Non Cholera Vibrio as Causative Agent Of Chronic Suppurative Otitis Media in Saqr Hospital, Ras Al khaimah, United Arab Emirates

Hanadi Elias Hassan Hamad Alneil

B.Sc.in Medical Laboratory Sciences
Ahalia University (1994)

A Dissertation
Submitted to University of Gezira in Partial Fulfillment of The Requirements for the Award of the Degree of Master of Science in Microbiology

Department of Medical Microbiology
Faculty of Medical Laboratory
University of Gezira

January 2016
Non Cholera Vibrio as Causative Agent Of Chronic Suppurative Otitis Media in Saqr Hospital, Ras Al khaimah, United Arab Emirates

Hanadi Elias Hassan Hamad Alneil

Supervision Committee:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Al Amin Mohamed Ibrahim</td>
<td>Min Supervisor</td>
<td></td>
</tr>
<tr>
<td>Dr. Bakri Yousif Mohamed Nour</td>
<td>Co- Supervisor</td>
<td></td>
</tr>
</tbody>
</table>

Date: 30 / 01 / 2016
Non Cholera Vibrio as Causative Agent Of Chronic Suppurative Otitis Media in Saqr Hospital, Ras Al Khaimah, United Arab Emirates

Hanadi Elias Hassan Hamad Alneil

Examination Committee:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Al Amin</td>
<td>Chairman</td>
<td>.........................</td>
</tr>
<tr>
<td>Mohamed Ibrahim</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr. Mohammed Seed Ahmed</td>
<td>External</td>
<td>................................</td>
</tr>
<tr>
<td>Dr. Albadawi Talha</td>
<td>Examiner</td>
<td></td>
</tr>
</tbody>
</table>

Date: 30 / 01 / 2016
Declaration

This dissertation is my original work and has not been presented for a degree in any other University or for any other award.

Signature..........................Date........................
Dedicated

This research work is dedicated
to
My Father and My Mother soul in heaven, and
to
my whole family (Husband, Sons and Sisters)
who gave me enormous support
throughout the entire period of study. Let the
Almighty God bless them abundantly.
Acknowledgements

First, all thanks of ALLAH for giving me the power and – willing to complete this study. I am deeply grateful and my heartfelt thanks to Dr. Alamin Mohammed Ibrahim the supervisor of this Dissertation and Dr Fatima Mohammed Ahmed there have always been a source of inspiration and helped me through their guidance, constructive criticism, remarks and valuable suggestions to improve the quality of this Dissertation. My very special recognition is conveyed to my husband Mohammed Ibrahim; beloved son Ammar & Khalid who share much of my joy and sorrow. Words cannot express my gratitude for their love, support, patience, constant encouragement and understanding that have sustained me during the course of my post-graduate studies. I also acknowledge the unfailing support from my beloved sister who have given undoubted trust, motivation and emotional support during the postgraduate study. I owe deep gratitude also
indebted to a great many people without whose help it would have been quite impossible for me to present this finished work those are: Ebtissam Ali, Fatima Obaid, Alphonsamma and Hessa Hassan
Non Cholera Vibrio as Causative Agent Of Chronic Suppurative Otitis Media in Saqr Hospital, Ras Al Khaimah, United Arab Emirates

Hanadi Elias Hassan Hamad Alneil

Abstract
Chronic suppurative otitis media is mostly caused by common bacteria, fungi and virus and very rarely caused by unusual organisms like Vibrio cholerae. V.cholerae belonging to the non-O1, non-O139 serogroups are a human pathogen and natural inhabitant of aquatic environments. These bacteria can cause gastroenteritis and extraintestinal infections such as ear, wound infections and fatal septicemia and are transmitted through contaminated food and water due to the rise in surface water temperatures. Infections caused by Vibrio cholera non-O1, non-O139 serogroups are generally disregarded because of the fact that it is an unusual pathogen for humans. However, non-O1, non-O139 V. cholerae infections are very rare in UAE and in this study patient with
(CSOM) has been presented and this indicate that halophilic marine vibrios may be pathogenic in UAE in persons exposed to Lakes or seawater. The clinical manifestation and strain characteristic of extraintestinal *V. cholerae* non-O1, non-O139 infections acquired on 2014 in UAE is described. The strain was obtained from the case of (CSOM) infection, which were sent to another hospital in the same area to compare the identification result. Strain was identified as *V. cholerae* and was tested for agglutination in polyvalent *V. cholerae* antiserum. Susceptibility of the *V. cholerae* isolates to penicillin, ampicillin, cefuroxime, ceftriaxone, gentamicin, ciprofloxacin, tetracycline and chloramphenicol was determined. The clinical strain available for characterization showed the typical biochemical characteristics of *V. cholerae*, but not agglutinated with the polyvalent O1 antiserum. Clinicians should be aware that 12 pathogenic vibrios can cause chronic ear infections or any types of infections rather than toxogenic vibrio cholera O1 in both immunocompromised and
healthy individuals whose are exposed to warm seawater.
النتيجة عن التهاب 139 أو 1 ضمة الكوليرا غير عامل مسبب لالتهاب الأذن الوسطي التقيحي المزمن بمستشفى صقر أمارة رأس الخيمة. دولة الإمارات العربية المتحدة

هنادي الياس حسن حمد النيل

ملخص الدراسة

ينتج التهاب الأذن الوسطي المزمن التقيحي غالبا نتيجة البكتيريا, الفطريات والفيروسات والنزيفات ونادرا جدا حدوثه نتيجة البكتيريا غير الكوليرا والتي تعيش طبيعيا في البيئات العادية أو الشائعة مثل ضمة المائية وهي من العوامل المسببة للامراض البشرية ويمكن لهذه البكتيريا أن تسبب التهاب المعدة والأمعاء والالتهابات خارج الأمعاء مثل الإذن والتهابات الجروح والتهابات الدموع والدموع القاتل وتنقل عن طريق الطعام والمياه الملوثة نتيجة لارتفاع الحرارة في أسطح هذه المياه ولكن هناك تجاهل للإصابات الناجمة عن الضم كالأمم 1 و139 عموما بسبب حقيقة إن المرض غير عادي في البشر ولكن عدوى ضمة الكوليرا غير 1 و139 نادرة جدا في دولة الإمارات العربية المتحدة وفي هذه الدراسة تم عرض الإصابة بهذه البكتيريا لمريض يعاني من التهاب الأذن الوسطى التقيحي المزمن نتيجة للإصابة بهذه السلالة وهذا يشير إلى إن
الضمات المحبة للملوحة البحرية قد تكون مسببة للإمراض في الإمارات العربية المتحدة في الأشخاص المعرضين للبحيرات أو مياه البحر وبالوصف السريري لمظهر وسمة الضمة الكوليراغير1و139 من التهابات الإضافية المكتشفة في عام 2014 في الإمارات العربية المتحدة تم الحصول على سلالة من عدوى الإذن الوسطى وقد تم إرسالها إلى مستشفى آخر في نفس المنطقة لمقارنة النتيجة وقد تم تحديد وتأكيد ضمة الكوليرا المعزولة وتم اختبارها بمتعدد التكافؤ المصلي لضمة الكوليرا ولكن لم تتلتصق الضمة مع المصل وكذلك تم تحديد قابلية الكوليرا الضمة للمضادات الحيوية البنسلين-امبيسيلين-سيفوركسيم-سيفترياكسون-جنتاميسين-سيبروفلوكساسين-التراسيكلين والكلور مفينيكول يجب على الأطباء ان يكونوا على علم بان ضمات الكوليرا الأولى عشر المسببة للإمراض يمكن ان تسبب التهابات الإذن المزمنة او اي نوع من انواع العدوى بدلا من الكوليرا الضمة المولدة للذيفان في الإفراد قليلي المناعة أو الأصحاء الذين يتعرضون لمياه البحر الساخنة.
## Table Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supervision Committee</td>
<td>I</td>
</tr>
<tr>
<td>Examination Committee</td>
<td>II</td>
</tr>
<tr>
<td>Declaration</td>
<td>IIIi</td>
</tr>
<tr>
<td>Dedicated</td>
<td>IV</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>V</td>
</tr>
<tr>
<td>Abstract English</td>
<td>VI</td>
</tr>
<tr>
<td>Abstract Arabic</td>
<td>VII</td>
</tr>
<tr>
<td>Table Contents</td>
<td>VII</td>
</tr>
<tr>
<td>List of Abbreviations</td>
<td>XI</td>
</tr>
<tr>
<td>List of Tables</td>
<td>XII</td>
</tr>
<tr>
<td>List of Figures</td>
<td></td>
</tr>
</tbody>
</table>

