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The American University in Cairo

School of Science and Engineering

**MOLECULAR CHARACTERIZATION OF EXTENDED-
SPECTRUM Beta LACTAMASE (ESBL) PRODUCING
Klebsiella pneumoniae AND *Escherichia coli* AMONG
HOSPITALIZED PATIENTS IN OMAN**

A Thesis Submitted to:

The Biotechnology Program

In partial fulfillment of the requirements for the degree of Master
of Biotechnology

By: Lobna Mohamed Mourad

Bachelor in Biology

Supervisor: Dr. Guillermo Pimentel

Academic Advisor: Dr. Rania Siam

October 2010

DEDICATION

I would like to dedicate this thesis to my parents who have always been supportive of my career and ambitions; thank you Mom and Dad for all your faith in me and your encouragement. Special dedication to my best friend AHMED, thank you for always being there, believing in me, and for your endless support. Thank you Amira for your encouragement and constant motivation. Finally, I would like to dedicate this thesis to each and every single person who contributed in this study, believed in me, and supported my dreams and goals.

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ABSTRACT

The American University in Cairo

Molecular Characterization of Extended-Spectrum- β -Lactamases (ESBL) Producing
Klebsiella pneumoniae and *Escherichia coli* among Hospitalized Patients in Oman

Lobna Mohamed Mourad

Advisers: Dr. Guillermo Pimentel and Dr. Rania Siam

Hospital-acquired infections due to ESBL-producing gram-negative rods are a worldwide healthcare problem causing substantial patient morbidity and mortality. In the Middle East, a significant increase in incidence and prevalence has been reported recently due to the misuse of antibiotics and lack of coherent antimicrobial resistance (AR) surveillance programs. The aim of the study was to determine the level of genotypic diversity and mechanism of AR of *E. coli* and *K. pneumoniae* ESBL-producing isolates from nosocomial infections among patients in Oman. 35 *E. coli* and 14 *K. pneumoniae* isolates were used in the study. Antibiotic susceptibility testing (AST) and ESBL screening was conducted via disk diffusion and E-test following CLSI (Clinical and Laboratory Standards Institute) guidelines. ESBL producers were screened for *bla*_{CTX-M}, *bla*_{SHV}, *bla*_{OXA}, and *bla*_{TEM} resistance markers via PCR. All PCR amplicons were sequenced to determine their allelic variants. In order to demonstrate overall genotypic diversity, Pulsed-Field Gel Electrophoresis (PFGE) analyses were done separately for all *E. coli* and *K. pneumoniae* isolates. 40 (80%) isolates were determined to be ESBL producing bacteria (27 *E. coli* and 13 *K. pneumoniae*). The highest level of AR (>70%) was against tetracycline, ampicillin, nalidixic acid, cephalothin, and cefpodoxime. The lowest level of AR was against chloramphenicol, amikacin and ticarcillin-clavulanic acid.

Resistance against imipenem was not detected. Similarity of *K. pneumoniae* isolates ranged from 61% to 100%. Three *K. pneumoniae* clusters (n= 7; 58%) had \geq 80% similarity suggesting high level of similarity. *E. coli* PFGE analyses showed an overall similarity of 64% with 4 clusters (n= 14; 54%) showing 80% similarity. No correlation was demonstrated between the AR pattern and genotypic similarity for either species. Percentages of isolates with genetic markers for *bla*_{CTX-M}, *bla*_{SHV}, *bla*_{TEM}, and *bla*_{OXA} were 73%, 24%, 68%, and 60% respectively. DNA sequencing analyses revealed that the most common AR mechanism in these ESBL isolates is due to *bla*_{CTX-M-15} marker. In addition, SHV-1, SHV-11, SHV-12, TEM-1, and OXA-1 contribute to the overall AR mechanisms in nosocomial ESBL isolates from Oman. This is the first study characterizing the AR mechanism of ESBL's isolates from hospital-acquired infections in Oman. The results showed that hospital-acquired *E. coli* isolates from Oman are more diverse than *K. pneumoniae*. The *bla*_{CTX-M-15} is the most abundant mechanism conferring ESBL phenotype on *E. coli* and *K. pneumoniae*, while the ESBL-SHV-type was the least abundant.

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I. LITERATURE REVIEW

In the early 1980s, third-generation cephalosporins were an important therapeutic tool in treating severe bacterial infections due to β -lactamase-mediated resistance (Colodner, 2005; Perez *et al.*, 2007). However, after the emergence of cephalosporin-resistant (including ceftazidime) *Escherichia coli* and *Klebsiella pneumoniae* strains, the efficacy of these antibiotics in saving lives was significantly compromised. According to Knothe *et al.* (1983) there are three main characteristics that cause result in resistance to beta-lactam antibiotics: (1) production of beta-lactamases from chromosomal or plasmid genes; (2) changes in the active site(s) resulting in the loss of the ability to bind with the antibiotics; and (3) “Trapping” of the non-hydrolyzed antibiotics by highly induced lactamases, which prevents drug transportation and uptake. The β -lactamase enzymes were discovered in the early 1980s. These new beta-lactamase enzymes, called extended-spectrum-beta-lactamases (ESBLs), were found in common enteric bacilli and showed distinct hydrolytic properties. The first ESBL enzymes discovered were shown to be mainly derived from the original TEM and SHV β -lactamases as a result of point mutations in the original enzymes resulting in the enzymes’ “extended spectrum” of action (Falagas and Karageorgopoulos, 2009). For example, significant resistance was characterized by single amino acid changes such as: Gly238 \rightarrow Ser; Glu240 \rightarrow Lys; Arg164 \rightarrow Ser; Arg164 \rightarrow His; Asp179 \rightarrow Asn, and; Glu(Asp)104 \rightarrow Lys (Perez *et al.*, 2007). Currently, the presence of these ESBLs is becoming a major health problem for hospitalized patients, tertiary hospitals, facilities that offer long-term care, and even in the community (Perez *et al.*, 2007).

Beta-lactamases are known to be one of the most heterogeneous groups of resistance enzymes, with over 700 different beta-lactamases described. Although β -lactamases show a great deal of amino acid variability, they are known to share a common overall topology. The β -lactamases are globular proteins made up of alpha-helices and beta-pleated sheets (Perez *et al.*, 2007). ESBLs are known to have the capability of hydrolyzing several classes of antibiotics, such as penicillins, first-, second-, third-, and fourth-generation cephalosporins and the monobactam antibiotic aztreonam (Perez *et al.*, 2007). However, ESBL derivatives from the TEM and SHV families are inhibited by β -lactamase inhibitors that are available commercially, such as clavulanic acid, sulbactam, or tazobactam. The inhibition of ESBLs by β -lactamase inhibitors is a crucial property that it is used in the laboratory in the form of a phenotypic test that identifies whether a certain bacterial isolate possesses an ESBL or not (Perez *et al.*, 2007).

1. TEM β -lactamases:

The first TEM β -lactamase gene, designated ESBL gene as *bla*_{TEM-1}, was discovered in *E. coli* and *Salmonella enterica* serovar Paratyphi soon after the introduction of ampicillin for clinical use in 1965 (Mroczkowska and Barlow, 2008). The TEM-1 enzyme itself was first isolated from a Greek patient named Temoniera, from a blood culture of an *E. coli* isolate.

Since that time, the TEM-1 β -lactamase has been shown to occur world-wide and is currently thought to be the most common resistance mechanism against β -lactam antibiotics in Gram-negative bacilli (Sturenburg and Mack, 2003). During the 1970s, *bla*_{TEM-1} became widespread among Enterobacteriaceae and by the early 1980s was known to be the most prevalent resistance gene among clinical microbial populations.

The TEM-1 β -lactamase is known to confer resistance primarily against penicillins, including ampicillin (Mroczkowska and Barlow, 2008): up to 50-60% of plasmid-mediated ampicillin resistance in *E. coli* is due to TEM-1 β -lactamase. TEM-1 is known to be located on Tn3, a promiscuous transposon, in which it appears that several rearrangement and transposition events resulted in the migration of the TEM-1 gene to other bacterial strains, such as *Haemophilus influenza* and *Neisseria gonorrhoeae* (Sturenburg and Mack, 2003).

TEM-1 as well as its biochemical twin, TEM-2, hydrolyze penicillins and first-generation cephalosporins; however, they cannot hydrolyze the oxyimino cephalosporins (Sturenburg and Mack, 2003). In 1987, TEM-3 was the first TEM variant reported to have an increased activity against the extended-spectrum cephalosporins. Since then the number and variety of extended-spectrum TEM variants has increased rapidly. TEM is one of the most commonly found ESBL enzyme types in *E. coli* and *K. pneumoniae*, in which over 160 different variants are found in this family (Colodner, 2005; Mroczkowska and Barlow, 2008).

The point mutations that are clustered in particular areas of the enzyme are what determine the broader spectrum of resistance. As described by Joris *et al.* (1991) these point mutation sites are adjacent to four evolutionarily conserved structural regions that form the active site of the enzyme (Sturenburg and Mack, 2003). It has been shown that in the progenitor enzyme all these elements are very close to the active site, in which it is thought that the amino acid substitutions result in the enlargement of the active site resulting in novel enzyme-substrate interactions that take place between the large oxyimino substituents of the extended-spectrum cephalosporins (Sturenburg and Mack,

2003). In fact, the accelerated evolution of the *bla*_{TEM} sequence and phenotypic diversity presents it as a good model for studying the principles of evolutionary biology with clinical applications (Mroczkowska and Barlow, 2008).

Genetic recombination as well as protein engineering have demonstrated novel β -lactamases phenotypes. For example, ceftazidime resistant mutants can be generated using a wide variety of techniques such as insertional mutagenesis, site-directed and site-saturation mutagenesis, random replacement mutagenesis, DNA shuffling, and random insertional deletion strand exchange mutagenesis (Babic *et al.*, 2006). These experiments are mainly used in order to show how many substitutions can be generated to alter the TEM-1 phenotype. In some cases these experiments predicted the emergence of novel phenotypes before they appeared clinically (Babic *et al.*, 2006).

The fact that some *bla*_{TEM} alleles confer resistance against cephalosporins and penicillins might indicate the possible increased prevalence of these alleles due to their advantageous phenotypes. Therefore, it is predicted that ESBL *bla*_{TEM} alleles would either co-exist with *bla*_{TEM-1} or replace it as it is the most frequently found allele in clinical settings where cephalosporins are mainly used (Babic *et al.*, 2006). However, neither of these predictions has yet proven to be true among clinical bacterial isolates, and *bla*_{TEM-1} is still the most common allele conferring resistance to first-line cephalosporins (Mroczkowska and Barlow, 2008).

2. SHV β -lactamases:

The SHV-type ESBL is known to be one of the most common ESBL types in clinical isolates compared to other ESBL types. The designation SHV refers to the sulfhydryl

variable, inhibition of the SHV activity by *p*-chloromercuribenzoate is substrate-related; and varies depending on the substrate used for the assay; however, studies never confirmed the validity of this hypothesis (Paterson and Bonomo, 2005).

The SHV β -lactamase family originally was found in *Klebsiella* spp.. The progenitor of the SHV family, SHV-1, is universally present in *K. pneumoniae*. It has been shown that in most *K. pneumoniae* strains, the SHV-1 gene can be found on the bacterial chromosome, which may suggest that this gene may have evolved in *Klebsiella* and was subsequently incorporated into a plasmid that resulted in its spread to other enterobacterial species (Sturenburg and Mack, 2003). SHV-1 confers resistance to ampicillin, piperacillin, and ticarcillin, which are broad-spectrum penicillins; however, they do not confer resistance to the oxyimino substituted cephalosporins.

