the United States, China, Japan and Korea is by *Vibrio parahaemolyticus* (Merward *et al.*, 2011; Liu *et al.*, 2004, Su and Liu, 2007). This bacterium has been frequently involved in outbreaks of food-borne diseases worldwide (Crump *et al.*, 2008, Dalsgaard *et al.*, 2009).

V. parahaemolyticus has been involved in several outbreaks of Seafood-borne gastroenteritis. Although, not all the strains are considered as pathogenic. Both pathogenic and non-pathogenic forms of the organism can be isolated from marine and estuarine environments and from fish and shellfish dwelling in these environments (Sakazaki *et al.*, 1968).

The most common occurring *Vibrio* species in the marine environments and seafoods are *Vibrio alginolyticus* (Gopal *et al.*, 2005; Colakoguet *et al.*, 2006). This is an opportunistic pathogen and its pathogenicity is thought to be similar to that of *V. parahaemolyticus* (Gonzalez – Escalona *et al.*, 2005). *Vibrio alginolyticus* can be easily isolated from fish, clams, crabs, oyster, mussels and shrimp, as well as seawater throughout the world often at high numbers. Results of many surveys have revealed that the *Vibrio alginolyticus* is one of the most commonly isolated *Vibrios* and according to some published reports, it can cause the symptoms of gastroenteric disease (Oliver and Kaper, 1997).

Genus *Aeromonas* comprise non-motile psychrophilic and motile mesophilic Gram negative bacteria and include 15 species, being distributed ubiquitously in aquatic environments and are of increasing importance as seafood and waterborne pathogens (Janda *et al.*, 2010).

Spoilage of fish is mainly due to the activity of psychrotrophic gram-negative bacteria such as *Shewanella putrifaciens* and *Psuedomonas* spp. *Shewanella putrifaciens* has been identified as the specific spoilage bacteria of marine temperate-water fish stored aerobically in ice. Some *Psuedomonas* spp. are the specific spoilers of iced stored tropical freshwater fish (Lima dos santos, 1978; Gram *et al.*, 1990) and are also, together with *Shewanella putrifaciens*, spoilers of marine tropical fish stored in ice (Gillespie and Mac Rae, 1975; Gram *et al.*, 1990). At ambient temperature, motile *Aeromonads* are the specific spoilers at aerobically stored freshwater fish (Gorzyka and PekPoh Len, 1985; Gram *et al.*, 1990). Barile *et al.*, (1985) found that a large population of the flora at ambient-stored Mackerel consisted of *Shewanella putrifaciens*; this indicates that the bacterium may also take part in spoilage of fish stored at ambient temperature (Ofred, 2009).

The aim of this study is to screen seafoods and to determine the incidence of clinically important *Vibrios* and related species in seafood samples collected from the Eastern province of Saudi Arabia. And to investigate the occurrence of *Vibrio* and related species in seafood products and to make recommendations to reduce risk associated with the consumption of seafood.

Materials and Methods

This study was carried out during the period from January 2015 until May 2016. A total of 448 samples comprising Fishes (353), Prawns (49) and Cuttlefishes (46) were randomly collected from Dammam retail and wholesale fish markets. The collected samples were individually kept in sterile polyethylene bags embedded with ice and transported rapidly to the laboratory in an insulated box.

Sample processing for bacteriological examination

Bacteriological analysis of collected sea foods were performed within 2 hours of sampling. The detection of pathogenic *Vibrio* species was achieved according to the standard methods adopted from Online Bacteriological Analytical Manual, USFDA (BAM 8th edition Online). Approximately 25 g of each sample were homogenized in 225 ml of Alkaline Peptone Water (APW) with 2% NaCl and incubated overnight at 35 ± 2°C. After incubation, a loop full of the pre-enriched culture was streaked on to Thiosulphate Citrate Bile salt Sucrose (TCBS) Agar. After

incubation at 35 ± 2°C for 18 – 24 hours, presumptive colonies both suspicious and non-suspicious for *Vibrios* were sub cultured into Tryptone Soya Agar plates with and without 2% added NaCl. After incubation the purified colonies were characterized by biochemical tests which included Gram staining, Motility, Oxidase production and then identified at the genus and species level using API 20NE (Biomerieux, France) test kits. The identification was obtained by using the identification software “api web”.