### Chapter One: Introduction

1.1 Definition of ear infection and cause 1

1.2 Case history 2

1.1.2 Research 3

1.1.3 Research Hypothesis 3

1.3 Study Objectives 3

### Chapter Two: Human pathogenic Vibrio species

2.1 History of Vibrio sp 4
<table>
<thead>
<tr>
<th>2.3</th>
<th>Description of the Genus</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4</td>
<td>Epidemiology &amp; Transmission</td>
<td>6</td>
</tr>
<tr>
<td>2.5</td>
<td>Pathogencity</td>
<td>6</td>
</tr>
</tbody>
</table>

**Chapter Three: Materials and Methods**

<table>
<thead>
<tr>
<th>3.1</th>
<th>Study Design</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1.1</td>
<td>Study area</td>
<td>15</td>
</tr>
<tr>
<td>3.1.2</td>
<td>Sample Collection</td>
<td>15</td>
</tr>
<tr>
<td>3.2</td>
<td>Analysis required</td>
<td>15</td>
</tr>
<tr>
<td>3.3</td>
<td>Ethical consideration</td>
<td>15</td>
</tr>
<tr>
<td>3.4</td>
<td>Methodology</td>
<td>16</td>
</tr>
<tr>
<td>3.4.1</td>
<td>Instruments preparation</td>
<td>16</td>
</tr>
<tr>
<td>3.4.2</td>
<td>Equipment and general materials</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>3.4.3</td>
<td>Reagent and stains</td>
</tr>
<tr>
<td></td>
<td>3.4.4</td>
<td>Media preparation</td>
</tr>
<tr>
<td></td>
<td>3.4.5</td>
<td>Control used</td>
</tr>
<tr>
<td>3.5</td>
<td>Methods of identification</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>3.4.1</td>
<td>Culture of swab</td>
</tr>
<tr>
<td></td>
<td>3.4.2</td>
<td>Gram stain(Direct)</td>
</tr>
<tr>
<td></td>
<td>3.4.3</td>
<td>Biochemical Identification</td>
</tr>
</tbody>
</table>

**Chapter Four: Results**

| 4.1 | Results | 22 |

**Chapter Five: Discussion, Conclusion and**
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1 Discussion</td>
<td>29</td>
</tr>
<tr>
<td>5.2 Conclusion</td>
<td>30</td>
</tr>
<tr>
<td>5.3 Recommendations</td>
<td>31</td>
</tr>
<tr>
<td>References</td>
<td>32</td>
</tr>
</tbody>
</table>
List of Abbreviations

CA                          Chocolate agar
CLED                     Cystine lactose electrolyte
deficient                Chronic Supportive Otitis Media
CSOM                    Chronic Supportive Otitis Media
ENT                        Ear Nasal Throat
GS                          Gram stain
LBA                       Leaked Blood Agar
MAC                      MaCconkey agar
MHA                        Mueller Hinton agar
NAV                    Non agglutinable V.cholera
NCV                       Non Cholera Vibrio
NOVC                 Non O1 Vibrio Cholera
OM                         Otitis media
OPD                    Out Patients Department
SBA                       Sheep Blood Agar
SDA                        Sabourad Dextrose Agar
TC                         Cholera Toxin
TCBS                   Thiosulphate citrate bile salts
sucrose agar
TM                         Tympanic Membrane
TSI                  Triple Sugar Iron agar
List of Tables

Table. 1: Different types of Microorganisms isolated from ear discharge under study

TABLE. 2: classification of Vibrio species that may be found in human clinical specimens.

Table. 3: Pathogenic Vibrio species associated with different clinical syndromes.

Table:4 Probability of identification and confidence level of V. cholerae .

Table:5 Automated biochemical identification system by VITEK2

Table 6. Result of antibiotic susceptibility patterns of V. cholera strain.
List of Figures

Figure 1: A mixed growth of organisms include a hemolytic strain of *V. cholerae* on a sheep blood agar plate from the original sample.

Figure 2: A mixed growth of organisms include strain of *V. cholerae* on a MAC agar plate from the original sample.

Figure 3: A mixed growth of organisms include strain of *V. cholerae* on TCBS agar plate from the original sample.

Figure 4: A mixed growth of organisms include strain of *V. cholerae* on a CA plate from the original sample.

Figure 5: Mixture of organisms include of *V. cholerae* (comma shape) on direct Grams stain

Figure 6: Show gram negative curve shaped (comma) in indirect GS from BA

Figure 7: A positive oxidase test (as shown here) results in the development of a dark purple color within 10 seconds. *V. cholerae* is oxidase-positive, E.Coli is oxidase-negative and Pseudomonase is oxidase-positive
Figure 8: Antisera to the polyvalent antiserum (O1 serogroup of *V. cholerae*) and monovalent does not show agglutination of strain (left). A normal saline control down (right) does not show agglutination.

Figure 9: TSI which contains sucrose in addition to glucose and lactose gives reactions of A/A, no gas, and no H2S.

Figure 10: A positive string test, shown here with *V. cholerae*, is a rapid and simple method for distinguishing between the genus *Vibrio* (positive).

Figure 11: A hemolytic strain of *V. cholerae* on a sheep blood agar plate (haemolysis test).

Figure 12: Biochemical reaction of Vibrio cholera strain by standard biochemical reaction API 20E (manufactured in Franc Biomerieux).

Figure 13: Agar Disk Diffusion, Mueller-Hinton susceptibility test agar.

Figure 14: Pure cultures of vibrio cholera. A on sheep blood agar. B on CLED medium. C on MaCconkey agar. D on TCBS.

Figure 15: Pure culture of vibrio choler strain on Mueller Hinton Agar.

Figure 16: Vitek 2 Compact System.
Figure 17: 0.5 McFarland standard tube (readymade) and vortex device (Grant bio).

Figure 18: Dispensater (dispensette).

Figure 19: polyvalent antiserum (O1 antisera) and monovalent antisera.
Chapter One

1. Introduction

1.1 Definition of ear infection and causes:

Ear infection is a common problem for both children and adults but the magnitude is different in different countries{1}. About 65-330 million people suffer from ear infection worldwide and 60% of them had significant hearing impairment{2}. Ear infection is commonly known as Otitis. There are three type of ear infection, Otitis externa is commonly known as outer ear infection, Otitis media known as middle ear infection, and Inner ear infection includes labrynthitis and vestibular neuritis{3}.Otitis media is an inflammation of the middle ear that affects the tympanic membrane{4} and ear discharge is one of the commonest symptoms of ear infection{5} It may be acute, chronic suppurative or serous. Though ear infection can be caused by viruses and fungi, but the major causes of ear infection are bacteria which are found in the skin of the external ear and enter into the middle ear through a chronic perforation{6,7}.The etiologies and prevalence of ear infection is different in different geographical areas {8,9}.Literatures reported that the geographical area and respiratory infections may affect the type of OM pathogens.{10} Chronic supportive otitis media (CSOM) is one type of OM, it is defined as persistent or intermittent infected discharge {11}from the middle ear for at least two weeks and is associated with a (TM) perforation that is usually painless {12,13} and often results in partial or total loss of the tympanic membrane {11}.Generally CSOM results from an acute ear infection that is not diagnosed promptly or is inadequately treated and Infrequently it can result from chronic otitis media with effusion{14} or caused by dysfunction of the Eustachian tube and bacterial infections {15}.Contaminated water may also play a role in the pathogenesis of CSOM, swimming or bathing in unclean water can lead to middle-ear contamination with bacteria and infection when TM perforation exists {16,17}. CSOM is mostly caused by bacteria but fungi and virus can also be a cause CSOM.{18} The aetiological pattern shows mixed infections of Gram –negative, Gram-positive aerobe and anaerobe bacteria as several studies worldwide have reported{19}.The most common bacterial pathogens in OM are Streptococcus pneumoniae, Haemophilus influenza, Moraxella catarrhalis, Staphylococcus aureus, Escherichia coli, Klebsiella spp, Pseudomonas aeruginosa, Proteus spp {20,21}and
Streptococcus pyogenes. In addition increasing numbers of patients have CSOM due to anaerobic bacteria such as Peptostreptococcus, Fusobacterium, Prevotella, and Porphyromonas being the most common anaerobic isolates. Also there are uncommon microorganisms isolated from cases of ear infections such as Kerstersia gyiorum, Mycoplasma pneumoniae, Chlamydia trachomatis, Mycobacterium tuberculosis (is a rare type of infection), Syphilitic otitis media (rare today) is caused by Treponema pallidum and Vibrio species other than V.cholera O1 and O139. Vibrio species other than vibrio cholera serotype O1 and O139 strain are seldom isolated from extra intestinal sites. We report here the isolation of a strain of non agglutinable V.cholera (NAV) from a case of CSOM from an obese 14-year-old boy after swimming in a pool in their farmer. To the best of our knowledge this is unusual organism reported from CSOM due to this strain in our area. Vibrio cholera other than vibrio cholera O1 and O139 is an unusual cause of CSOM, infection with V. cholera other than vibrio cholera O1 and O139 can occur after even mild, brief exposure to seawater, freshwater or ingestion of raw seafood and the interval between exposure to seawater and onset of clinical infection can be prolonged. However, little is known about the occurrence and characteristics of V. cholerae other than vibrio cholera O1 and O139 infections and otitis media or Otitis externa due to these strains especially in our area.