In 1983, three *K. pneumoniae* and one *Serratia marcescens* strains from patients in West Germany showed transferable resistance to cefotaxime and other new cephalosporins. Later it was shown that mutations in SHV-1 resulted in the emergence of the new plasmid-encoded β -lactamase, SHV-2, in which the amino acid at position 238 was changed from glycine to serine resulting in enhancement of the affinity of the SHV-1 β -lactamase to the oxyimino cephalosporins. In addition, this mutation led to a noticeable rise in the MIC to cefotaxime as well as slight rise in the MIC to ceftazidime (Sturenburg and Mack, 2003). Since that time, additional SHV ESBL variants have been reported containing various amino acid alterations, such as those in positions 179, 205, and 240, that contribute to an increased ESBL phenotype. Currently, there are over 40-SHV ESBL enzymes where changes in amino acid sequences have conferred the ability to hydrolyze the new cephalosporins (Sturenburg and Mack, 2003).

SHV ESBL-producing bacteria have been detected in various members of the Enterobacteriaceae. In addition, *Pseudomonas aeruginosa* and *Acinetobacter* spp. isolates have caused significant nosocomial outbreaks due to the production of ESBL by SHV enzymes (Paterson and Bonomo, 2005). Unlike TEM, only SHV-10 was reported as an SHV variant with an inhibitor resistant phenotype, where in position 130 the amino acid serine is replaced by glycine (Sturenburg and Mack, 2003). It was shown that SHV-2 and SHV-5 have the ability to hydrolyze cefepime where patients engaged in a retrospective study and infected with ESBL-producing bacteria with susceptibility to cefepime (Sturenburg and Mack, 2003).

3. CTX-M β -lactamases:

The CTX-M-type ESBLs now exceed over 40 different types, in which they are divided into five different clusters based on amino acid identities: group CTX-M-1 includes CTX-M-1, -3, -10, -12, -15, -28, -30 and FEC-1; group CTX-M-2 includes CTX-M-2, -4, -5, -6, -7, -20, and Toho-1; group CTX-M-8 includes only CTX-M-8; group CTX-M-9 includes CTX-M-9, -13, -14, -16, -17, -19, -21, -24, -27, and Toho-2, and finally; group CTX-M-25 includes CTX-M-25 and CTX-M-26 (Pitout *et al.*, 2005). CTX-M (cefotaxime) β -lactamases possess strong hydrolytic activity against cefotaxime. Bacterial isolates that possess CTX-M enzymes usually show MIC (minimum inhibitory concentration) levels against cefotaxime in the resistant range ($>64 \mu\text{g/ml}$), but MIC levels against ceftazidime in the susceptible range (2 to $8 \mu\text{g/ml}$). However, it has been reported that some CTX-M positive isolates have the ability to hydrolyze ceftazidime, conferring resistance against this particular cephalosporin (Paterson and Bonomo, 2005). It has also been shown that cefepime is efficiently hydrolyzed by the CTX-M enzymes,

for which MIC levels against cefepime are higher than for other ESBL-producing bacteria.

Unlike clavulanic acid, tazobactam shows a 10-fold inhibitory effect against CTX-M β -lactamases. It has been observed in several species that they harbor either both CTX-M and SHV ESBLs or CTX-M and AmpC β -lactamases. Toho-1 and Toho-2 β -lactamases are structurally related to the CTX-M β -lactamases. Similar to CTX-M β -lactamases, Toho-1 and Toho-2 hydrolytic activity is stronger against cefotaxime than ceftazidime (Paterson and Bonomo, 2005).

There has been a rapid spread of the CTX-M β -lactamases, and they have now been detected on every continent. Previously, CTX-M β -lactamases were mainly found in three different geographical regions: South America, Eastern Europe, and, the Far East (Paterson and Bonomo, 2005). Although it was observed in the past that CTX-M β -lactamases were infrequently detected in Western Europe and North America, the presence of CTX-M has been reported in recent years in these regions. Subsequently, the CTX-M β -lactamases have become widespread in India and China, and it is speculated by some that the CTX-M type of β -lactamases is the most frequent ESBL type. Several studies have shown the presence of identical CTX-M β -lactamases in different geographic areas, such as CTX-M-3 that was discovered in Poland and Taiwan (Paterson and Bonomo, 2005).

4. OXA β -lactamases:

OXA β -lactamases are named after their ability to hydrolyze oxacillin. They are predominantly found in *P. aeruginosa* but have been reported in several Gram-negative

species (Paterson and Bonomo, 2005). The hydrolysis rate of OXA β -lactamases for cloxacillin and oxacillin is known to be more than 50% compared to that of benzylpenicillin.

The OXA ESBLs were discovered originally in *P. aeruginosa* samples from a hospital in Ankara, Turkey (Paterson and Bonomo, 2005). Novel derivatives of OXA-10, OXA013 and OXA-18 were discovered in France in *P. aeruginosa* isolates from hospital-acquired infections. It has been shown that the ESBL OXA-type β -lactamases evolution from the parent enzymes with narrower spectra resembles the evolution of the TEM- and SHV-type ESBLs (Paterson and Bonomo, 2005).

OXA-1 is the most common OXA β -lactamase with a prevalence rate of 1 to 10% in isolates of *E. coli* that possess an OXA β -lactamase (Paterson and Bonomo, 2005). Most of the OXA β -lactamases do not significantly hydrolyze the extended-spectrum cephalosporins and hence they are not considered to be ESBLs. However, OXA-10 is known to weakly hydrolyze cefotaxime, aztreonam, and ceftriaxone, resulting in most organisms having a reduced susceptibility to these antibiotics. OXA-11, -14, -15, -16, -17, -18, -19, -28, -31, -32, -35, and -45, are all OXA ESBLs that confer resistance to cefotaxime, and in some cases ceftazidime and aztreonam (Paterson and Bonomo, 2005).

5. Overview of ESBL Producing Bacteria in the Middle East:

Since the 1980s, ESBL-producing bacteria have rapidly spread across many different geographical regions. This spread may be a result of the transmission of plasmids among unrelated Enterobacteriaceae, a phenomenon observed in French hospitals (Moubareck *et al.*, 2005), or by the clonal dissemination of the enzyme-producing microorganisms. In

several cases, transfer of ESBL-producing bacteria between hospitals followed by their clonal expansion and/or horizontal transfer of plasmids with ESBL genes may take place. Although ESBL-producing bacteria have been reported in many countries, few studies providing antibiotic resistance (AR) data in the Middle East have been published. The majority of the studies performed to date represent only hospitalized patients where the transfer rate of ESBL from patient fecal flora to community subjects is still unknown (Moubareck *et al.*, 2005). For example, a 5-year study in Lebanon (1997 - 2001), at Saint George Hospital, showed that among 4,299 *E. coli* and 1,248 *K. pneumoniae* isolates, 2% and 20%, respectively, were ESBL positive (Moubareck *et al.*, 2005). However, no epidemiological studies on ESBL-producing bacteria have been conducted recently in Lebanon. Another study done by Moubareck *et al.* (2005) revealed that out of 118 isolates, 72 (61%) were shown to be ESBL positive with *E. coli* being the most abundant ESBL carrier. Molecular data showed that CTX-M-15 was the most prevalent ESBL produced, while SHV-5a was detected in the remaining isolates (Moubareck *et al.*, 2005).

In Egypt, recent studies on Enterobacteriaceae revealed 70% AR rates to third-generation cephalosporins (Khalaf *et al.*, 2009). A survey from 2001 to 2002 covering medical centers in Northern and Southern European countries, Saudi Arabia, Lebanon, Egypt, and South Africa showed that the highest incidence of ESBL-producing bacteria was in Egypt. Recently, CTX-M-type ESBLs have been reported in Egypt, with CTX-M-15 being the most prevalent ESBL reported in the Middle East and North Africa (Khalaf *et al.*, 2009). Also, CTX-M-14 has been detected in Egypt and Tunisia. A study by Khalaf *et al.* (2009) showed that all 5 clinical isolates tested were ESBL positive and resistant to cefotaxime (CTX). Molecular analyses identified *bla*_{CTX-M-14} in *K.*

pneumoniae and *Enterobacter cloacae* and *bla*_{CTX-M-15} in *E. coli*. This study is considered to be the first report of CTX-M-14 in Egypt, North Africa, or the Middle East for *K. pneumoniae* and *E. coli* (Khalaf *et al.*, 2009).

In Saudi Arabia, a study by Al-Agamy *et al.* (2009) revealed that out of a total of 400 *K. pneumoniae* isolates collected from two hospitals in Riyadh, 55% were ESBL positive. PCR analyses showed that 97.3%, 84.1%, and 34.1% of ESBL isolates were positive for SHV, TEM, and CTX-M β -lactamase genes, respectively. Also CTX-M-1 and CTX-M-9 were identified with a prevalence of 60% and 40%, respectively (Al-Agamy *et al.*, 2009). In Kuwait, a study by Ensor *et al.* (2008) showed that CTX-M-15 is the predominant ESBL gene among *K. pneumoniae* and *E. coli* isolates from both hospital and community patients. Another study in Oman by Al-Lawati *et al.* (2000) showed that 2 out of 13 *E. coli* isolates and 3 out of 20 *Klebsiella* spp. isolates were ESBL positive, in which they were resistant to aztreonam and ceftazidime, but sensitive to ceftazclav.

II. PROJECT OBJECTIVES

Very minimal ESBL surveillance and molecular characterization studies has been carried out in the Middle East, which is a serious threat to the public health because of the increasing incidence of ESBL infections in the region. This study from Oman can be the start of a more thorough surveillance system throughout the Middle East. Isolates for this study were extracted from hospitalized patients with nosocomial infections in 2006. The objectives of the study are the following:

- To determine the percentage of *E. coli* and *K. pneumoniae* isolates that produce ESBL enzymes.
- To determine the AR mechanism of ESBL isolates by sequencing four resistant markers (*bla*_{CTX-M}, *bla*_{SHV}, *bla*_{TEM}, and *bla*_{OXA}) responsible for this phenotype.
- To compare DNA and amino acid sequence of ESBL producing isolates from hospital-acquired infections in Oman to isolates from other geographical regions.
- To determine the amount of genetic variability present in *E. coli* and *K. pneumoniae* isolates by molecular fingerprinting using Pulsed Field Gel Electrophoresis (PFGE).

III. MATERIALS AND METHODS

1. Specimen Collection, Identification and Archiving:

Bacterial isolates were donated for this study by Dr. Suleiman Busaidy, Director of the Central Public Health Laboratory (CPHL) in Oman. Isolates were taken from patient blood cultures at Public Health Hospitals as part of routine care for suspected nosocomial infections (sepsis). Highly-resistant Gram-negative rods identified by clinical laboratories were transferred to Dr. Busaidy's lab for further confirmation. Isolates were shipped to NAMRU-3 in chocolate agar slants at room temperature for advanced molecular characterization.

At NAMRU-3 samples were processed for ESBL identification and long-term archiving. All isolates were cultured on MacConkey agar (Becton Dickinson, USA) in a non-CO₂ incubator at 37°C for 24 hours. Phenotypic identification was carried out using the 5-biochemical differential tubes test (Kligler's Iron Agar, Motility Indole Ornithine Medium, Lysine Iron Agar, Simmons Citrate Agar and Urea Broth) (Becton Dickinson, USA), as per NAMRU-3 standard operating procedures (Manual of Clinical Microbiology, ASM, 2009). Confirmation of *K. pneumoniae* identity was carried out using the Analytical Profile Index for Enterobacteriaceae (API-20E) (BioMerieux, France). Following confirmation, isolates were inoculated aseptically using sterile cotton swabs (Becton Dickinson, USA) into 1.8 mL tubes and preserved in BrainHeart Infusion broth with 15% glycerol and stored at -70°C.