Results and Discussion

This study was conducted to determine the incidence of *Vibrio* and related species in Seafoods (353 Fishes, 49 Prawn and 46 Cuttlefish) for sale in wholesale and retail outlets of Fish markets in Eastern province of Saudi Arabia. The results related to the incidence and identification at the species level of *Vibrio* and related species from Fishes, Prawn and Cuttlefish are summarized in Table 1 and Table 2. The overall percentages of prevalent bacterial families are shown in Table 3.

Table 1: List of Bacterial species Isolated from Fishes, Prawn and Cuttlefish

FISHES	PRAWN	CUTTLEFISH
<i>Vibrio alginolyticus</i>	<i>Vibrio alginolyticus</i>	<i>Vibrio alginolyticus</i>
<i>Vibrio parahaemolyticus</i>	<i>Vibrio parahaemolyticus</i>	<i>Vibrio parahaemolyticus</i>
<i>Vibrio fluvialis</i>	<i>Vibrio cholerae</i>	<i>Vibrio vulnificus</i>
<i>Vibrio vulnificus</i>	<i>Vibrio vulnificus</i>	<i>Vibrio cholerae</i>
<i>Vibrio cholerae</i>	<i>Shewanella putrefaciens</i>	<i>Aeromonas hydrophila</i>
<i>Aeromonas hydrophila</i>	<i>Aeromonas hydrophila</i>	<i>Aeromonas sobria</i>
<i>Aeromonas sobria</i>	<i>Pseudomonas alcaligenes</i>	<i>Photobacterium damsela</i>
<i>Aeromonas salmonicida</i>	<i>Photobacterium damsela</i>	<i>Plesiomonas shigelloides</i>
<i>Pasteurella pneumotropica</i>	<i>Pasteurella pneumotropica</i>	<i>Pasteurella pneumotropica</i>
<i>Pasteurella aerogenes</i>	<i>Pasteurella aerogenes</i>	<i>Pseudomonas luteola</i>
<i>Pseudomonas luteola</i>		
<i>Pseudomonas alcaligenes</i>		
<i>Brevundimonas vesicularis</i>		
<i>Brevundimonas diminuta</i>		
<i>Plesiomonas shigelloides</i>		
<i>Photobacterium damsela</i>		
<i>Shewanella Putrefaciens</i>		
<i>Burkholderia cepacia</i>		

Table 2: Prevalence of Bacterial species among Fishes, Prawn and Cuttlefish

S.No	Bacterial Isolates	Name of the Sample			Total Prevalence
		Fishes	Prawn	Cuttlefish	
01.	<i>Vibrio alginolyticus</i>	94	21	15	130
02.	<i>Vibrio parahaemolyticus</i>	27	10	08	045
03.	<i>Vibrio cholerae</i>	29	01	01	031
04.	<i>Vibrio vulnificus</i>	16	02	03	021
05.	<i>Vibrio fluvialis</i>	12	---	---	012
06.	<i>Photobacterium damsela</i>	16	04	03	023
07.	<i>Aeromonas hydrophila</i>	26	03	07	036
08.	<i>Aeromonas sobria</i>	06	---	02	008
09.	<i>Aeromonas salmonicida</i>	01	---	---	001
10.	<i>Pasteurella aerogenes</i>	05	01	---	006
11.	<i>Pasteurella pneumotropica</i>	04	02	01	007
12.	<i>Pseudomonas alcaligenes</i>	03	01	---	004
13.	<i>Pseudomonas luteola</i>	02	---	01	003
14.	<i>Brevundimonas diminuta</i>	08	---	---	008
15.	<i>Brevundimonas vesicularis</i>	01	---	---	001
16.	<i>Shewanella putrefaciens</i>	13	03	---	016
17.	<i>Plesiomonas shigelloides</i>	06	---	01	007
18.	<i>Burkholderia cepacia</i>	06	---	---	006
TOTAL NUMBER OF ISOLATES		275	48	42	365