1.2 Case history:
A 14-year old, obese, Omani boy living in Ras Al khaimah -UAE, presented to the department of outpatient - ENT because of ears pain with purulent exudates from 3 weeks prior, no fever, diarrhoea, nausea, or vomiting was reported when presented to ENT department. The patient was a known case of Otitis media and his past medical history was remarkable for recurrent episodes of otitis media since early childhood, he had no travel history for more than 2 years but his social history was significant for visiting their ranch and swimming in the pool weekly. On the initial physical exam, otoscopy of the both ears revealed CSOM with exudates and large perforation (active) with granular edge discharge. Ear swab samples sent to the microbiology department for culture. Blood was obtained along with the swabs as a routine for hematology and biochemistry tests. His hemoglobin was 16.3 g/dl, blood glucose 4.9 mmol/l. and laboratory tests revealed liver function tests within the reference ranges, stool sample was collected after three days of ear report for routine
and culture. Ear discharge yielded a mixed culture of microorganisms included the strain under the study. The organisms were obtained listed in Table(1). Systemic antibiotic therapy administered for 10 days and the patient responded to treatment and improved.

1.1.2 Research Question:
Is the pattern characteristic and susceptibility of the *Vibrio cholerae* non O1 isolate from CSOM in Saqr hospital –Ras Alkhaimh – UAE 2014 different from six strains were previous isolated in Denmark1994-1998 ?

1.1.3 Research Hypothesis:
The pattern characteristic and susceptibility of *Vibrio cholerae* non O1 isolate from CSOM in Saqr hospital -Ras Alkhaimh – UAE 2014 is different from six strains were previous isolated in Denmark1994-1998.

1.3 Study Objectives:

1.1.3 General objective:
2. To aware the clinicians and technicians about unusual pathogens, especially in refractory cases of ear infections.
3. To review the literatures and provide a better understanding of the majority of vibrio cases reported in UAE.

1.1.2 Specific objectives:
1. To compare the isolated strain with other strains isolated in Denmark 1994 especially the portal of entry of the organisms.
2. To characterize the *Vibrio cholerae* non-O1 strain isolated biochemically and serologically.
3. To determine the susceptibility patterns of the strain isolate by disk diffusion.
Chapter Two

2. Literature review

2.1 History of Vibrio species:

Historically, vibrios (Vibrio spp.) were the first bacteria to be isolated and identified from the environment. In 1854, Vibrios were described by the Italian medical student Pacini (30, 31) and became an important argument in the contemporary debate of germ theory vs. miasma theory i.e. identifying the causative agent of disease as an organism or as polluted vapor in the air. However, a few years earlier, John Snow had isolated the bacterium V. cholerae after a Cholera outbreak tracked to a contaminated drinking water well in London. Robert Koch, originator Of Koch’s postulates, isolated V. Cholerae during an outbreak in Egypt and India in 1883 and suggested that the bacterium was the causative agent of pandemic cholera, the most feared disease at that time. John Snow declared that cholera could not be tracked back further than 1769, but this may be due to the fact that epidemics in Asia were not documented in Europe (32).

2.2 Taxonomy and serological classification:

The genus Vibrio is a major taxa of culturable heterotrophic bacteria (33, 34), according to Bergey’s Manual of Systematic Bacteriology (35), classified as belonging to the phylum Proteobacteria, class Gammaproteobacteria, order Vibrionales and family Vibrionaceae. In 2012 classification Vibrionaceae, is the only family in the order Vibrionales. The family of Vibrionaceae includes the genus Vibrio (111 species) (36), 12 of these Vibrio species are associated with human infections(37,38) and other 10 genera; Aliivibrio (6 species), Allomonas (1 species), Beneckea (11 species), Catenococcus (1 species), Enterovibrio (4 species), Grimontia (1 species), Listonella (3 species), Lucibacterium (1 species), Photobacterium (24 species) and Salinivibrio (4 species) (36). Other bacterial orders in this class include; Aeromondales and Enterobacteriales. The taxonomy is widely debated however, as the gene sequencing of the 16S rRNA, normally used as accurate genetic identification, is unreliable, since several Vibrio spp. have nearly identical 16S rRNA(39). The Vibrio spp. consist of both human pathogenic and non pathogenic strains and which inhabit the same environmental niche(37), among them, Vibrio cholerae is the most important
species in the genus. It is divided into 3 major subgroups: V. cholerae O1, V. Cholerae O139, and V. cholerae non-O1(37). Differences in the sugar composition of the heat-stable surface somatic “O” antigen is the basis of the serological classification of Vibrio cholerae, the bacterium is classified into 206 “O” serogroups (40), based on this surface O antigen of the lipopolysaccharide (41).

2.3 Description of the Genus:

Members of the genus Vibrio are defined as halophilic facultative anaerobes (41; 42; 43), small straight, slightly curved or comma-shaped Gram-negative rods 0.5 to 0.8 μm in width and 1.4 to 2.6 μm in length (44). They are motile with only single polar flagella (38,45) enclosed in a sheath (44). Na+ stimulates the growth of all Vibrio species and Na+ is an absolute requirement for most species (37). They are capable of respiratory and fermentative metabolism (38,45), all Vibrio species can ferment D-glucose, producing acid but rarely gas (37) (only a single species V. furnissii producing gas). All human pathogenic Vibrio species except V. metschnikovii reduce nitrate to nitrite (38,45) and are oxidase positive (except for 2 species). The Vibrio species of medical importance grow well on common media including blood agar (where they may be beta-hemolytic (V. cholerae non-O1 and some V. cholerae O1 strains of the eltor biotype), alphahemolytic (V. vulnificus and many others) or non-haemolytic (37)), chocolate and Mueller-Hinton agars (45). Vibrio strains usually grow well on MacConkey agar (sometimes with a reduced plating efficiency) and will appear as colorless (Lactose-negative) colonies. Vibrio cultures often do not grow well on more selective plating media for enteric organism (37), although they grow well on thiosulphate-citrate bile salts sucrose medium (TCBS) which considered as selective media for vibrio (37). However, not all Vibrio species of medical importance grow well on TCBS agar (45). Vibrio cholerae grows as yellow colonies because it ferments sucrose whereas other Vibrio species are sucrose negative and appear as green colonies (43). The string test and susceptibility towards the 0/129 Vibriostatic agent differentiates Vibrio species from closely related organisms such as Aeromonas and Plesiomonas species (46).
2.4 Epidemiology & Transmission:
Substantial evidence has been provided showing that *Vibrio* species are natural inhabitants of marine aquatic environments in both temperate and tropical regions (41;42;43). *Vibrio* infections are seasonal and the number of patients has a highest peak during summer months (48). Transmission of many *Vibrio* infections is often implicated in food-borne disease which is primarily through the consumption of undercooked seafood or exposure to warm seawater in coastal areas (49;50,51). Water plays an important role in its transmission and epidemiology and there appears to be a positive correlation between water temperature and the numbers of human pathogenic vibrios isolated, as well as the number of reported infections. Pathogenic vibrios are found more frequently in environments whose water temperature exceeds 10 °C for at least several consecutive weeks (52; 53; 54). Salinity and nutrient concentration in Pathogenic Vibrio species have halophilic characteristics and occur most frequently in water ranging in salinity from 5 % to 30 %. (52; 55; 59; 61;63). *Vibrio* species have been isolated from waters showing a broad range of salinities and varying pH values. *V. cholerae* and *V. mimicus* are the only species found in fresh water (less than 5 % salinity) (53; 56;58; 60; 66). Thus, due to the ubiquitous nature of *Vibrio* species in the aquatic environment, the presence of *Vibrio* species in bathing water cannot be controlled by water quality control measures such as wastewater treatment and disinfection. Human carriers and their skin shedding appear to be of only limited importance in the epidemiology of *Vibrio* infections associated with recreational water use.