2. Antibiotic Susceptibility Testing (AST):

AST was conducted and interpreted following CLSI (Clinical and Laboratory Standards Institute) guidelines and/or manufacturer's instructions (CLSI, Wayne, PA USA). Tables 1 and 2 show the antibiotics tested using Kirby-Bauer and/or E-test (AB Biodisk, Solna, Sweden) methods, respectively, to determine the AR profile of each isolate.

Briefly, a bacterial suspension was prepared using 0.85% saline solution and turbidity adjusted to the equivalent of a 0.5 McFarland Standard. A sterile cotton swab was dipped into the bacterial suspension and used to inoculate Mueller-Hinton agar plates in three directions to ensure uniform growth of bacteria. Inoculated plates were left for 15 minutes to dry, antibiotic discs or E-test strips were placed on the agar surface using an automatic dispenser, and the plate incubated in a non-CO₂ incubator at 37°C for 18-20 hours. ESBL production was detected using the double-disk approximation method described by Jarlier *et al.* (1988). Utilizing CAZ or CTX in combination with clavulanic acid, the zone of inhibition was measured. A zone of inhibition ≥ 5 mm, when compared to CAZ or CTX without clavulanic acid, indicates an ESBL positive isolate (CLSI, Wayne, PA USA).

3. Pulsed-Field Gel Electrophoresis (PFGE):

Methods to prepare bacterial plugs and DNA restriction digestion for ESBL-producing *E. coli* and *K. pneumoniae* isolates were similar. Colonies were scraped from a MacConkey agar plate using a sterile cotton applicator and diluted in a cell suspension buffer (CSB) (1 M Tris, pH8.0, 0.5 M EDTA, pH 8.0) until an optical density of 0.5-0.55 was achieved using a turbidity meter (Dade Behring, CA, USA). An aliquot of bacterial suspension was gently mixed with an equal volume of melted agarose (pulsed field grade), and dispensed into pre-formed plug molds. PFGE plugs were made by transferring 400 µl of bacterial suspension into 1.5 ml reaction tubes that were mixed with 20 µl of proteinase K and 400 µl of the melted 1% SeaKem Gold Agarose(SKG): 1% sodium dodecyl sulfate (SDS) (47.5 ml of TE buffer, 2.5 ml 20% SDS and 0.5 g SKG). Immediately, the mixture was dispensed into the wells of the plug molds and left to solidify for 20 minutes. After the plugs solidified, they were transferred into 50 ml falcon tubes containing 5 ml cell lysis buffer (CLB) (1 M Tris, pH8.0, 0.5 M EDTA, pH 8.0, 5 g Sarcosyl) and 25 µl proteinase K. The BD Falcon 50-ml conical tubes were incubated at 54°C in a shaker for 2 hours with vigorous shaking. After incubation, the lysis buffer was discarded and plugs were washed twice with 10-ml sterile water and four times with 10-ml TE buffer. Each wash was for 20 minutes and after the final wash the plugs were stored in TE buffer at 4°C until digestion.

Bacterial DNA was digested using the *XbaI* enzyme (New England BioLabs, Ipswich, MA, USA). *Salmonella enterica* ser. Braenderup (Tenover *et al.*, 1995) was used as control and ladder to determine DNA fragment sizes and genetic relationships among isolates based on PFGE patterns. Briefly, each plug was cut with a razor blade

into a 2-mm slice and placed into a 1.5-ml tube with 200 µl of incubation buffer (180 µl sterile water and 20 µl 10X NE buffer (New England BioLabs, Ipswich, MA, USA)). Tubes were incubated at 37°C in a water bath for 15 minutes. The restriction solution was prepared by mixing 1755 µl of distilled-sterile water, 200 µl 10X NE buffer (New England BioLabs, Ipswich, MA, USA), 20 µl 100X BSA (New England BioLabs, Ipswich, MA, USA), and 25 µl 20,000 U/ml XbaI (New England BioLabs, Ipswich, MA, USA). After incubation, the 200-µl incubation buffer was discarded and 200 µl of restriction solution was added to the plugs. Tubes were incubated in a water bath at 37°C for 4 hours. Later, the restriction solution was discarded and 200 µl of 0.5 X TBE were added to the tubes. After restriction, the plugs were loaded into a 1% SKG agarose gel (0.5 X TBE). Plugs were placed at the bottom of a 10-well comb with control plugs at both ends. The plugs were dried using tissue paper to ensure no excess buffer remained in the plugs. Agarose gel was poured into the casting gel tray, left to solidify for 30 minutes and loaded into the buffer chamber of a CHEF DRII or DRIII pulsed field apparatus (Bio-Rad, Hercules, CA, USA) containing 0.5X TBE at 14°C. PFGE running conditions were as following: 6.0 V/cm for 19 hours for *K. pneumoniae* and 20 hours for *E. coli* at switch times from 2.2-54.2 seconds. PFGE patterns were observed by staining the gel with ethidium bromide for 20 minutes and de-staining three times with distilled water for 20 minutes each. Photos were taken with the Gel Doc XR System (Bio-Rad, Hercules, CA, USA). The banding pattern of the gels was then analyzed and dendograms were generated using the BioNumerics software.

4. Detection of Antimicrobial Resistance Alleles via PCR and Sequencing:

DNA extraction was carried out using the SV Minipreps Promega DNA Purification System (Promega, Madison, WI, USA) according to the manufacturer's instructions. Amplification of the *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M}, and *bla*_{OXA} genetic markers was carried out in a final volume of 25 µl using the following reaction: 10X buffer (Promega, Madison, WI, USA), 10 µM dNTP mix (Promega), 30µM PCR-specific forward primer, 30µM PCR-specific reverse primer, 5U/µl Taq polymerase (Promega, Madison, WI, USA), 25 µM MgCl₂ (Promega, Madison, WI, USA), 2 µl DNA and Molecular Biology Grade water. The PCR reactions were conducted in a GeneAmp PCR System 9700 (Applied Biosystems, Carlsbad, CA, USA). The amplicon-specific primers are shown in Table 3 (Chen *et al.*, 2004).

PCR Cycling Conditions

Genes	Initial denaturation (1 cycle)	Amplification cycles (30 times)	Final extension (1 cycle)
<i>bla</i> _{TEM}	94°C 5 min	94°C 30 sec, 52°C 45 sec and 72°C 45 sec	72°C 7 min
<i>bla</i> _{SHV}	95°C 10 min	95°C 30 sec, 55°C 1 min and 72°C 1 min	72°C 7 min
<i>bla</i> _{CTX-M}	94°C 5 min	94°C 30 sec, 57°C 45 sec and 72°C 45 sec	72°C 7 min
<i>bla</i> _{OXA}	94°C 5 min	94°C 30 sec, 52°C 45 sec and 72°C 45 sec	72°C 7 min

PCR amplicons were analyzed by gel electrophoresis on 1.5% agarose gel (Bio-Rad, Hercules, CA, USA) using 1X TBE (Promega, Madison, WI, USA) as running buffer. The gels were run at 100V for 40 minutes followed by staining for 20 minutes on

ethidium bromide and destaining for 30 minutes with distilled water. Gels were visualized and photographed under UV light.

5. Sequencing Reactions:

Only isolates that showed a single PCR amplicon were selected for sequencing analyses. Forward and reverse sequencing was carried out for all amplified targets. After amplification, the PCR amplicons were purified and sequenced. In the purification step, 10 μ l of the PCR product was added to 5 μ l of ExoSAP-IT reagent (USB, Cleveland, Ohio) and the mixture was incubated in a thermocycler for 15 minutes at 37°C followed by 15 minutes at 80°C. The purified PCR product was then subjected to cycle sequencing using the Big Dye Fluorescent System (Applied Biosystems, Carlsbad, CA, USA). The reaction was carried out in a total volume of 20 μ l with the following reagents: 2 μ l of the purified PCR product, 5X sequencing buffer (Applied Biosystems, Carlsbad, CA, USA), Big Dye Terminator V3.15X (Applied Biosystems, Carlsbad, CA, USA), molecular biology grade water, and either 3 μ M forward primer or 3 μ M reverse primer. The sequencing reaction was performed under the following conditions: initial denaturation at 96°C for 10 sec, followed by 25 cycles of 96°C for 10 sec, 50°C for 5 sec and 60°C for 4 min. Reactions were kept at 4°C or stored at -20°C until purification of the sequenced products using the Performa Gel Filtration Cartridge (Edge Biosystems, Gaithersburg, Maryland, USA) according to manufacturer's instructions. 10 μ l of sequenced products were loaded into the ABI 3100 (Applied Biosystems, Carlsbad, California, USA) automated sequencer plate.

6. Sequence Data Analyses:

The nucleotide sequences were compared using BLAST and analyzed using the BioEdit Clustal W program (Hall TA., 1999) as well as the BioNumerics software (Applied Maths, Inc. Austin, TX). Sequences were first assembled, aligned, and edited using the BioNumerics software. After that, sequences were imported to BioEdit where they were aligned with a known sequence and edited further. The nucleotide sequence was then converted to the amino acid sequence. The amino acid sequences were analyzed using the website <http://www.lahey.org/studies/>, which is maintained by the Lahey Clinic, in order to assign a specific TEM, SHV, CTX-M, or OXA allele to each isolate.

IV. RESULTS

1. Antimicrobial Susceptibility and ESBL Screening

50 Gram negative isolates were collected in 2006 from blood cultures of patients with suspected nosocomial infections at Public Health Hospitals in Oman. Out of 50 isolates (36 *E. coli* and 14 *K. pneumoniae*) tested, 40 (80%) were confirmed as ESBL-producing. Out of the 40 isolates, 27 (75%) *E. coli* and 13 (92.8%) *K. pneumoniae*, are ESBL producing species, which was determined using the double-disk approximation method as described in the materials and methods section. Antibiotic susceptibility testing showed that the highest level of AR (>70%) was against five different antibiotics, AM, CF, CPD, NA, and TE (Figure 3), in which the results were interpreted according to the measurement of the zone of inhibition using the CLSI standard guidelines. 47 (94%) isolates showed resistance against ampicillin (AM), which implies that it should not be used in treating nosocomial infections caused by ESBL-producing bacteria. Also 46 (92%) of the isolates were resistant to cephalothin (CF) also suggesting the poor antimicrobial activity of this particular agent against ESBL bacteria. Cefpodoxime (CPD), nalidixic acid (NA), and tetracycline (TE) all demonstrated high resistance ranging from 82% to 74%. On the other hand, the lowest level of AR ($\leq 16\%$) was shown against chloramphenicol (C), amikacin (AN) and ticarcillin-clavulanic acid (TIM) where 16% to 6% of the isolates were resistant to these three antimicrobial agents, suggesting the effectiveness of these antibiotics against ESBL-producing bacteria (Figure 3). The decreased percentage of resistance against ticarcillin-clavulanic acid (TIM) can highly be due to the fact that ticarcillin is combined with clavulanic acid, which is a β -lactamase inhibitor, and hence less number of bacteria species survived. This is also the case with ampicillin/sulbactam (SAM); however, the a higher percentage of resistance was

observed for this compound and this can be due to either the strength of ampicillin as an antimicrobial agent or decreased effectiveness of sulbactam as a β -lactamase inhibitor compared to that of clavulanic acid.