Fig. 1: Dammam Fish Market in the Eastern Province of Saudi Arabia

Out of 448 samples analysed, 262 (58.48 %) samples were found to be contaminated with *Vibrionaceae* with the prevalence of *Vibrio alginolyticus* (130), *Vibrio parahaemolyticus* (45), *Vibrio cholerae* (31), *Photobacterium damsela* (23), *Vibrio vulnificus* (21) and *Vibrio fluvialis* (12). The ranges of incidence levels of *Vibrionaceae* were from Fishes (194 out of 353), Prawn (38 out of 49) and Cuttlefish (30 out of

46). From these 194 *Vibrionaceae* isolated in Fishes, 94 samples were found to be contaminated with *Vibrio alginolyticus*, followed by 29 samples with *Vibrio cholerae*, 27 samples with *Vibrio parahaemolyticus*, 16 samples with *Vibrio vulnificus*, 16 samples with *Photobacterium damsela* and 12 samples with *Vibrio vulnificus*.

Table 3: The Overall Percentage of Prevalent Bacterial Families among Fishes, Prawn and Cuttlefish

S. No	Bacterial Isolates	Bacterial Family	Overall % of Prevalence in Samples			No. % for Total 448 Samples
			Fishes (353)	Prawn (49)	Cuttlefish (46)	
01.	<i>Vibrio alginolyticus</i>	<i>Vibrionaceae</i>	194 (54.95 %)	38 (77.55 %)	30 (65.21 %)	262 (58.48 %)
	<i>Vibrio parahaemolyticus</i>					
	<i>Vibrio cholerae</i>					
	<i>Vibrio vulnificus</i>					
	<i>Vibrio fluvialis</i>					
	<i>Photobacterium damsela</i>					
02.	<i>Aeromonas hydrophila</i>	<i>Aeromonadaceae</i>	33 (9.34 %)	3 (6.12 %)	9 (19.56 %)	45 (10.04 %)
	<i>Aeromonas sobria</i>					
	<i>Aeromonas salmonicida</i>					
03.	<i>Pasteurella aerogenes</i>	<i>Pasteurellaceae</i>	9 (2.54 %)	3 (6.12 %)	1 (2.17 %)	13 (2.90 %)
	<i>Pasteurella pneumotropica</i>					
04.	<i>Pseudomonas alcaligenes</i>	<i>Pseudomonadaceae</i>	5 (1.41 %)	1 (2.04 %)	1 (2.17 %)	7 (1.56 %)
	<i>Pseudomonas luteola</i>					
05.	<i>Brevundimonas diminuta</i>	<i>Caulobacteriaceae</i>	9 (2.54 %)	0	0	9 (2.00 %)
	<i>Brevundimonas vesicularis</i>					
06.	<i>Shewanella putrefaciens</i>	<i>Shewanellaceae</i>	13 (3.68 %)	3 (6.12 %)	0	16 (3.57 %)
07.	<i>Plesiomonas shigelloides</i>	<i>Enterobacteriaceae</i>	6 (1.69 %)	0	1 (2.17 %)	7 (1.56 %)
08.	<i>Burkholderia cepacia</i>	<i>Burkholderiaceae</i>	6 (1.69 %)	0	0	6 (1.33 %)
TOTAL PREVALENCE OF BACTERIAL ISOLATES			275 (77.90 %)	48 (97.95 %)	42 (91.30 %)	365 (81.47 %)

Prawns were found to be contaminated by *Vibrionaceae* in 38 samples. Out of these, 21 samples were found with *Vibrio alginolyticus*, followed by 10 samples with *Vibrio parahaemolyticus*, 4 samples with *Photobacterium damsela*, 2 samples with *Vibrio vulnificus* and 1 sample with *Vibrio cholerae*. None of the Prawn samples were found to be contaminated with *Vibrio fluvialis*.

30 samples of Cuttlefish were found to be contaminated by *Vibrionaceae* with the prevalence of *Vibrio alginolyticus* in 15 samples, followed by 8 samples with *Vibrio parahaemolyticus*, 3 samples with *Vibrio vulnificus*, 3 samples with *Photobacterium damsela* and 1 sample with *Vibrio cholerae*. No cuttlefish were found to be contaminated with *Vibrio fluvialis*.