1. Pathogenicity:
The infections caused by Vibrio species can be classified as intestinal or extra intestinal, although this division is not absolute (37,45) (Table 2).
Table. 2.1 Classification of Vibrio species that may be found in human clinical specimens

Occurrence in human clinical specimens

<table>
<thead>
<tr>
<th>Species</th>
<th>Intestinal</th>
<th>Extra intestinal</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. alginolyticus</em></td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td><em>V. carchariae</em></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>V. cholerae</em> O1</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td><em>V. cholerae</em> Non-O1</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td><em>V. cincinnatiensis</em></td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td><em>V. damsel</em></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>V. fluvialis</em></td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td><em>V. furnissii</em></td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td><em>V. hollisae</em></td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td><em>V. metschnikovii</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>V. mimicus</em></td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td><em>V. parahaemolyticus</em></td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td><em>V. vulnificus</em></td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

Key:

a Modified from Kelly et al. (1991)

b The symbols +, ++, +++ and ++++ give the relative frequency of each organism in specimens from implicated infections; - = not found.

The most important one is Vibrio cholerae. *Vibrio cholerae* (toxigenic strains) (69, 70) produce the cholera toxin (CT) (70) including serogroups O1 and O139 (Bengal) and causing epidemic and pandemics cholera (69,70) or extraintestinal infections(37) while remaining serogroups of V. cholerae they so-called *Vibrio cholerae* non-O1 and non-O139 (VC non O1 and nonO139) 'NAG' (non-agglutinable) 0r 'NCV' (non-cholera vibrios ,biochemically resemble V. cholerae serotype 01(62)but do not produce (CT) (70),they may produce other toxins (72) and rarely cause epidemics (but not epidemic cholera (69,70)) and extraintestinal infections. In recent years, there has been an increase in the number of reports of infections involving non-O1, non-
O139 *V. cholerae* (NOVC) (57), isolated from blood, wounds, ears, infected eyes and gall bladder and they are also reported from patients with meningitis, pneumonia and infection of the reproductive organs and urinary tract. (37) Non-choleragenic vibrios, including the other serogroups of the *V. cholerae* species, and other species of *Vibrio* *V. alginolyticus*, *V. parahaemolyticus*, *V. cholerae*, *V. carlariae*, *V. mimicus*, *cincinnatiensis*, *V. damsela*, *V. fluvialis*, *V. furnissii*, *V. hollisae*, *V. vulnificus*, *V. metschnikovii* (37,45)). Table 3

<table>
<thead>
<tr>
<th>Species</th>
<th>Gastro-intestinal tract</th>
<th>Wound</th>
<th>Ear</th>
<th>Primary septicemia</th>
<th>Bacteremia</th>
<th>Lung</th>
<th>Meninges</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. cholerae</em> 01</td>
<td>++</td>
<td>(+)</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td><em>V. cholerae</em> non-01</td>
<td>+ +</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
<td>(+)</td>
<td>*</td>
<td>(+)</td>
</tr>
<tr>
<td><em>V. parahaemolyticus</em></td>
<td>+ +</td>
<td>+</td>
<td>(+)</td>
<td>*</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td><em>V. vulnificus</em></td>
<td>+</td>
<td>+ +</td>
<td>*</td>
<td>+ +</td>
<td>+</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td><em>V. fluvialis</em></td>
<td>++</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td><em>V. alginolyticus</em></td>
<td>*</td>
<td>+ +</td>
<td>+</td>
<td>*</td>
<td>(+)</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td><em>V. damesela</em></td>
<td>*</td>
<td>++</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td><em>V. furnissii</em></td>
<td>(+)</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td><em>V. hollisae</em></td>
<td>+ +</td>
<td>*</td>
<td>*</td>
<td>(+)</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td><em>V. mimicus</em></td>
<td>+ +</td>
<td>+</td>
<td>+</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td><em>V. metschnikovii</em></td>
<td>(+)</td>
<td>*</td>
<td>*</td>
<td>(+)</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td><em>V. cincinnatiensis</em></td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>(+)</td>
<td>*</td>
<td>(+)</td>
</tr>
</tbody>
</table>

+ +, most common site of infection; +, other sites of infection; (+), rare sites of infection;* infection remains to be firmly established.

Pathogenic *V. cholerae*(toxigenic strains) is the most important species in the genus *Vibrio*. It has caused many epidemics of cholera and millions of death (74) and it is produces a heat-sensitive enterotoxin that causes the characteristic cholera symptoms, including "rice water stool." The species comprises several somatic (O) antigen groups, including O-group-1 (75), which is associated with El Tor (the most common
biotype) or classic biotypes. *V. cholerae* O1 may have several serotypes, including Inaba, Ogawa, and Hikojima. (57, 75) *V. cholerae* non-O1 also can cause gastrointestinal disease, though typically less severe than that caused by *V. cholerae* O1 (75). Serotype O139 is an exception, and produces classic cholera symptoms. (47)

**5.2.1 V. Cholerae O1:**

*V. cholerae* O1 is the primary causative agent of cholera[49] and it is the organism responsible for seven pandemics of Cholera.[64] and did not occur in aquatic environment unless they had been contaminated with faeces from patients with cholera, direct person-to-person transmission of cholera is not an important mode of transmission[67]. In patients with severe cholera or “cholera gravis”, there is massive diarrhoea with large volumes of “rice water stool” (Clear fluid with flakes of mucus) passed painlessly. There is usually vomiting and little desire to eat. If left untreated, the patient becomes prostrate with symptoms of severe dehydration, electrolyte imbalance, painful muscle cramps, watery eyes, loss of skin elasticity and anuria (absence of urine excretion). (37) Severe infections may result in death if the dehydration is not treated aggressively with fluid and electrolyte replacement [64]. The incubation period of cholera is usually 2 to 3 days (range from 6 hours to 5 days). Severe illness has been associated with; high-dose exposure [65] (the infectious dose of *V. cholerae* is more than $10^6$organisms) [67], low gastric acidity and blood group O.[65]

**5.2.2 V. cholerae O139:**

Toxigenic *V. cholerae* serogroup O139 (Synonym: *V. cholerae* O139 Bengal) is a relatively new organism that causes epidemic cholera (72), it was first identified in 1992 (47) as the cause of a new epidemic of cholera in India and Bangladesh.[71]. The symptoms are typical of cholera but the organism does not react in *V. cholerae* O1 antisera or in O2 – 138 antisera. Thus, it was named O139 (37). Unlike *V. cholerae* O1, strains of O139 produce a capsule (68) as do some strains of *V. cholerae* non-O1 (73). It has been speculated that the emergence of this strain is the beginning of the eighth Cholera pandemic (37).
5.2.3 V. Cholerae non-O1:
V. cholerae non-O1 strains (synonym: ‘nag vibrios”; non-agglutinating vibrios, “non-cholera vibrios; V. cholerae “non-O1, non-O139”; and V. cholerae non-O1, 139) do not agglutinate in O1 or O139 antisera but are otherwise typical strains of V. cholerae in their biochemical reactions (37). They usually do not produce cholera toxin but can produce other toxins (77; 37). They can cause a severe, cholera-like disease, but they are usually isolated from patients with mild diarrhoea, extraintestinal infections, seafood, and the environment (37). V.cholerae non-O1 strains have also caused septicaemia in patients with cirrhosis or other underlying diseases (73). Strains have also been isolated from ears, wounds, the respiratory tract and urine (78, 73).