No resistance was detected against imipenem (IPM), in which none of the isolates was resistant to IPM suggesting the effectiveness of this antimicrobial agent against ESBL bacteria (Figure 3). Therefore, in terms of susceptibility, 100% of all the isolates were susceptible to IPM (Figure 1). In terms of intermediate resistance, Ticarcillin-clavulanic acid (TIM) was shown to have the highest number of intermediate isolates (n= 22), which also suggests the poor antimicrobial activity of this agent against ESBL bacteria (Figure 2). Based on E-test, 76% of the isolates conferred resistance to ceftriaxone (TX), while 50% of the isolates were susceptible to cefepime (PM) (Table 4). The E-test results indicate that less resistance has been observed against fourth generation cephalosporins (PM) compared to the antimicrobial agent that belongs to the third generation cephalosporins (TX).

2. Characterization of ESBL Genes

The forty isolates that tested positive for ESBL production were then analyzed to identify the type of ESBL present. Plasmid DNA was extracted and PCR utilized for the four major type markers. The percentages of isolates carrying the plasmid-encoded genetic markers, *bla*_{CTX-M}, *bla*_{SHV}, *bla*_{TEM}, and *bla*_{OXA} were 73%, 24%, 68%, and 60% respectively. The band sizes for *bla*_{CTX-M}, *bla*_{SHV}, *bla*_{TEM}, and *bla*_{OXA} were 766 bp, 720 bp, 650 bp, and 600 bp respectively (Figures 4, 5, 6, & 7).

Twenty-one *E. coli* and 8 *K. pneumoniae* isolates were positive for *bla*_{CTX-M} (Figure 4). These CTX-M type ESBL genes were then sequenced using the Big Dye protocol to identify the subtypes present. DNA sequencing analyses using the BioEdit and BioNumerics software packages, revealed that all *bla*_{CTX-M} positive isolates carried the CTX-M-15 allele (Figures 8 a & b). Isolate 06-012018 initially tested negative for any ESBL phenotype (i.e. no resistance to 3rd or 4th-generation cephalosporins). However, PCR and sequencing analyses subsequently demonstrated that it was positive for only CTX-M-15, which implies that the isolate did not possess the ESBL phenotype due to the ESBL gene not being expressed (Table 5).

Isolates of *K. pneumoniae* (n=10) were the only ones with *bla*_{SHV} markers (i.e. no *E. coli* isolates possessed the SHV type of ESBL), which is usually the case presented by the *Klebsiella* spp. (Figure 5). Subsequent sequencing analyses using the Lahey Clinic webpage, amino acid sequences revealed that 4 isolates possessed the SHV-11 subtype, 4 isolates the SHV-12 subtype, and the SHV-1 subtype was detected in 2 isolates (Figures 11 a & b). This indicates that sequencing analyses showed that only 8 isolates possessed the ESBL SHV-type, since SHV-1 is not categorized as an ESBL.

According to the PCR amplification, thirty-two isolates were positive for *bla*_{TEM} allele (Figure 6). All of these isolates (16 *E. coli* and 6 *K. pneumoniae*) carried the *bla*_{TEM-1} subtype showing that no ESBL TEM-type isolates have identified, since the TEM-1 is the parent enzyme from which ESBL TEM-types later originated (Figures 9 a & b). In addition, sequencing analyses revealed that all OXA-positive isolates (18 *E. coli* and 4 *K. pneumoniae*) were shown to possess the most common OXA allele, OXA-1 allele (Figure 7, 10 a & b).

3. Genotypic Analyses

PFGE was carried out in order to establish the genotypic fingerprint of the *E. coli* and *K. pneumoniae* isolates separately. As shown in the materials and methods section, the protocol for preparing the PFGE gels for both species was the same; however, there were only slight differences in the running conditions of the *E. coli* and *K. pneumoniae* gels. The PFGE banding pattern for both species was analyzed using the BioNumerics software, in which the DNA fragment sizes and genetic relationships were determined using the *Salmonella enterica* ser. Braenderup as control and ladder to determine. According to the PFGE gel analysis, similarity between the *K. pneumoniae* isolates ranged from 61% to 100% (Figure 13). PFGE results for *K. pneumoniae* revealed that all isolates clustered in two main clusters and a single outlier. Only two *K. pneumoniae* isolates (06-012053 and 06-012055) were shown to be indistinguishable (i.e. 100% similarity). However, in order to claim that these two particular isolates are identical more thorough epidemiological work needs to be carried out. As for *E. coli* isolates, they clustered in 4 main groups revealing an overall similarity of 64% (Figure 12). Similar to *K. pneumoniae*, only 2 *E. coli* isolates (06-012012 and 06-012029), were shown to be indistinguishable. For both species, there was no significant correlation found between the AR patterns and genotypic (as judged by PFGE similarity). The lack of correlation is due to the fact that the PFGE investigated the genotypic properties of the isolates, while the AR patterns tested for the ESBL phenotype.

V. DISCUSSION

Studies that characterize the AR mechanisms of ESBL-producing bacteria are essential to monitor the evolution and spread of ESBL genes and to establish a sound AR surveillance system that guides antibiotic prescription and use policies. ESBL-producing organisms represent a major threat to public health, but may be effectively controlled, partly by continuing epidemiological studies and solid pharmaceutical research aimed at enhancing the development of new β -lactam antibiotics effective against ESBL-producing bacteria. The Middle East region currently lacks thorough epidemiological studies investigating ESBL prevalence as well as robust surveillance systems. Therefore, more ESBL characterization studies are needed in order to monitor the spread of ESBL genes in the region and control the spread of ESBL-producing bacteria throughout hospitals and in the community.

This study was conducted as part of a larger study that investigated the increasing distribution of ESBL-producing bacteria among countries in the Persian Gulf and particularly illustrated the increasing percentage of ESBL-producing bacteria in Oman. Oman is a Middle Eastern country with a total population of 2.577 million, out of which 1.844 million are Omanis. Around 600,000 expatriates are known to live in Oman, most of whom are from Pakistan, Egypt, The Philippines, Bangladesh and India - all developing countries where the prevalence of ESBLs is known to be high (Encyclopedia of the Nations, 2010).

In this study, 80% (40/50) of analyzed Gram-negative bacilli tested positive for the ESBL phenotype. Compared to other published regional studies this percentage is substantially high, suggesting the possible misuse of antibiotic therapy by the general

public. A study at the intensive care unit (ICU) at the Royal Hospital in Oman showed that every 100 patients treated in the ICU received an antibiotic treatment for 916 days average, which were mainly third-generation cephalosporins, ciprofloxacin, amikacin, and imipenem. According to this study the antibiotic consumption increased by 50% from 1996 to 2000. With this substantial antibiotic use, it is not surprising to observe the noticeable change in the microbial ecology in terms of high incidence of resistance among the bacteria (Al-Lawati *et al.*, 2000). Also as noted by Al-Tawfiq *et al.* (2010) that a study from Oman reported that 31% of the urinary tract community *E. coli* were resistant to ciprofloxacin. According to Al-Tawfiq *et al.* (2010) the high incidence of antimicrobial resistance in the Middle East in general is due to number of reasons, such as the wide availability of over-the-counter antibiotics, patient-self medication, lack of public awareness in proper antibiotic use. Another explanation for the high percentage of ESBL bacteria could be due to the regular movement of immigrants from developing countries conducting manual labor in Oman.

The isolates provided for this study were collected in 2006 from the blood of hospitalized patients suffering from nosocomial infections at public health hospitals in Oman. Both ampicillin (a penicillin) and cephalothin (a cephalosporin) showed the highest levels of resistance among the isolates, 94% and 92%, respectively. Both of these antibiotic groups contain the characteristic beta-lactam ring that is targeted by the different beta-lactamases produced by most Gram-negative and very few Gram-positive bacteria. Alternatively, 100% of the tested strains were shown to be susceptible to imipenem, which suggests the effectiveness of imipenem as a course of treatment for patients suffering from nosocomial infections with ESBL positive bacterial species.

Based on results from the E-test method, which allows the determination of the MIC for each antibiotic tested, cephalothin was shown to have the highest level of resistance (90% of strains); while, 50% of isolates were susceptible to cefepime. The majority of isolates susceptible to cefepime were *E. coli*, with only one *K. pneumoniae* isolate (06-012047) being susceptible. According to such findings it can be deduced that cefepime would be more effective than cephalothin in the course of treatment against β -lactamase-producing *E. coli*. In other words, the highest resistance levels found were against a first-generation antibiotic, while the fourth-generation antibiotic was shown to be more effective against *E. coli* isolates. This type of surveillance can help guide treating physicians on which antibiotics are most appropriate and effective.

The main goal of performing PFGE on the isolates was to establish a genotypic map and to determine whether or not PFGE genotype correlated with ESBL profile. The *E. coli* dendrogram contained 26 isolates that ranged in similarity from 64% to 100%. The 26 isolates clustered into 4 main clusters, out of which 14 isolates were shown to have 80% or more similarity, resulting in 54% of the total number of *E. coli* isolates being $\geq 80\%$ similar. Isolates 06-012012 and 06-012029 were shown to be indistinguishable with 100% similarity. As for *K. pneumoniae*, the similarity among the 12 isolates ranged from 61% to 100%, forming three main clusters. Among these three clusters, 7 isolates (58%) were shown to be $\geq 80\%$ similar. Also, isolates 06-012053 and 06-012055 were shown to have 100% similarity making these two isolates indistinguishable, which according to Tenover *et al.*, indicates that these two isolates represent the same strain and suggest as well that the isolates might have been part of an outbreak (Tenover *et al.*, 1995). Since 54% of the *E. coli* isolates and 58% of the *K. pneumoniae* isolates were shown to be

≥80% similar, the isolates for both bacterial species are considered to be closely related. According to Tenover *et al.* isolates are assigned to the closely related category when the PFGE pattern differs by 2 or 3 fragments from that of an outbreak. Such pattern changes can be due to a single genetic event, such as a deletion, an insertion, or a point mutation (Tenover *et al.*, 1995). In this study it was determined that PFGE genotype (pattern) did not correlate with antibiotic resistance profile (including the E-test). In other words, no correlation could be established between the PFGE genotype and the ESBL phenotype. This lack of correlation may be due to the fact that PFGE typing is based on differences detected in the complete genome, while the ESBL typing only targets the specific ESBL genetic markers.

Studies conducted in other regions have shown significant genotypic diversity among isolates. According to Mendonca *et al.* (2009) a study in Portugal showed that 108 *K. pneumoniae* isolates identified 65 different PFGE profile types out of which only two turned out to have 100% similarity. In another study in Spain, Sorlozano *et al.* (2007) showed that a different PFGE pattern was obtained for each of the 8 clinical isolates studied. In Mexico, Mosqueda-Gomez *et al.* (2008) identified 54 different genotypes out of the 121 isolates typed, in which 21 isolates were shown to be unique while the other 100 clustered in 33 different genotypes with each containing two to four isolates. Such data from other studies show that the isolates in this study reveal a close genetic relatedness as well as genotypic similarities unlike other regions. Similarly, the PFGE results of a study in neonatal intensive care unit of a Kuwaiti hospital by Dashti *et al.* (2010) showed that all isolates have an identical banding pattern, which indicates that the bloodstream infections have been caused by a single clone of ESBL producing *K.*

pneumoniae. PFGE is a very useful laboratory tool that helps identifying accurately an outbreak situation. Since outbreak isolates are of the same species and known to be both genetically and epidemiologically related, they generally share the same PFGE banding pattern in terms of number of bands and band size.