Seafood products harvested from contaminated waters or which have been improperly preserved after harvesting are known to play an important role in infections by *Vibrio* spp. (Baffone *et al.*, 2000). The percentage of incidence is slightly lower when compared to the observations of TulayElalMuset *et al* (2014) who isolated *Vibrio* species in 67 % of total count of the seafood samples with overall incidence in the samples as *Vibrio alginolyticus* (37 isolates) followed by *Vibrio parahaemolyticus* (28 isolates), *Vibrio cholerae* (1 isolate) and *Vibrio vulnificus* (1 isolate). Hadi *et al* (2004) detected the presence of potentially pathogenic *Vibrio* species with overall incidence in the samples as 11 % for *Vibrio alginolyticus*, 7.6 % for *Vibrio fluvialis*, 6 % for *Vibrio vulnificus*, 4.7 % for *Vibrio parahaemolyticus* and 4.6 % for *Vibrio cholerae*. Adeleye *et al* (2009) reported the occurrence of the *Vibrio* species in the seafood samples as *Vibrio alginolyticus* (31.8 %), followed by *Vibrio harveyi* (27.3 %), *Vibrio mimicus* (22.7 %), *Vibrio parahaemolyticus* (11.4 %) and *Vibrio cholerae* (6.8 %).

Vibrio alginolyticus was the most frequently isolated *Vibrio* spp. (52.5 %) in the study conducted by Wafaa MK Bakr *et al* (2011), followed by *Vibrio parahaemolyticus* (14.1 %) and *Vibrio mimicus* (11.5 %) from seafoods. Toti *et al* (1996), has been reported *Vibrio alginolyticus* to be the most common species in Europe and North America. On the other hand, Gopal *et al* (2005), studied for the abundance of *Vibrio* spp. in multiple shrimp farm environments from the east and west coast of India. This study revealed the dominance of *Vibrio alginolyticus* (19 %), followed by *Vibrio parahaemolyticus* (13 %), *Vibrio cincinnatiensis* (7 %) in west coast samples, compared

with east coast samples which accounted for *Vibrio alginolyticus* (4 %) and *Vibrio parahaemolyticus* (3 %). Thararatchitov *et al* (2009), reported that the contamination of raw seafood by *Vibrio alginolyticus* was most frequent (61.5 %), followed by *Vibrio parahaemolyticus* (43.6 %), *Vibrio cholerae* (35.9 %), *Vibrio mimicus* (23.1 %) and *Vibrio vulnificus* (2.6 %). The present study revealed that the most dominant *Vibrio* spp. isolated from analysed seafoods was *Vibrio alginolyticus*, which was in agreement with the studies conducted by different researchers from all over the world.

In contrast, Okonko *et al* (2011), isolated 53 *Vibrio* species in seafoods with the predominance of *Vibrio cholerae* (47.2 %), *Vibrio parahaemolyticus* (18.9 %), *Vibrio mimicus* (15.1 %), *Vibrio fluvialis* (13.2 %), *Vibrio alginolyticus* (3.8 %) and *Vibrio vulnificus* (1.9 %). *Vibrios* are responsible for a number of clinical conditions such as cholera, gastroenteritis, septicaemia and wound infections. Twelve *Vibrio* species have been documented as potential food-borne disease agents in humans: *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Vibrio alginolyticus*, *Vibrio furnissi*, *Vibrio fluvialis*, *Vibrio damsel*, *Vibrio mimicus*, *Vibrio hollisae*, *Vibrio cincinnatiensis*, *Vibrio harveyi* and *Vibrio metschnikovii* (Shikongo *et al*, 2012).