5.2.4 V. Parahaemolyticus:
V. parahaemolyticus was first identified as a foodborne pathogen in Japan in the 1950s. By the late 1960s and early 1970 V.parahaemolyticus was recognised as a cause of diarrhoeal disease worldwide (76). The disease is usually mild and self-limiting but can be fatal. Rehydration is usually the only treatment needed but in some severe cases the patient will require hospital admission, antimicrobial therapy may be beneficial (37). There is now ample evidence implicating V. parahaemolyticus in extraintestinal infections including infections of the ear, eye, blood, and wounds (79). It has also been associated with pneumonia (80) resulting from inhalation of contaminated aerosol. The illness caused by V. parahaemolyticus food poisoning is a gastroenteritis characterized by watery diarrhea (81), diarrhea is usually watery but can sometimes be bloody (37) and abdominal cramps in most cases with nausea, vomiting, low-grade fever, chills and headache. The incubation period is usually between 12 and 24 hours (81, 37) and usually resolves in three days (82). The infection is typically acquired through consumption of contaminated seafood, these could be raw, inadequately cooked or that have been cross-contaminated by improper handling, poor temperature control of storage favors bacterial proliferation. The total dose of greater than one million may cause disease, this dose may be markedly lowered by coincident consumption of antacids or presumably by food with buffering capacity (83). One volunteer feeding study done in Japan estimated that 2 x 10⁵ to 3 x 10⁷ cells have to be ingested for disease (84). V. parahaemolyticus is a slightly halophilic bacterium, the optimum growth NaCl concentrations range from 2 to 4% and poor growth is exhibited in media below 0.5% NaCl and the bacterium is
inactivated rapidly in distilled water and growth at levels of 10% NaCl is inhibited (85). The organism grows at a temperature range between 5 and 43°C with optimum growth at 37°C. The optimum pH range for growth is 7.8 to 8.6 although it can grow in the pH range of 4.8 to 11(82). Similar to other vibrios, _V. parahaemolyticus_ may be overlooked if plated on nonselective medium; therefore it should be plated on TCBS. On TCBS _V. parahaemolyticus_ isolates appear as distinct green colonies. Virulence can be determined by b-hemolysis of red blood cells by using Wagatsuma blood agar, although newer methods use DNA gene probes [86]. _V. parahaemolyticus_ is also grouped according to antigens by (2005) 75 combinations of the O and K antigens had been identified of which 11 belong to the pandemic Clone (87,88).

5.2.5 Vibrio alginolyticus:

_V. alginolyticus_ is very common in the marine environment(45) and as other Vibrio spp, _V. alginolyticus_ appears on a seasonal basis that was rarely found in winter and abundant in summer (89). Studies by Baross and Liston showed that the minimum growth temperature for _V. alginolyticus_ is 8 (90). It is a halophilic organism recognized by Miyamoto et al(91) and named Oceanomonas alginolytica and was renamed _V. alginolyticus_ by Sakazaki at 1968 (92) the first recognized as being pathogenic in humans in 1973.(93) It is very similar to _V. parahaemolyticus_ on their biochemical properties and also isolated from similar types of marine samples. Acetoin production and fermentation of arabinose are important features which distinguish _V. alginolyticus_ from _V. parahaemolyticus_ (89). _V. alginolyticus_ cause soft tissue infections following exposure to seawater (45), wounds, ear infections are also seen with this organism. It appears that a majority of patients with otitis associated with _V. alginolyticus_ have predisposing conditions, including chronic otitis media and rupture or tubulation of the tympanic membrane. Infections are usually self-limiting, moderate severity and short duration. Antibiotic treatment is only occasionally necessary. Septicemia and gastroenteritis were thought to be a rare presentation of _V. alginolyticus_ infection but it accounted for 12% of infections(94). Other clinical syndromes reported in association with _V. alginolyticus_ infection include chronic diarrhea in a patient with AIDS (95), conjunctivitis(96), post-traumatic intracranial infection, central nervous system disease, osteomyelitis have also been reported (97). Resistance to tetracycline and chloramphenicol has been reported in a few isolates of _V. alginolyticus_ but all strains appear to be sensitive to
Thus, prevention, early detection and initiation of treatment of *V. alginolyticus* infections are very important to keep human healthily.

5.2.6 **Vibrio vulnificus:**

*Vibrio vulnificus* is an autochthonous estuarine and marine bacterium that can be found in temperate and tropical climates worldwide (99). It was first isolated by the US Centers for Disease Control (CDC) in 1964 but misidentified as a virulent strain of *V. parahaemolyticus*. In 1976, it was referred to as “lactose fermenting vibrio”, which was one of main characteristics that distinguished this organism from two other closely related species (V. *parahaemolyticus* and V. *alginolyticus*) (100). The bacterium was formally described as *Vibrio vulnificus* by Farmer et al in 1979 (101). The epithet ‘vulnificus’ derives from the Latin ‘wound’, as the first cases on infections caused by *V. vulnificus* involved wound infections. *V. vulnificus* is an opportunistic pathogen that can cause wound infections and primary septicemia. This bacterium has less often been described as a cause of gastroenteritis and its role as a primary cause of gastrointestinal disease remains to be determined (102).

The primary septicaemic form is the major form of infection with *V. vulnificus* which involves a rapidly progressing septicemia with few gastrointestinal signs, the incubation period is from seven hours to several days, the most frequent symptoms are fever, chills, nausea and cardiovascular hypotension. This form of disease is predominantly associated with the consumption of raw bivalve shellfish containing the organism by individuals with underlying chronic disease, particularly liver disease (103). In these individuals, the microorganism enters the blood stream resulting in septic shock, rapidly followed by death in many cases (about 50%). Over 70% of infected individuals have distinctive bulbous skin lesions. For predisposed persons, septicemia can presumably occur with doses of less than 100 total organisms (104). Wound infections occur in connection with puncture wounds after handling of raw seafood or trauma and exposure to saline environments that harbor the organism (103). *V. vulnificus* is similar phenotypically to *V. parahaemolyticus* (in cultural characteristics and sensitivity to processing procedures) (99). The two most distinctive characteristics of *V. vulnificus* are fermentation of lactose and production of β-D-galactosidase and these biochemical tests for them can be used to distinguish it from the related *V. parahaemolyticus* (100). Also it differs principally in salt requirement and tolerance, growing in media containing between 0.1 and 5% NaCl. Same as *V. parahaemolyticus*, the organism grows optimally at 37°C although it can
grow at a temperature range between 8 and 43°C. The pH range for growth of *V. vulnificus* is 5 to 10, with an optimum at 7.8.(105)

5.2.7 *Vibrio mimicus*:

*V. mimicus* are a non-halophilic Vibrio named according to its similarity to *V. cholerae* [106]. They are found in aquatic ecosystem, including seawater, freshwater, brackish water (107) and they have been isolated from a variety of clinical disorders associated with exposure to aquatic environments (106). Reviewed the clinical and epidemiological characteristics of infections associated with *V. mimicus*, the majority of isolates were from stool samples, *Vibrio mimicus* are responsible for gastroenteritis after consumption of raw or uncooked seafood containing *Vibrio mimicus* and are closely related phylogenetically to *Vibrio cholerae* (107). A few ear infections arose after exposure to seawater. It is notable that a common feature of all the sporadic incidents was their location at which warm seawater could be expected (106). *Vibrio mimicus* produce colonies of 2 to 3 mm in diameter on blood agar, and colonies on thiosulphate citrate bile salt sucrose (TCBS) are of green color (108). Phenotypically, most of the features of *V. mimicus* are similar to *V. cholerae* and the main trait differentiating them biologically is sucrose fermentation (109).

5.2.8 *Vibrio fluvialis*:

*V. fluvialis* is a halophilic Vibrio first identified in 1975 in a patient with diarrhea in Bahrain(110). It is biochemically similar to Aeromonas hydrophila but can be differentiated from this organism by its ability to grow well on media containing 6% to 7% sodium chloride. The largest series of *V fluvialis* infections involved 500 patients in Bangladesh, half of whom were young children(111). In that series, patients presented with diarrhea (100%, 75% bloody), vomiting (97%), abdominal pain (75%), dehydration (67%) and fever (35%). *V fluvialis* rarely causes wound infections or primary septicemia (94,112).

5.2.9 *V. hollisae*:

*Vibrio hollisae* is a halophilic vibrio species, first described by Hickman et al (113) and recently reclassified as *Grimontia hollisae* by Thompson et al (114). It is primarily known to cause moderate to severe cases of gastroenteritis in healthy people and is rarely isolated from extraintestinal sites such as blood samples (37) and wound infections(94,115). The organism is reported usually or poorly grow on TCBS agar and MacConkey agar and does grow well on sheep blood agar and marine agar (113).
5.2.10 V. damsela (Photobacterium damsel):
Photobacterium damsela(formerly Vibrio damsela) is a halophilic gram-negative bacillus similar to V vulnificus that strictly causes soft tissue infections following exposure the wounds to brackish water or injury by saltwater animals(116). Infections can be fulminate and are frequently fatal even in immunocompetent hosts(117).