Determining the AR mechanism of ESBL-producing strains is crucial because it helps monitor the spread and transmission of these ESBL-producing genes as well as maintaining a sound surveillance system that could provide guidance in appropriate courses of treatment to those suffering such bacterial infections. According to these analyses, all *bla*_{CTX-M} positive isolates contained specifically the CTX-M-15 allelic variant. This allele has been shown to be the most common CTX-M type allele in several Middle Eastern countries, such as Kuwait, Lebanon, Egypt, and Saudi Arabia (Ensor *et al.*, 2009; Moubareck *et al.*, 2005; Al-Agamy *et al.*, 2006; Khalaf *et al.*, 2009; Al-Agamy *et al.*, 2009). Given the population mobility in and out of Oman from countries where CTX-M is known to be prevalent, our high positive percentage (73%) could be related to these regional movement events.

It has been observed that there is a high prevalence of CTX-M groups in both hospital and community settings (Pitout *et al.*, 2005), which obscures treatment for several diseases such as urinary tract infections (Ehlers *et al.*, 2009). According to Ehlers *et al.* the occurrence of the CTX-M ESBLs affects public health by facilitating the spread of infection from the hospital to the community. Therefore, such ESBL-producing bacteria, especially the ones carrying CTX-M-type markers, require strict and efficient AR surveillance systems and careful treatment policy guidance in order to prevent increasing local AR rates (Ehlers *et al.*, 2009).

Based on sequence analyses for *bla*_{SHV}, SHV-11, SHV-12, and SHV-1 were the only allelic variants detected. Previous studies have shown that *K. pneumoniae* isolates are most likely to have SHV genes as the predominant AR mechanism (Sturenburg and Mack, 2003). A study in Iran by Shahcheraghi *et al.* (2007) demonstrated that SHV was the dominant ESBL enzyme in *K. pneumoniae* strains. Another study also in Iran by Feizabadi *et al.* (2010) revealed that the SHV types characterized were SHV-5, SHV-11, and SHV-12: all found in *K. pneumoniae* strains. According to Lee *et al.* (2006), *K. pneumoniae* produces class A chromosomal β -lactamases that are resistant to ampicillin, ticarcillin, amoxicillin, and carbenicillin (Lee *et al.*, 2006). In clinical *K. pneumoniae* isolates, three families of chromosomal β -lactamases have been identified, SHV, LEN, and OKP. Plasmid-mediated SHV-type ESBLs are very common in *K. pneumoniae* isolates, in which SHV-12 is known to be common in south-east Asia (Lee *et al.*, 2006). In the study by Lee *et al.* the ESBL incidence was more common in *K. pneumoniae* isolates containing the *bla*_{SHV-11} gene than it is in isolates containing the *bla*_{SHV-1} gene. Also it was demonstrated that a number of the *K. pneumoniae* isolates carrying the *bla*_{SHV-11} gene, also produced the ESBL SHV-12 subtype. However, the abundance of SHV-12 in chromosomal *bla*_{SHV-11} gene-containing strains remains unknown (Lee *et al.*, 2006). Also it is worth mentioning that SHV-1 is not considered an ESBL type since it is the parent enzyme from which precursors of ESBL SHV-type later evolved. According to Lee *et al.* it is possible to identify a chromosomal SHV as well as a plasmid SHV in the same isolate; therefore, the three SHV-types identified could have co-existed in a single isolate if chromosomal DNA was extracted as well. Since *bla*_{SHV} is known to be more common in chromosomal DNA, this represents a limitation in the study because if

additional to plasmid DNA, chromosomal DNA was extracted, the percentage of SHV-positive isolates might have been higher.

Several studies have shown the abundance of SHV-1, -11, and -12 types in southwestern Asia and the Middle East. Bali *et al.* (2010) showed that SHV-12 in *K. pneumoniae* and *E. coli*, is one of the most common ESBLs reported in Turkey. On the other hand, in Palestine Hussein *et al.* (2009) demonstrated that among 60 Gram-negative bacterial isolates from Palestinian hospitals in 2006, both bla_{SHV-1} and bla_{SHV-12} were identified among ESBL types found. As for bla_{TEM} , analyses revealed that all isolates were the TEM-1 subtype, which is not considered to be an ESBL as the TEM-1 subtype is the parent enzyme from which more TEM precursors with ESBL phenotype later originated. However, it is important to mention that such a result could be due to the use of a particular set of primers that were unable to discriminate between the different TEM families. In other words, the upstream and downstream primers are used to discriminate between closely related TEM subtypes; however, in this case only the primer detecting the core region of the gene was used and hence all isolates were characterized as TEM-1. Our study suggest that TEM is not a significant factor conferring the ESBL phenotype on *E. coli* and *K. pneumoniae* isolates from hospital-acquired infections in Oman. Due to the fact that bla_{TEM-1} was shown to be the most common allele in this study, it can be hypothesized that the bacteria might have a selective advantage expressing bla_{TEM-1} more than other alleles in the bla_{TEM} family. As was shown in studies in Turkey, Palestine, and Kuwait, only bla_{TEM-1} was identified from the TEM family (Hussein *et al.*, 2009; Bali *et al.*, 2010; Dashti *et al.*, 2010). In Turkey 50% of the isolates were positive for TEM (Bali *et al.*, 2010), while a study in Saudi Arabia revealed that 84.1% of the isolates were TEM

positive (Al-Agamy *et al.*, 2009). The lack of ESBL TEM allele in several countries of the Middle East region suggest decline of the ESBL TEM incidence compared to the rapid increase of CTX-M prevalence. Regarding *bla*_{OXA}, analysis showed that all the isolates were OXA-1 which is known to be the most common OXA β -lactamase especially in *E. coli*. Also OXA-1 is known to be the most common the Middle East region, such as in Palestine, OXA-1 was identified in hospital isolates (Hussein *et al.*, 2009).

Since the AR mechanisms identified were all located in plasmids, this facilitates the transfer and spread of these AR mechanisms in a geographical location. However, more epidemiological and molecular characterization studies need to be carried out in order to address this concept as well as monitor the spread of ESBL bacterial isolates in the region.

VI. CONCLUSION

Antibiotic resistance among Gram-negative bacteria represents a global challenge with the increased resistance to β -lactam antibiotics. According to Hanson (2010), there are two main strategies by which scientists can overcome the problem of resistance; the first is to develop new combinations of antibiotics faster than the development of resistance. The second strategy is to develop the technology that would enable scientists to carry out thorough and rapid surveillance of resistant bacteria both in community and hospital settings. The rapid detection and identification of resistance can lead to judicious antibiotic use, which will minimize the existence and development of more resistance.

This study revealed that a high incidence of ESBL-producing *E. coli* and *K. pneumoniae* was present in hospital-acquired infections in Oman. Genotypic analysis showed that both the *E. coli* and *K. pneumoniae* isolates were highly diverse suggesting that it is more than a single strain circulating. DNA sequencing analysis revealed that *bla*_{CTX-M-15} is the most prevalent ESBL producing gene found in hospital-acquired *E. coli* and *K. pneumoniae* isolates in Oman. The other ESBL genes revealed by the analyses were *bla*_{TEM-1}, *bla*_{SHV-1}, *bla*_{SHV-11}, *bla*_{SHV-12}, and *bla*_{OXA-1}. This study highlighted the importance of the need to improve the surveillance system of antibiotic resistance and resistance mechanisms in the Middle East. In addition, it is crucial to organize public awareness campaigns since they are necessary to encourage responsible antibiotic use.

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VIII. TABLES AND FIGURES

Antibiotics Tested via the Disk Diffusion Method

Antibiotic Discs	Antibiotic Name	Antibiotic Concentration
C	Chloramphenicol	30 µg
CRO	Ceftriaxone	30 µg
AN	Amikacin	30 µg
SXT	Trimethoprim-sulfamethoxazole	1.25/23.75 µg
TE	Tetracycline	30 µg
CIP	Ciprofloxacin	5 µg
GM	Gentamicin	10 µg
AM	Ampicillin	10 µg
NA	Nalidixic Acid	30 µg
IPM	Imipenem	10 µg
FEP	Cefepime	30 µg
ATM	Aztreonam	30 µg
SAM	Ampicillin/Sulbactam	10/10 µg
CF	Cephalothin	30 µg
TIM	Ticarcillin-clavulanic acid	75/10 µg
CPD	Cefpodoxime	10 µg
CAZ	Ceftazidime	30 µg
CTX	Cefotaxime	30 µg

Table 1: table showing the 18 different antibiotics (AB Biodisk, Solna, Sweden) tested via the disk diffusion method and the drug concentration for each particular antibiotic. The ATCC[®] controls (*E. coli* 25922, *S. aureus* 25923, *P. aeruginosa* 27853) were used to test the efficiency of the antibiotic discs.

Antibiotics Tested via E-test

E-Test Strips	E-Test Name
TZ	Ceftazidime
PM	Cefepime
CE	Cephalothin
TX	Ceftriaxone

Table 2: table showing the antibiotics (AB Biodisk, Solna, Sweden) tested via E-test. The ATCC[®] control (*E. coli* 25922) was used to test the efficiency of the antibiotic strips.

Forward and Reverse Primers Utilized for PCR of ESBL Genes

ESBL Genes	Forward (5'—3')	Reverse (5' – 3')	Size (bp)
<i>bla</i> _{TEM-1}	CAG CGG TAA GAT CCT TGA GA	ACT CCC CGT CGT GTA GAT AA	643
<i>bla</i> _{SHV-1}	GGC CGC GTA GGC ATG ATA GA	CCC GGC GAT TTG CTG ATT TC	714
<i>bla</i> _{CTX-M}	AAC CGT CAC GCT GTT GTT AG	TTG AGG CTG GGT GAA GTA AG	766
<i>bla</i> _{OXA-1}	AAT GGC ACC AGA TTC AAC TT	CTT GGC TTT TAT GCT TGA TG	595

Table 3: this table shows the primers used to amplify the four specific gene markers. The TEM primer amplifies the *bla*_{TEM} gene (858 bp), SHV primer amplifies the *bla*_{SHV} gene (861 bp), CTX-M primer amplifies the *bla*_{CTX-M} gene (876 bp), and OXA primer amplifies the *bla*_{OXA} gene (869 bp) (Chen *et al.*, 2004).