Prevalence of *Aeromonadaceae* in analysed samples was around 45 out of 448 total samples. The most dominant isolates were *Aeromonas hydrophila* (36), followed by *Aeromonas sobria* (8) and *Aeromonas salmonicida* (1). The ranges of incidence levels were from Fishes (33 out of 353), Cuttlefish (9 out of 46) and Prawn (3 out of 49). In these 33 *Aeromonads* contaminated Fishes, 26 samples were found to be contaminated with *Aeromonas hydrophila*, followed by 6 samples with *Aeromonas sobria* and 1 sample with *Aeromonas salmonicida*. Total *Aeromonads* isolated from Cuttlefish is around 9 with the prevalence of *Aeromonas hydrophila* in 7 samples followed by *Aeromonas sobria* in 2 samples. *Aeromonas salmonicida* was not found in any of the analysed samples of Cuttlefish. Only 3 Prawn samples (out of 49) were found to be contaminated with *Aeromonas hydrophila*. Both *Aeromonas sobria* and *Aeromonas salmonicida* were found to be absent in analysed Prawn samples.

The genus *Aeromonas* comprises of two different groups of bacteria. One is non-motile psychrophilic *Aeromonas salmonicida* and the other group comprising of three mesophilic motile spp.,

Aeromonas hydrophila, *Aeromonas caviae* and *Aeromonas sobria* (Praveen *et al.*, 2014). AlphonsaVijaya Joseph *et al* (2013) reported the prevalence of *Aeromonads* in seafoods and its environments and assigned the isolates of *Aeromonads* in to five species namely, *Aeromonas hydrophila* (33.3 %), *Aeromonas enteropelogenes* (26.6 %), *Aeromonas punctate* (20 %), *Aeromonas caviae* (13.3 %) and *Aeromonas aquarorium* (6.6 %). Out of 99 fish samples 22 (22.22 %) were found positive, in which *Aeromonas hydrophila* (66.6 %), *Aeromonas sobria* (27.27 %), *Aeromonas caviae* (9.09 %) were found positive (Leitao and Silveira, 1991). And out of 536 fishes and 278 prawns analysed, total of 319 strains of *Aeromonas hydrophila* were isolated (Vivekanandhan *et al.*, 2005).

Differences in the occurrence level of *Aeromonas hydrophila* in the seafood of different parts of the world can be in regard to secondary contamination while handling, storage and transportation. Water has frequently been shown to be contaminated with *Aeromonas* species and it is likely that contaminated water may have contributed to the high incidence (Vivekanandhan *et al.*, 2005). The dominance of *Aeromonas hydrophila* in seafood samples over other species is in accordance with earlier studies, where it was the predominant species in the fish samples analysed (Hatha *et al.*, 2005; and Yoganath *et al.*, 2009).

In contrast Yucel *et al* (2005), isolated 132 *Aeromonas* species from the marketed fishes in Ankara, turkey; predominant being *Aeromonas caviae* (66 %), followed by *Aeromonas hydrophila* (22.6 %) and *Aeromonas veronii* biovar *sobria* (11.6 %). A study of 87 fish samples was done and reported that out of 87 fish samples 60 (69 %) were found positive for *Aeromonas* species. Out of 60 isolates obtained, *Aeromonas veronii* biovar *sobria* (48), *Aeromonas hydrophila* (10) and *Aeromonas caviae* were found in 2 isolates. Some of the species of motile *Aeromonads* became emerging pathogens due to their ability to produce virulence factors, not only at optimum temperature but also under cold storage conditions (Neyts *et al.*, 2000). *Aeromonas* spp. are normal inhabitants of the aquatic medium. Due to the incidence of human disease caused by *Aeromonas* after consumption of the contaminated foods, it was considered as a human pathogen. Their ability to produce extracellular haemolysin, enterotoxins, cytotoxins, lipases and proteases are responsible for the spoilage of foods and pathogenicity (Farag, 2006).