5.2.11 V.furnisii :
This species was formerly classified as the aerogenic (able to produce gas from glucose) ,it is biovar of Vibrio fluvialis but further taxonomic study indicated that separate species status was warranted (118,49). This organism has most commonly been isolated from stool samples (119).The importance of V furnissii as an enteric pathogen remains unclear.

5.2.12 Vibrio metschnikovii:
V. metschnikovii is the name used for a group of organisms known in the older literature as both V. metschnikovii and as V. proteus, oxidase negative and do not reduce nitrate to nitrite .V. metschnikovii was redefined in 1978 by Lee et al (120) and is now one of the Vibrio species that is easy to isolate and identify. The original strain(s) of V. metschnikovii was isolated in 1884 from cultures of fecal samples from cholera patients. In 1981 Jean-Jacques et al (121) described a case of bacteremia due to V. metschnikovii in a patient with an inflamed gall bladder in 1985 Farmer et al (122).

5.2.13 V.cincinatiensis:
Vibrio  cincinnatiensis sp.(Latin adj., derived from the Society of Cincinnati from which the city of Cincinnati, Ohio, was named).The organism was first isolated from the cerebrospinal fluid and blood of a patient presenting with confusion to the University of Cincinnati in 1986. The patient drank alcohol heavily but had no evidence of liver disease .It is halophilic Vibrio species and tolerates 6% but not 8% NaCl supplement to the growth medium,colonies on nutrient agar are cream color, round, smooth, and glossy. Yellow colonies are produced on thiosulfate-citrate-bile saltssucrose agar(123). Subsequently, it has been isolated from faeces (intestine),the ear, a leg wound, animals and water
Chapter Three
3. Materials and methods

3.1 Study Design:
Case study was identified on the definition of the case history in the topic, the study was conducted to characterize the Vibrio cholerae non-O1 strain isolated biochemically and serologically and to determine the susceptibility pattern of the strain isolate by disk diffusion.

3.1.1 Study area:
The study strain was isolated in Microbiology department - Saqr hospital - Ras Alkhaimah – UAE

3.1.2 Sample Collection:
Present study was carried out in patient attending the outpatient department of ENT, Saqr Hospital, Ras AlKhaimah, UAE, on 12 November 2014 with clinical evidence of CSOM. A baseline data of case were recorded including history, general examination, Otorhinolaryngological examination investigations and treatment received in the past. Age, sex, side or type of pathology was made.

Four sterile transport swabs were collected by the clinician from both ear of the patient (Two swabs were taken from left and right ear on the first day of attendance of the patient to ENT- OPD before any medication and two after 10 days of medication with Ciprofloxacin to check the effectiveness of treatment, the swabs were send to the microbiology laboratory for bacteriological processing.

3.2 Analysis required:
Each swab was cultured on Sheep blood agar, chocolate agar, maCconkey agar, SDA and CLED, and subculture on MHA, TCBS and TSI, incubated at 37 C° aerobically and on LBA and incubated at 37 C° anaerobically and gram stain.

1. Ethical consideration:
Permission for conducting this research was taken from ethical committee researches, director and the head of medical laboratories of Saqr hospital - Ras Alkhaimah - UAE.
3.4 Methodology:

3.4.1 Instruments preparation:
Incubator, autoclave sterilizer machine, Vitek 2 Compact System, light microscope, safety cabinet and vortex device. All available used documents of daily quality control measures and calibration.

3.4.2 Equipment and general materials:
Bunsen burn with gas cylinder, 0.5 McFarland standard tube, fixed micropipette, commercial sterile micropipette tips, sterile plate, sterile disposable loops, Sterile test tubes, Sterile cotton Swab, sterile glass spreader, slide. Dispensator (Dispensette) and filter paper.

3.4.3 Reagent and stains:
Set of Gram stain, oxidase reagent, API 20 E, 0.5% aqueous solution of sodium deoxycholate, polyvalent and monovalent antiserum and individuals antibiotics susceptibility discs. Availability and validity (lot number and expiry date) checked in the microbiology lab daily.

3.4.4 Media preparation:
Commercial sterile readymade agar media used includes Sheep Blood agar, MacConkey agar, Chocolate agar, CLED, SDA, Mueller Hinton Agar and prepared TCBS, Leaked blood agar and TSI media sterilized and used.

3.4.5 Control strains:
E. coli ATCC 25922 and S. aureus ATCC 25923 and Pseudomonas ATCC.

3.5 Methods of identification:

3.4.1 Culture of swab:
The material of each swab was inoculated on non-selective medium include blood agar, macConkey agar, chocolate agar for aerobic bacteria, SDA Dextrose Agar for fungus and Leaked blood agar (LBA) for anaerobic bacteria, those media are used as a routine in our hospital for all swabs collected from ears and figure (1-4). After the result of direct Grams stain, the material from the original swab was inoculated on selective medium TCBS (thiosulfate citrate bile salts sucrose agar, is the selective medium). Anaerobic culture was placed in Filde’s jar were maintained up to 48 hours at 37°C while BA, CA, MAC, SDA and TCBS cultures were maintained up to 24 hours at 37°C CO2 aerobically according to criteria described by Barrow and Feltham (2003). After incubation period the plates were examined for growth of an
organisms. The organism under study was isolated from the culture dishes and were identified by culture characters, morphology, direct and indirect Grams stain, Oxidase test, motility and Vitek 2 machine as *V. cholerae* subculture on Mac Conkey’s agar, TCBS, CLED and subculture every 48hrs on sheep blood agar to avoid dry and loose of strain and keep also as stock culture (prepare in slant test tube of tryptone soy agar supplemented with 1% NaCl (w/v) and incubate at room temperature) to perform more study, to describe the patron of strain by [McCartney 1996], colonial morphology, Biochemical reactions, haemolysis in blood agar, serological and other clinical investigations are not done routinely as part of identification of *V. cholerae*, API E20 to compare the result with previous result was performed by Vitek 2 Compact System and to determine the susceptibility patterns of the strain isolate by disk diffusion and compare the result of The organism under study with 8 strains were isolated from ears discharge in Denmark 1994. Antimicrobial susceptibility test was performed on Mueller Hinton Agar because there’s no penal of antimicrobial susceptibility testing by the Vitek 2

3.4.2 Gram stain(Direct):
Direct GS was done from ear discharge and indirect GS from overnight growth from Sheep blood agar medium after bacterial growth.

3.4.3 Biochemical Identification:

3.4.3.1 oxidase test:
3 drops of the oxidase reagent (1% tetramethyl- p-phenylenediamine) placed on a piece of filter paper in a Petri dish. A small amount of fresh growth of test strain Picked up from the Mueller Hinton agar(MHA) with a disposable sterile loop and smeared across the wet filter paper. Positive and negative controls tested at the same time(Pseudomonas as a positive control strain and E. Coli as negative control strain) .

3.4.3.2 Identification by VITEK2:
1. Definition:
It is an automated phenotypic microbiology identification system utilizing growth-based technology through accommodating colorimetric reagent cards that are incubated and interpreted automatically (VITEK2 Compact System, BIOMERIEUX, France).
2. Principle of Identification by VITEK2:

Obtained gram negative, oxidase positive and motile isolates were selected for Identification biochemically confirmatory tests by using VITEK2 Compact System.

1. transferring one pure colony using a sterile swab to a tube containing 3 ml sterile saline solution mixed till making a suspension between 0.5-0.63 Mcfarland turbidity range that was measured using a turbidity meter called Densichek TM device with control.

2. Identification cards were then inoculated with microorganism suspensions using an integrated vacuum apparatus.

3. A test tube containing the microorganism suspension was placed into a special rack (Cassette) and the identification card was placed in the neighboring slot while inserting the transfer tube into the corresponding suspension tube.

4. The filled cassette was placed into a vacuum chamber station.

5. After the vacuum was applied and air was re-introduced into the station, the organism suspension was forced through the transfer tube into micro-channels that filled all the test wells.

6. After that, the device (VITEK2 Compact) performed about 47 biochemical reactions giving a final report of all applied biochemical reactions and the final identification of isolated colonies with its probability percentage after 6 hours.

3.4.3.3 Triple Sugar Iron agar (TSI):

1-TSI slant inoculated by stabbing the butt and streaking the surface of the medium with24-hour growth of test strain from (MHA) medium.

2- Incubated overnight at (37°C) for (24 hours).