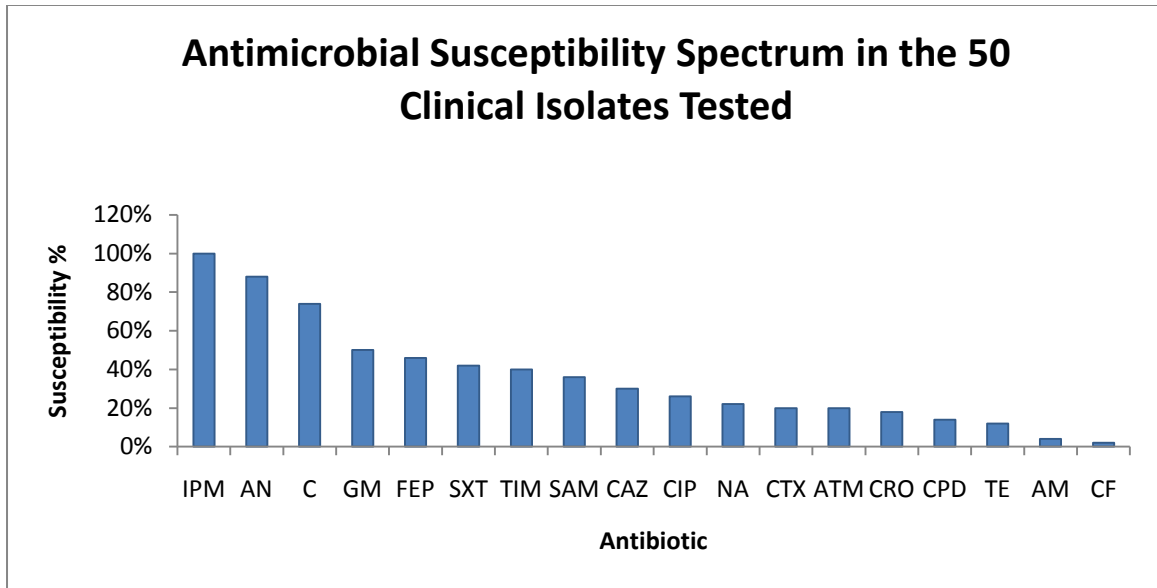


Figure 1: A bar graph showing the susceptibility of the 50 isolates against the 18 different antibiotics tested, measured by the zone of inhibition using the CLSI standards. All isolates (100%) were susceptible to IPM. AN was the second antibiotic to which 88% of the isolates were susceptible. On the other hand, the majority of the isolates presented the least susceptibility against AM and CF.

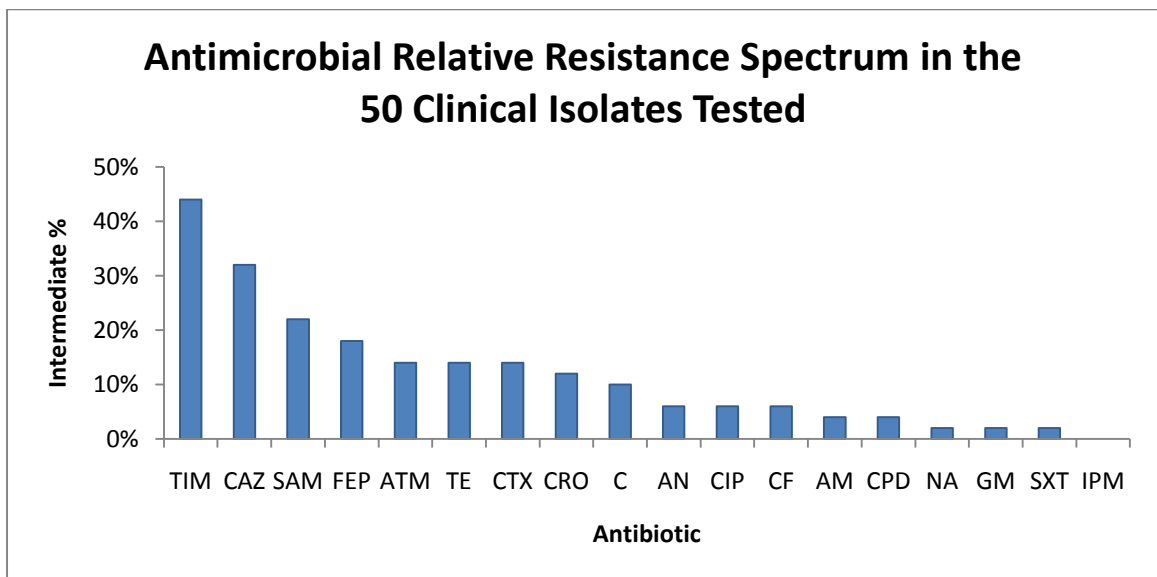


Figure 2: A bar graph showing the percentage of the isolates that are found in the intermediate region between the susceptibility and resistance, measured by the zone of inhibition using the CLSI standards. According to the data 44% of the isolates were resistant to TIM.

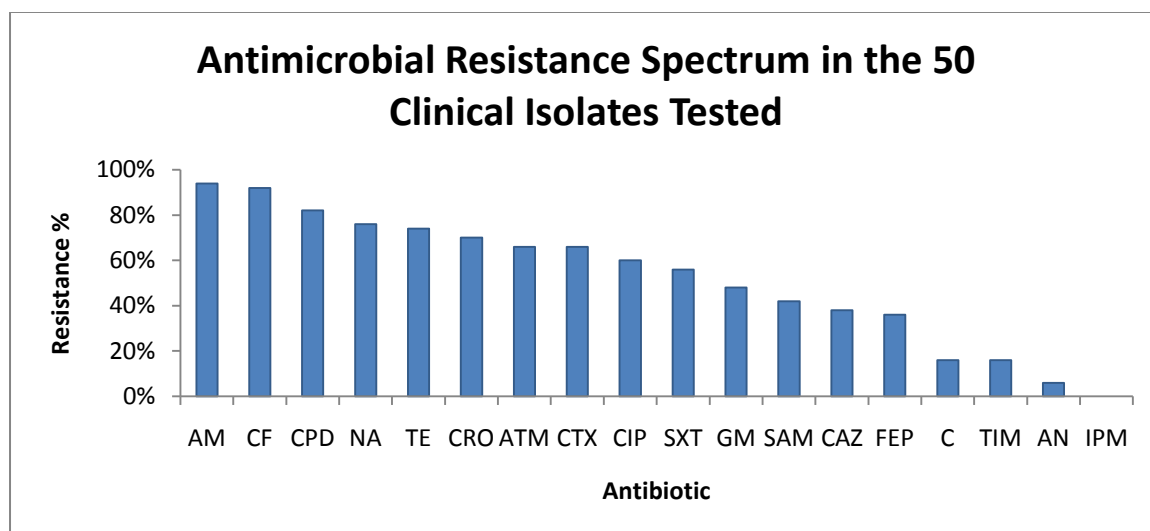


Figure 3: A bar graph showing the resistance of the 50 isolates against the 18 different antibiotics tested, measured by the zone of inhibition using the CLSI standards. According to the data, 94% of the isolates were resistant to AM, while 92% were resistant to CF making it the second in terms of resistance. On the other hand, all isolates (100%) showed no resistance to IPM. Also, only 6% of the isolates were resistant to AN making it the second antibiotic after IPM to have the highest efficacy.

Antimicrobial Resistance Spectrum Tested via E-Test

E-Test	Name	Resistance (%)	Intermediacy (%)	Susceptibility (%)
TZ	Ceftazidime	24%	40%	36%
PM	Cefepime	20%	30%	50%
CE	Cephalothin	90%	8%	2%
TX	Ceftriaxone	76%	4%	20%

Table 4: The E-test data shows the four different antibiotics tested and the percentages of the isolates resistant, intermediate, and susceptible to each particular antibiotic, by determining the minimum inhibitory concentration using the CLSI standards. 76% of the isolates were resistant to TX, while 50% of the isolates were susceptible to PM.

Amplification of CTX-M:

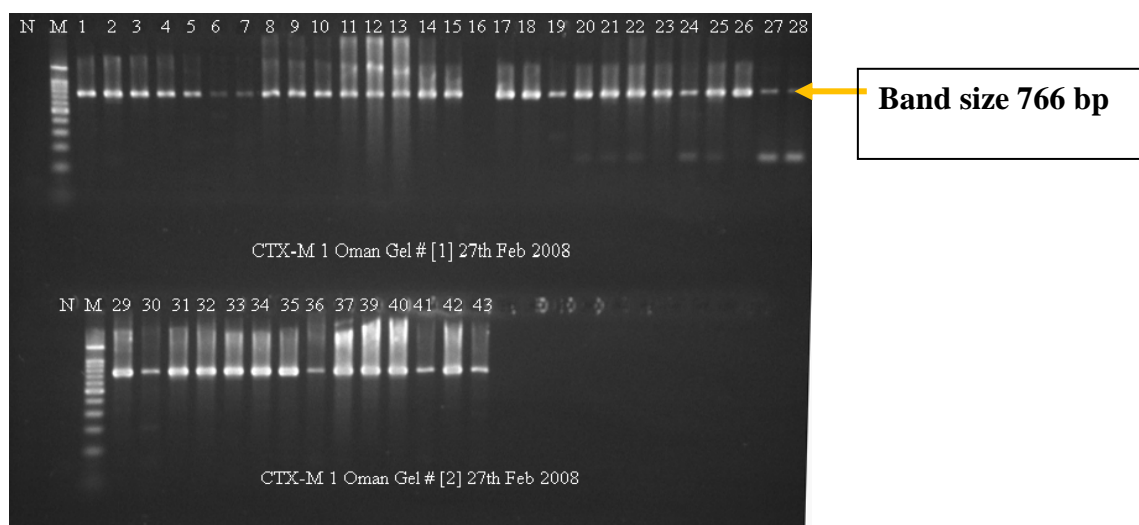


Figure 4: PCR gel showing the amplification of the *bla*_{CTX-M} gene. Isolates were categorized to be positive for *bla*_{CTX-M} if a single sharp band has been amplified. 73% of the 40 ESBL positive isolates possessed the *bla*_{CTX-M} gene. A 100 bp DNA ladder was used as a molecular marker (M).

Amplification of SHV:

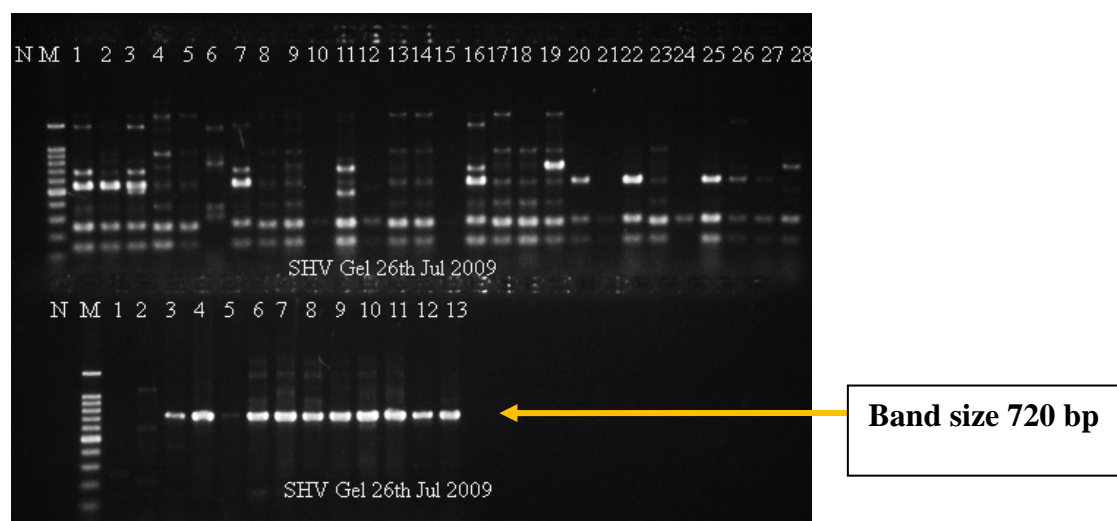


Figure 5: PCR gel showing the amplification of the *bla*_{SHV} gene. Isolates were categorized to be positive for *bla*_{SHV} if a single sharp band has been amplified. 24% of the 40 ESBL positive isolates possessed the *bla*_{SHV} gene. A 100 bp DNA ladder was used as a molecular marker (M).