Presence of *Pasteurellaceae*, *Caulobacteriaceae* and *Pseudomonadaceae* in analysed samples was around 13, 9 and 7 out of 448 total samples respectively. The isolates of *Pasteurella* spp were *Pasteurella pneumotropica* (7) and *Pasteurella aerogenes* (6). The ranges were from Fishes (9 out of 353), Prawn (3 out of 49) and Cuttlefish (1 out of 46). In Fishes, 5 samples were found to be contaminated with *Pasteurella aerogenes* and 4 samples were found with *Pasteurella pneumotropica*. In Prawns, 2 samples were found with *Pasteurella pneumotropica* and 1 sample was contaminated with *Pasteurella aerogenes*. But only 1 Cuttlefish was found to be contaminated with *Pasteurella pneumotropica*, whereas no samples were found with *Pasteurella aerogenes*. Two species of *Brevundimonas* namely *Brevundimonas diminuta* (8) and *Brevundimonas vesicularis* (1) which belongs to the family of *Caulobacteriaceae* were found to be contaminated in Fishes (9 out of 353) alone. Both the samples of Prawn and Cuttlefish were not found with *Brevundimonas* spp. Both of the *Brevundimonas* spp. are non-lactose fermenting environmental gram negative bacilli previously assigned to the genus *Pseudomonas* (Xiang and Roberto, 2005). All the above organisms can act as Opportunistic pathogens in humans with poor health and people with compromised immune systems.

Pseudomonas alcaligenes (4) and *Pseudomonas luteola* (3) are the two species which belongs to the family of *Pseudomonadaceae* were found to be contaminated in Fishes (5 out of 353), Prawn (1 out of 49) and Cuttlefish (1 out of 46). In these, 3 samples of Fishes and 1 sample of Prawn were found to be contaminated with *Pseudomonas alcaligenes* whereas, 2 samples of Fishes and 1 sample of Cuttlefish were found with *Pseudomonas luteola*. *Pseudomonas* spp. are one of the Specific Spoilage bacteria in fresh fish that produces various metabolites which are associated with spoilage. It can produce hypoxanthine from inosine or inosine monophosphate which come from the autolytic changes in dead fish and use them as a biosynthetic material to grow. The resulting products of such metabolic activities by these bacterial species render the fish unsuitable for human consumption (Huss, 1995).

Shewanella putrifaciens were isolated from 16 out of 448 samples, which includes Fishes (13 out of 353) and Prawn (3 out of 49), except Cuttlefish. It can be found in freshwater, brackish and salt water ecosystems. It is a main food spoilage bacterium in marine fish and is known to cause the rotting smell associated with dead fish because of its production of

trimethylamine. Infections from *Shewanella putrifaciens* mainly occurs in soft tissues such as skin, Intra-abdominal areas or in the blood (Pagani 2003; Mc Nair, 2010). *Plesiomonas shigelloides* were found in 6 samples of Fishes and 1 sample of Cuttlefish. It was absent in Prawn. *Plesiomonas shigelloides* is an emerging pathogen that is widespread in the aquatic environment. The natural reservoirs of this organism are water, fish and seafood in temperate and tropical climates (Miller *et al.*, 2006; Herrera *et al.*, 2006). In humans, it has been implicated in gastrointestinal infections (Escobar *et al.*, 2012). *Burkholderia cepacia* found to be contaminated only in Fishes at the range of 6 out of 353 samples. It was not found in both Prawn and Cuttlefish. *Burkholderia cepacia*, formerly known as *Pseudomonas cepacia*, is a gram-negative bacillus commonly found in soil, vegetation and water. It has emerged as an important opportunistic pathogen in immune compromised patients (Govan *et al.*, 1996; Holmes *et al.*, 1999)

Conclusion

The results of this present investigation revealed that the contamination of seafoods by *Vibrio* and related species in the Eastern Province of Saudi Arabia is considerably high. These contaminated seafoods can act as reservoirs of human pathogens which are serious threat to the seafood consuming community. Pathogenic species of the genus *Vibrio* and related species pose a considerable public health threat as the causative agents of both sporadic and epidemic human infections. The source of the organism may be from the environment where the fish caught, secondary contamination during catching, unhygienic handling and transportation may also contribute for its distribution. In view of the findings of this research work it is strongly recommended that fresh seafoods should be properly stored at low temperature, good hygienic conditions and use of clean water during processing must be strictly followed. Moreover, illness is associated by the ingestion of raw or undercooked seafoods. But *Vibrios* can be easily destroyed by heat and therefore proper cooking is sufficient to eliminate most *Vibrios*.

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