3- Examined the reaction after incubation period.

3.4.3.4 String test:

The test performed on a glass microscope slide by suspended 24-hour growth of test strain from (MHA) medium in a drop of 0.5% aqueous solution of sodium deoxycholate.

3.4.3.5 Wet mount

Dark-field and phase-contrast microscopy used for screening motility of suspected isolate strain. Saline suspension of organism microscopically examined.
1-Serological Identification of V. cholerae:
It is a rapid and specific method of identifying *V. cholerae*.

3.4.4.1 Slide agglutination procedure:
1. Agglutination test for *V. cholerae* somatic O antigens is carried out in a clean glass slide.
2. By inoculating sterile loop removed a portion of the growth from the surface of MHA (figure 14), emulsified in a small drop of physiological saline and mix thoroughly for about 30 seconds.
3. Then add a small drop of polyvalent and monovalent antiserum to the suspension.
4. After mixed the suspension and antiserum well.
5. Then observed for any agglutination.

3.5 Additional screening tests
3.5.1 Hemolysis Testing (Plate hemolysis):
Blood agar plates containing 5% sheep blood (readymade media), streaked by test strain and incubated at 37°C 24 hours. QC strains (strongly hemolytic control strain and Strains that give incomplete hemolysis included in test)

3.5.2 API 20 E:
Confirmation for Vibrio cholera strain was identified biochemically by Vitek2 compact and other manual test by standard biochemical reaction API 20E (manufactured in Franc Biomerieux). API 20E is an identification system for Enterobacteriacea and other non-fastidious gram negative rods which uses 20 standardized and miniaturized biochemical test and database.

3.5.2.1 Principle of test:
The API 20E (Analytical profile index) strips consist of 20 micro tubes containing dehydrated substrates. These tests were inculcated with a bacterial suspension that reconstitutes the media during incubation. Metabolism produces color changes that are either spontaneous or revealed by the addition of reagents. The reaction was read according to the profile index and the identification is obtained by referring to the analytical profile index or using the identification software.
3.5.2.2. **Procedure:**

1. Single isolated colony was picked up by the sterile loop from pure on MHA and transferred into prepared suspension is sterile ready for used (NaCl 0.85% medium 5 ml). Mixed well.
2. Inoculated the suspension in strips contain twenty micro tubs of biochemical reaction by sterile syringe.
3. Anaerobic condition was maintained by adding a drop of sterile mineral oil to the line indicated. All the other the wells were half filled by suspension organism except CIT, GEL, VP, which were completely filled.
4. The strips were incubated at 37 °C for 24 hours.
5. After incubation period were removed from the incubator and necessary indicators added (TDA) Games, V1P + V2P Nitrat1 + Nitrat2).
6. Then the result was recorded by sub-mention of the figure using API20E sheet by reading using software for selected a good identification of microorganism . (Figure 12)

3.6. **Antibiotic susceptibility testing:**

Antimicrobial susceptibility tests were done on Mueller-Hinton agar (Oxoid, England) using disk diffusion method. Antimicrobials agents were used in this study they included ampicillin (10 μg), tetracycline (30 μg), chloramphenicol (30 μg), ceftriaxone (30 μg), cefturoxime(μg), erythromycin (25μg ), gentamicin (10 μg), ciprofloxacin (5 μg), penicillin G (10 μg) and cotrimoxazole (25 μg). The drug susceptibility pattern was interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2006, formerly known as National Committee for Clinical Laboratory Standards/NCCLS). Reference strains of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used for quality control for antimicrobial susceptibility tests.

3.6.1 **Procedure for Agar Disk Diffusion:**

3.6.1.1 Mueller-Hinton susceptibility test agar:

Mueller-Hinton agar medium is the only susceptibility test medium that has been validated by NCCLS.
3.6.1.2 McFarland turbidity standard:
The McFarland0.5 standard is used to adjust the turbidity of the inoculums for the susceptibility test (readymade).

3.6.1.3 Preparation of inoculums:
1. With an inoculating loop selected the colonies from non inhibitory agar medium (blood agar) and transferred to sterile a test tube contain 3 ml of sterile saline.
2. The bacterial suspension and the McFarland standard agitated on a vortex prior to measure.
3. hen first calibrated the reader device by using 0.5 McFarland standard and after measured the density of bacterial suspension.

3.6.1.4 Inoculation procedure
1. After adjusting the turbidity of the inoculums suspension, dipped asterile cotton swab into the suspension and Pressed firmly against the inside wall of the tube just above the fluid level.
2. Then Streaked the swab over the entire surface of the MHA medium.
3. The above mentioned antibiotics discs were placed individually on the surface of the solidified MHA agar with sterile forceps and gently pressed down onto the agar, after allowed to diffuse into the agar for 10-15 min and Incubated the plate at 37°C for 18hrs.
4. After incubation, measured the diameter of the zones of complete inhibition (including the diameter of the disk) with a ruler on the under surface of the plate and record it in millimeters.
5. After the strain was characterized as sensitive, intermediate or resistance based on the diameter of the inhibition zones was measured around the disc as described by the National Committee for Clinical Laboratory Standards.
Chapter Four

4. Results

4.1 Results:

Mixed growth of organisms was obtained from BA, CA, MCA and LBA included the strain under study, pure culture of the strain under study on TCBS and no growth on SDA and mixed growth of mixture of anaerobic organisms, the organisms was obtained are listed in Table(4-1).

Table 4.1. Types of Microorganisms isolated from ear discharge under study

<table>
<thead>
<tr>
<th>NO</th>
<th>Organisms</th>
<th>Gram stain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>V. cholera</td>
<td>G-ve comma shape</td>
</tr>
<tr>
<td>2</td>
<td>Pseudomonas pseudoalcaligenes</td>
<td>G-ve bacilli</td>
</tr>
<tr>
<td>3</td>
<td>Enterococcus species</td>
<td>G+ve cocci</td>
</tr>
<tr>
<td>4</td>
<td>Bacteroides species (Anaerobic)</td>
<td>G-ve bacilli (pale pink)</td>
</tr>
</tbody>
</table>

1- On Sheep Blood agar:
On Sheep BA the colonies show beta haemolytic due to haemolysin enzyme. (Figure - 14A)

2-On Mac Conkey’s agar:
Strain grow as pale, non-lactose-fermenting colonies

3-On Chocolate agar:
medium-sized convex, smooth colonies.

4-On TCBS agar:
It is grow as medium-sized convex, smooth, yellow colonies.

5-On CLED:
medium-sized convex, smooth, transparent colonies.

6-On Leaked blood agar
NO growth of vibrio only mixture of anaerobic bacteria.
7-On SDA:  
No growth.

8-Direct Gram stain:  
Demonstrated typical small, curved gram-negative rods mixed with other negative rods.

9-Indirect Gram stain:  
Gram negative with a rod curved like a comma on the observation under a microscope with magnification 1000 X.

10- oxidase test:  
A dark purple color appeared on the filter paper within 10 seconds indicated a positive reaction.

11- Wet mount:  
The organisms with typical small, curved rods (comma shape) and darting (“shooting star”) motility.

12- Phenotypic Characterization of Vibrio cholera by VITEK2 Compact system.  
The strain isolated was identified as V. cholerae by the automated VITEK 2 compact system using the ID-GN card (bioMe´rieux) with 99% probability (biotype profile number 0425601150400223) and a ‘excellent identification’ confidence level
13. Slide agglutination
The strain do not agglutinated in polyvalent antiserum (O1 antisera) nor monovalent antisera.

14. Triple Sugar Iron agar (TSI):
Triple sugar iron agar A/A, no gas, no H2S

15. String test:
A mucoid “string” is formed when an inoculated loop was drawn slowly away from the suspension. Positive and negative controls tested at the same time (Klebsiell Pneumonia as a positive control strain and Aeromonas as negative control strain)

16. Hemolysis Testing (Plate hemolysis):
Hemolytic colonies surrounded by a clear zones around them where red blood cells have been totally lysed

17. API 20 E:
Typical biochemical reaction when compared with vitek2 compact.

18. Antibiotic susceptibility patterns of V. cholera strain:
Table 4.2. Result of antibiotic susceptibility patterns of V. cholera strain.
Measured zone diameter (mm) and susceptibility status of V. cholera non O1

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>V. cholera nonO1</th>
<th>MIC (Zone Chart)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Interpretation</td>
<td>R</td>
</tr>
<tr>
<td>Augmentin</td>
<td>26 (S)</td>
<td>13</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>30 (S)</td>
<td>14</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>30 (S)</td>
<td>13</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>35 (S)</td>
<td>12</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>21 (S)</td>
<td>13</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>30 (S)</td>
<td>15</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>25 (S)</td>
<td>12</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>35 (S)</td>
<td>15</td>
</tr>
<tr>
<td>penicilin G</td>
<td>25 (S)</td>
<td>17</td>
</tr>
<tr>
<td>Trimethopim sulfamethoxazole</td>
<td>30 (S)</td>
<td>10</td>
</tr>
</tbody>
</table>

S: Susceptible; R: Resistant
Figure 4-1: Biochemical reaction of Vibrio cholera strain by standard biochemical reaction API 20E (manufactured in Franc Biomerieux).