Amplification of TEM:

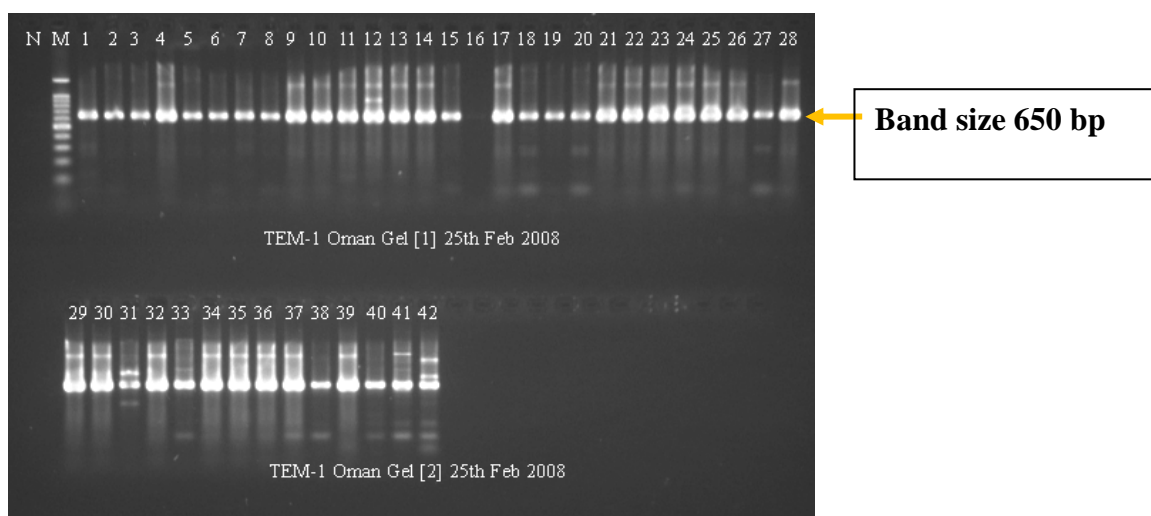


Figure 6: PCR gel showing the amplification of the *bla*_{TEM} gene. Isolates were categorized to be positive for *bla*_{TEM} if a single sharp band has been amplified. 68% of the 40 ESBL positive isolates possessed the *bla*_{TEM} gene. A 100 bp DNA ladder was used as a molecular marker (M).

Amplification of OXA:

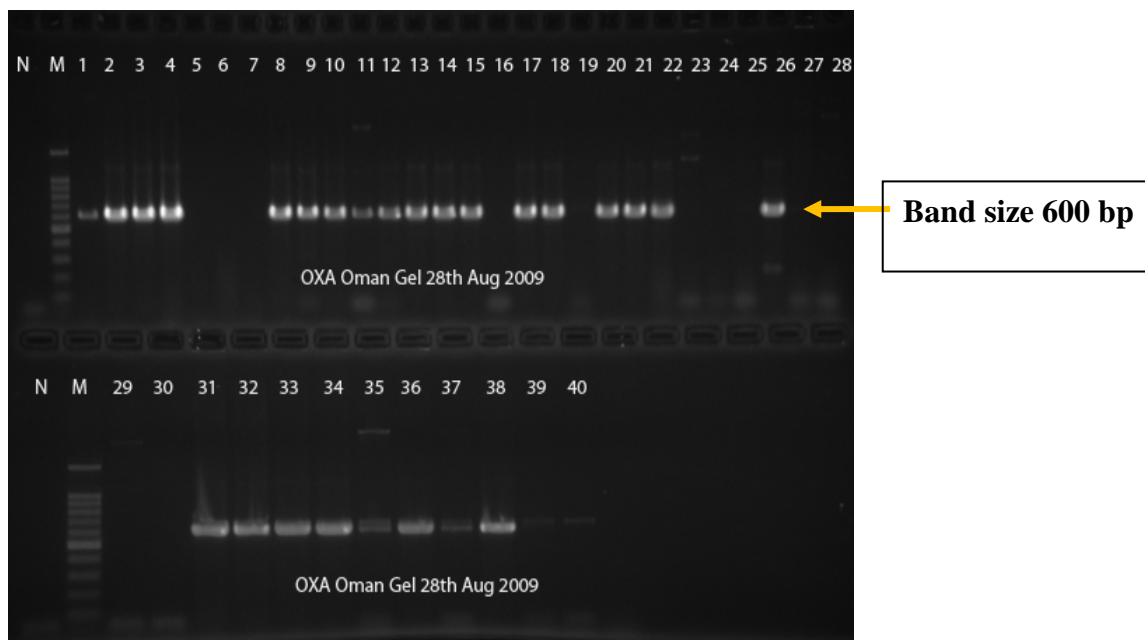


Figure 7: PCR gel showing the amplification of the *bla*_{OXA} gene. Isolates were categorized to be positive for *bla*_{OXA} if a single sharp band has been amplified. 60% of the 40 ESBL positive isolates possessed the *bla*_{OXA} gene. A 100 bp DNA ladder was used as a molecular marker (M).

CTX-M Nucleic Acid Sequence Alignment

CTXMF1_Oman(06-012014) -GCaACCGTCACGCTGTTGTTAGGAAGTGT GCCGCTGTAT GCGCAAACGG CGGACGTACA
CTXMF1_Oman(06-012015) GGCaACCGTC ACGCTGTTGT TAGGAAGTGT GCCGCTGTAT GCGCAAACGG
CGGACGTACA
CTXMF1_Oman(06-012016) GGCaACCGTC ACGCTGTTGT TAGGAAGTGT GCCGCTGTAT GCGCAAACGG
CGGACGTACA

CTXMF1_Oman(06-012014) GCAAAAACCTT GCCGAATTAG AGCGGCAGTC GGGAGGCAGA CTGGGTGTGG
CATTGATTAA
CTXMF1_Oman(06-012015) GCAAAAACCTT GCCGAATTAG AGCGGCAGTC GGGAGGCAGA CTGGGTGTGG
CATTGATTAA
CTXMF1_Oman(06-012016) GCAAAAACCTT GCCGAATTAG AGCGGCAGTC GGGAGGCAGA CTGGGTGTGG
CATTGATTAA

CTXMF1_Oman(06-012014) CACAGCAGAT AATTCGCAAA TACTTTATCG TGCTGATGAG CGCTTTGCGA
TGTGCAGCAC
CTXMF1_Oman(06-012015) CACAGCAGAT AATTCGCAAA TACTTTATCG TGCTGATGAG CGCTTTGCGA
TGTGCAGCAC
CTXMF1_Oman(06-012016) CACAGCAGAT AATTCGCAAA TACTTTATCG TGCTGATGAG CGCTTTGCGA
TGTGCAGCAC

CTXMF1_Oman(06-012014) CAGTAAAGTG ATGGCCGCGG CCGCGGTGCT GAAGAAAAGT GAAAGCGAAC
CGAATCTGTT
CTXMF1_Oman(06-012015) CAGTAAAGTG ATGGCCGCGG CCGCGGTGCT GAAGAAAAGT GAAAGCGAAC
CGAATCTGTT
CTXMF1_Oman(06-012016) CAGTAAAGTG ATGGCCGCGG CCGCGGTGCT GAAGAAAAGT GAAAGCGAAC
CGAATCTGTT

CTXMF1_Oman(06-012014) AAATCAGCGA GTTGAGATCA AAAAATCTGA CCTTGTTAAC TATAATCCGA
TTGCCGAAAA
CTXMF1_Oman(06-012015) AAATCAGCGA GTTGAGATCA AAAAATCTGA CCTTGTTAAC TATAATCCGA
TTGCCGAAAA
CTXMF1_Oman(06-012016) AAATCAGCGA GTTGAGATCA AAAAATCTGA CCTTGTTAAC TATAATCCGA
TTGCCGAAAA

CTXMF1_Oman(06-012014) GCACGTCAAT GGGACGATGT CACTGGCTGA GCTTAGCGCG GCCGCGCTAC
AGTACAGCGA
CTXMF1_Oman(06-012015) GCACGTCAAT GGGACGATGT CACTGGCTGA GCTTAGCGCG GCCGCGCTAC
AGTACAGCGA
CTXMF1_Oman(06-012016) GCACGTCAAT GGGACGATGT CACTGGCTGA GCTTAGCGCG GCCGCGCTAC
AGTACAGCGA

CTXMF1_Oman(06-012014) TAACGTGGCG ATGAATAAGC TGATTGCTCA CGTTGGCGGC CCGGCTAGCG
TCACCGGTT
CTXMF1_Oman(06-012015) TAACGTGGCG ATGAATAAGC TGATTGCTCA CGTTGGCGGC CCGGCTAGCG
TCACCGGTT
CTXMF1_Oman(06-012016) TAACGTGGCG ATGAATAAGC TGATTGCTCA CGTTGGCGGC CCGGCTAGCG
TCACCGGTT

CTXMF1_Oman(06-012014) CGCCGACAG CTGGGAGACG AAACGTTCCG TCTCGACCGT ACCGAGCCGA
CGTTAAACAC
CTXMF1_Oman(06-012015) CGCCGACAG CTGGGAGACG AAACGTTCCG TCTCGACCGT ACCGAGCCGA
CGTTAAACAC
CTXMF1_Oman(06-012016) CGCCGACAG CTGGGAGACG AAACGTTCCG TCTCGACCGT ACCGAGCCGA
CGTTAAACAC

CTXMF1_Oman(06-012014) CGCCATTCCG GGCGATCCGC GTGATACCAC TTCACCTCGG GCAATGGCGC
AAACTCTGCG
CTXMF1_Oman(06-012015) CGCCATTCCG GGCGATCCGC GTGATACCAC TTCACCTCGG GCAATGGCGC
AAACTCTGCG
CTXMF1_Oman(06-012016) CGCCATTCCG GGCGATCCGC GTGATACCAC TTCACCTCGG GCAATGGCGC
AAACTCTGCG

CTXMF1_Oman(06-012014) GAATCTGACG CTGGGTAAAG CATTGGGCGA CAGCCAACGG GCGCAGCTGG
TGACATGGAT
CTXMF1_Oman(06-012015) GAATCTGACG CTGGGTAAAG CATTGGGCGA CAGCCAACGG GCGCAGCTGG
TGACATGGAT

CTXMF1_Oman(06-012016) GAATCTGACG CTGGGTAAAG CATTGGGCGA CAGCCAACGG GCGCAGCTGG TGACATGGAT

CTXMF1_Oman(06-012014) GAAAGGCAAT ACCACCGGTG CAGCGAGCAT TCAGGCTGGA CTGCCTGCTT CCTGGGTTGT

CTXMF1_Oman(06-012015) GAAAGGCAAT ACCACCGGTG CAGCGAGCAT TCAGGCTGGA CTGCCTGCTT CCTGGGTTGT

CTXMF1_Oman(06-012016) GAAAGGCAAT ACCACCGGTG CAGCGAGCAT TCAGGCTGGA CTGCCTGCTT CCTGGGTTGT

CTXMF1_Oman(06-012014) GGGGGATAAA ACCGGCAGCG GTGGCTATGG CACCACCAAC GATATCGCGG TGATCTGGCC

CTXMF1_Oman(06-012015) GGGGGATAAA ACCGGCAGCG GTGGCTATGG CACCACCAAC GATATCGCGG TGATCTGGCc

CTXMF1_Oman(06-012016) GGGGGATAAA ACCGGCAGCG GTGGCTATGG CACCACCAAC GATATCGCGG TGATCTGGCC

CTXMF1_Oman(06-012014) AAAAGATCGT GCGCCGCTGA TTCTGGTCAC TTA^tCTTACC CAGCCTCAAC CTAAGGCAGA

CTXMF1_Oman(06-012015) AAAAGATCGT GCGCCGCTGA TTCTGGTCAC TTA^tCTTACC CAGCCTCAAC CTAAGGCAGA

CTXMF1_Oman(06-012016) AAAAGATCGT GCGCCGCTGA TTCTGGTCAC TTA^tCTTACC CAGCCTCAAC CTAAGGCAGA

CTXMF1_Oman(06-012014) AAGCCGTCGC GATGTAT^tAG CGTC

CTXMF1_Oman(06-012015) AAGCCGTCGC GATGTAT^tAG CGTC

CTXMF1_Oman(06-012016) AAGCCGTCGC GATGTAT^tAG CgTC

Figure 8a: the DNA sequence alignment of 3 *E. coli* isolates for the CTX-M gene. This alignment after the sequences have been imported from BioNumerics to BioEdit for DNA and amino acid sequence alignment.