Figure 4-2. Agar Disk Diffusion, Mueller-Hinton susceptibility test agar
Figure 4-3: Pure cultures of vibrio cholera. A on sheep blood agar. B on CLED medium. C on MacConkey agar. D on TCBS.

Figure 4-4: Pure culture of vibrio cholera strain on Mueller Hinton Agar.
Figure 4-5: Vitek 2 Compact System.

Figure 4-6: 0.5 McFarland standard tube (readymade) and vortex device (Grant bio).
Figure 4-7: Dispensater (dispensette).
Chapter Five

5. Discussion, Conclusion and Recommendations

5.1 Discussion:
In this case study the organism isolated from case of CSOM was determined and confirmed as *Vibrio* cholera Non-O1/non-O139 phenotypically by biochemical reaction, serology and antimicrobial susceptibility testing, it is a gram-negative, curved, rod-shaped, motile, ferment glucose produce acid but no gas and no H2S, oxidase positive, reduce nitrate to nitrite, grow well on thiosulphate-citrate bile salts sucrose medium (TCBS) selective media and grows as medium-sized convex, smooth, yellow colonies, produce hemolytic colonies surrounded by a clear zones around them (Beta heamolytic) on SheepBA, non-lactose-fermenting colonies on MAC agar, String test positive, grow in media deficiency or contain trace amounts of sodium chloride such as CLED medium, not agglutinated with polyvalent antisera for *Vibrio cholerae* O1 and O139; and monovalents for serotypes Inaba and Ogawa and which susceptible to all antimicrobial susceptibility testing was done {ampicillin (10 μg), tetracycline (30 μg), chloramphenicol (30 μg), ceftriaxone (30 μg), cefuroxime(μg), Erythromycin (25μg ), gentamicin (10 μg), ciprofloxacin (5 μg), penicillin G (10 μg), cotrimoxazole (25 μg)}. This case was presented to withdraw attention to V. species infections, especially V. cholera Non-O1/non-O139 strains which are very rarely seen and may also be a source of contamination of water, but should be taken into consideration in related cases and physicians, technicians should be aware of the possibility of *V. cholerae* other than O1 and O139 extra-intestinal infections, especially in patients seen during warm summers when water temperatures and recreational activities in water are high and to determine patron of antimicrobial susceptibility and portal of entry. This caus of V. cholera Non-O1/non-O139 serogroup a causative agent of ear infection which is responded well to Ciprofloxacin. However, timely antibiotic therapy often results in a favourable outcome. Most reported infections with non-O1/non-O139 V. cholerae have responded well to third-generation cephalosporins, piperacillin/tazobactam and fluoroquinolones. Moreover, in a study conducted in denmark, non-O1/non-O139 V. cholerae strains isolated from patients with ear infections were all susceptible to
ampicillin, ciprofloxacin, erythromycin, tetracycline, cefuroxime, chloramphenicol, ceftriaxone, gentamicin and cotrimoxazole but resistant to penicillin G while our strain susceptible to all antibiotics included Penicillin G. In patients with infections caused by non-O1/non-O139 V. cholerae and other strains of vibrio, the medical history often reveals a recent consumption of raw or undercooked seafood or exposure to contaminated water. However, in our report a review of the case history showed that there was no history of diarrhoea or vomiting and no strain of non-O1/non-O139 V. cholerae was detected in the stool specimen. The exact nature of the exposure in our patient swimming in a pool a weekly, the exposure to water might be a possible portal of entry of the strain especially our patient had recurrent episodes of otitis media since early childhood and when compared portal of entry of our strain with six strains isolated from infections of ears in Denmark between 1994-1998, seem that there are three strains isolated from patients are exposure to Seawater, one patient was exposure to fresh water/swimming in pool, one to fresh water and the last one unknown the source.

5.2 Conclusion
To the best of our knowledge, non-O1/non-O139 V. cholerae strain isolated from patient with ear infection after swimming in a pool is unusual organism and has not reported once before in UAE. As a marine microorganism, non-O1/non-O139 V. cholerae strain always causes human infection through exposure to seawater or ingestion of raw seafood. In our patient when we compare the portal of entry with organisms was isolated from otitis seems that most likely to be the Seawater is the main natural reservoir for non-O1 non-O139 V. cholerae since it requires trace amounts of sodium chloride for growth because it can also grow in fresh water and direct exposure to contaminated water that permitted the propagation of the organism, while when compare susceptibility pattern of our strain with six strains in Denmark we seem little different in the pattern, our strain sensitive to penicillin G while strains in Denmark were resistance to penicillin G and this result support our hypothesis because the pattern of susceptibility of Vibrio cholerae non-O1 strain isolate from CSOM in Saqr hospital – Ras Alkhaimah – UAE 2014 show slightly different in susceptibility from six strains was previous isolated in Denmark 1994-1998.
5.3 Recommendations

1. It is suggested that physicians should be aware of the possibility of *V. cholerae* non-O1, non-O139 extra intestinal infections especially in patients seen during warm summers, when water temperatures and recreational activities in water are high.

2. It is important to do further research on quality of river water, *Vibrio* species and its pathogenicity not only on clinical samples but also on seawater because it is the part of the transmission.

3. Clinicians should be aware that 12 pathogenic vibrios can cause chronic ear infections or any types of infections rather than toxogenic vibrio cholera O1 in both immunocompromised and healthy individuals whose are exposed to warm seawater.
References:


47. CWG. 1933. Large epidemic of cholera-like disease in Bangladesh caused by 
Vibrio cholerae O139 synonym Bengal. Cholera Working Group. Lancet 
342:387-390
48. Hlady WG, Klontz KC. The epidemiology of Vibrio infections in Florida, 
50. Levine WC, Griffin PM. Vibrio infections on the Gulf Coast: results of first 
51. Marano NN, Daniels NA, Easton AN, et al. Stool culturing practices for 
Vibrio species at clinical laboratories in Gulf Coast states. J Clin 
52. BOCKEMUHL, J., ROCH, K., WOHLERS, B., ALEKSIC, V., ALEKSIC, S. & 
WOKATSCH, R. (1986). Seasonal distribution of facultatively 
enteropathogenic vibrios (Vibrio cholerae, Vibrio mimicus, Vibrio 
parahaemolyticus) in the freshwater of the Elbe River at Hamburg. Journal of 
Applied Bacteriology 60, 435-442.
Vibrio cholerae serovars from surface waters in Western Colorado. Applied 
and Environmental Microbiology 51, 1216-1219.
parahaemolyticus and other halophilic vibrios associated with seafood in Hong 
Kong. Journal of Applied Bacteriology 66, 57-64.
in natural and cultivated oyster populations in the Pacific Northwest. 
Diagnostic Microbiology and Infectious Disease 9, 1-6.
56. NAIR, G., BALAKRISH, B. L., SARKAR, B. L., DE, S. P., 
Vibrio cholerae in the freshwater environs of Calcutta, India. Microbial 
Ecology 15, 203-216.
(2010) Non-O1, non-O139 Vibrio cholerae bacteraemia in a cirrhotic patient. J 
Med Microbiol 59:1260–1262


polysaccharide, which confers increased virulence. Microbial Pathology 16: 235 - 241.


75. Yamamoto, K., Y. Takeda, T. Miwatani, and J.P. Craig. 1983. Evidence that a non-O1 Vibrio cholerae produces enterotoxin that is similar but not identical to cholera enterotoxin. Infect. Immun. 41:896-901.


108. ‘IDENTIFICATION OF VIBRIO SPECIES’ Health Protection Agency (HPA). Issue no: 2 Issue date: 12.11.07 Issued by: Standards Unit, Evaluations and Standards Laboratory. Reference no: BSOP ID 19i2