CTX-M Amino Acid Sequence Alignment

CTXMF1_Oman(06-012014) ATVTLLLSV PLYAQTADVQ QKLAELERQS GGRLGVALIN TADNSQILYR ADERFAMCST

CTXMF1_Oman(06-012015) ATVTLLLSV PLYAQTADVQ QKLAELERQS GGRLGVALIN TADNSQILYR ADERFAMCST

CTXMF1_Oman(06-012016) ATVTLLLSV PLYAQTADVQ QKLAELERQS GGRLGVALIN TADNSQILYR ADERFAMCST

CTXMF1_Oman(06-012014) SKVM^aAAAVL KKSESEPNLL NQRVEIKKSD LVN^yYNPIAEK HVNGTMSLAE LSAAALQYSD

CTXMF1_Oman(06-012015) SKVM^aAAAVL KKSESEPNLL NQRVEIKKSD LVN^yYNPIAEK HVNGTMSLAE LSAAALQYSD

CTXMF1_Oman(06-012016) SKVM^aAAAVL KKSESEPNLL NQRVEIKKSD LVN^yYNPIAEK HVNGTMSLAE LSAAALQYSD

CTXMF1_Oman(06-012014) NVAMNK^lIAH VGGPASVTAF ARQLGDETFR LDRTEPTLNT AIPGDPRDTT SPRAMAQTLR

CTXMF1_Oman(06-012015) NVAMNK^lIAH VGGPASVTAF ARQLGDETFR LDRTEPTLNT AIPGDPRDTT SPRAMAQTLR

CTXMF1_Oman(06-012016) NVAMNK^lIAH VGGPASVTAF ARQLGDETFR LDRTEPTLNT AIPGDPRDTT SPRAMAQTLR

CTXMF1_Oman(06-012014) NLTGKALGD SQRAQLVTWM KGNTTGAASI QAGLPASWVW GDKTGSGGYG TTNDIAVIWP

CTXMF1_Oman(06-012015) NLTGKALGD SQRAQLVTWM KGNTTGAASI QAGLPASWVW GDKTGSGGYG TTNDIAVIWP

CTXMF1_Oman(06-012016) NLTGKALGD SQRAQLVTWM KGNTTGAASI QAGLPASWVW GDKTGSGGYG TTNDIAVIWP

CTXMF1_Oman(06-012014) KDRAPLILVT YFTQPQKAE SRRDVLA

CTXMF1_Oman(06-012015) KDRAPLILVT YFTQPQKAE SRRDVLA

CTXMF1_Oman(06-012016) KDRAPLILVT YFTQPQKAE SRRDVLA

Figure 8b: the amino sequence alignment of 3 *E. coli* isolates for the CTX-M gene. This alignment after the DNA sequences have been translated to the amino acid sequences on the BioEdit for further analysis using the Lahey Clinic webpage.

TEM Nucleic Acid Sequence Alignment

06-012015 TATGTAATGA TAATAATGGT TTCTTAGACG TCAGGTGGCA CTTTTCGGGG AAATGTGCGC
06-012023 TGATGTCTGA TAATAATGGT TTCTTAGACG TCAGGTGGCA CTTTTCGGGG AAATGTGCGC
06-012024 TGATGTCTGA TAATAATGGT TTCTTAGACG TCAGGTGGCA CTTTTCGGGG AAATGTGCGC

06-012015 GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA TGTATCCGCT CATGAGACAA
06-012023 GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA TGTATCCGCT CATGAGACAA
06-012024 GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA TGTATCCGCT CATGAGACAA

06-012015 TAACCCTGGT AAATGCTTCA ATAATATTGA AAAAGGAAGA GTATGAGTAT TCAACATTTT
06-012023 TAACCCTGGT AAATGCTTCA ATAATATTGA AAAAGGAAGA GTATGAGTAT TCAACATTTT
06-012024 TAACCCTGGT AAATGCTTCA ATAATATTGA AAAAGGAAGA GTATGAGTAT TCAACATTTT

06-012015 CGTGTGCGCC TTATTCCTT TTTGCGGCA TTTGCTTC CTGTTTTGC TCACCCAGAA
06-012023 CGTGTGCGCC TTATTCCTT TTTGCGGCA TTTGCTTC CTGTTTTGC TCACCCAGAA
06-012024 CGTGTGCGCC TTATTCCTT TTTGCGGCA TTTGCTTC CTGTTTTGC TCACCCAGAA

06-012015 ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT CAGTTGGGTG CACGAGTGGG TTACATCGAA
06-012023 ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT CAGTTGGGTG CACGAGTGGG TTACATCGAA
06-012024 ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT CAGTTGGGTG CACGAGTGGG TTACATCGAA

06-012015 CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC CCGAAGAACG TTTTCCAATG
06-012023 CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC CCGAAGAACG TTTTCCAATG
06-012024 CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC CCGAAGAACG TTTTCCAATG

06-012015 ATGAGCACTT TTAAGTTCT GCTATGTGGT GCGGTATTAT CCCGTGTTGA CGCCGGGCAA
06-012023 ATGAGCACTT TTAAGTTCT GCTATGTGGT GCGGTATTAT CCCGTGTTGA CGCCGGGCAA
06-012024 ATGAGCACTT TTAAGTTCT GCTATGTGGT GCGGTATTAT CCCGTGTTGA CGCCGGGCAA

06-012015 GAGCAACTCG GTCGCCGCAT AACTATTCT CAGAATGACT TGGTTGAGTA CTCACCAGTC
06-012023 GAGCAACTCG GTCGCCGCAT AACTATTCT CAGAATGACT TGGTTGAGTA CTCACCAGTC
06-012024 GAGCAACTCG GTCGCCGCAT AACTATTCT CAGAATGACT TGGTTGAGTA CTCACCAGTC

06-012015 ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC TGCCATAACC
06-012023 ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC TGCCATAACC
06-012024 ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC TGCCATAACC

06-012015 ATGAGTGATA AACTGCTGC CACTTACTT CTGACAACGA TCGGAGGACC GAAGGAGCTA
06-012023 ATGAGTGATA AACTGCTGC CACTTACTT CTGACAACGA TCGGAGGACC GAAGGAGCTA
06-012024 ATGAGTGATA AACTGCTGC CACTTACTT CTGACAACGA TCGGAGGACC GAAGGAGCTA

06-012015 ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC TTGATCGTTG GGAACCGGAG
06-012023 ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC TTGATCGTTG GGAACCGGAG
06-012024 ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC TTGATCGTTG GGAACCGGAG

06-012015 CTGAATGAAG CCATACAAA CGACGAGCGT GACACCACGA TGCCTGCAGC AATGGCAACA
06-012023 CTGAATGAAG CCATACAAA CGACGAGCGT GACACCACGA TGCCTGCAGC AATGGCAACA
06-012024 CTGAATGAAG CCATACAAA CGACGAGCGT GACACCACGA TGCCTGCAGC AATGGCAACA

06-012015 ACGTTGCGCA AACTATTAAC TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA
06-012023 ACGTTGCGCA AACTATTAAC TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA
06-012024 ACGTTGCGCA AACTATTAAC TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA

06-012015 GACTGGATGG AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC
06-012023 GACTGGATGG AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC
06-012024 GACTGGATGG AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC

06-012015 TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT CATTGCAGCA
06-012023 TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT CATTGCAGCA
06-012024 TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT CATTGCAGCA

06-012015 CTGGGGCCAG ATGGTAAGCC CTCCCCTATC GCAGTTATCT ACACGACGGG GAGTCAGGCA
06-012023 CTGGGGCCAG ATGGTAAGCC CTCCCCTATC GCAGTTATCT ACACGACGGG GAGTCAGGCA
06-012024 CTGGGGCCAG ATGGTAAGCC CTCCCCTATC GCAGTTATCT ACACGACGGG GAGTCAGGCA

06-012015 ACTATG
06-012023 ACTATG
06-012024 ACTATG

Figure 9a: the DNA sequence alignment of 3 *E. coli* isolates for the TEM gene. This alignment after the sequences alignment have been imported from BioNumerics to BioEdit for DNA and amino acid sequence alignment.

TEM Amino Acid Sequence Alignment

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06-012015 IIMVS*TS GG TFRGNVRGTP ICLFF*IHSN MYPLMRQ*PW *MLQ*Y*KRK SMSIQHFRVA
06-012023 IIMVS*TS GG TFRGNVRGTP ICLFF*IHSN MYPLMRQ*PW *MLQ*Y*KRK SMSIQHFRVA
06-012024 IIMVS*TS GG TFRGNVRGTP ICLFF*IHSN MYPLMRQ*PW *MLQ*Y*KRK SMSIQHFRVA

06-012015 LIPFFAAFCL PVFAHPETLV KVKDAEDQLG ARVGYIELDL NSGKILESFR PEERFPMMST
06-012023 LIPFFAAFCL PVFAHPETLV KVKDAEDQLG ARVGYIELDL NSGKILESFR PEERFPMMST
06-012024 LIPFFAAFCL PVFAHPETLV KVKDAEDQLG ARVGYIELDL NSGKILESFR PEERFPMMST

06-012015 FKVLLCGAVL SRVDAGQEQL GRRIHYSQND LVEYSPVTEK HLTGDMTVRE LCSAAITMSD
06-012023 FKVLLCGAVL SRVDAGQEQL GRRIHYSQND LVEYSPVTEK HLTGDMTVRE LCSAAITMSD
06-012024 FKVLLCGAVL SRVDAGQEQL GRRIHYSQND LVEYSPVTEK HLTGDMTVRE LCSAAITMSD

06-012015 NTAANLLLT IGGPKELTAF LHNMGDHSVTR LDRWEPELNE AIPNDERDTT MPAAMATTLR
06-012023 NTAANLLLT IGGPKELTAF LHNMGDHSVTR LDRWEPELNE AIPNDERDTT MPAAMATTLR
06-012024 NTAANLLLT IGGPKELTAF LHNMGDHSVTR LDRWEPELNE AIPNDERDTT MPAAMATTLR

06-012015 KLLTGELLTL ASRQQLIDWM EADKVAGPLL RSALPAGWFI ADKSGAGERG SRGIIAALGP
06-012023 KLLTGELLTL ASRQQLIDWM EADKVAGPLL RSALPAGWFI ADKSGAGERG SRGIIAALGP
06-012024 KLLTGELLTL ASRQQLIDWM EADKVAGPLL RSALPAGWFI ADKSGAGERG SRGIIAALGP

06-012015 DGKPSRIAVI YTTGSQATM
06-012023 DGKPSRIAVI YTTGSQATM
06-012024 DGKPSRIAVI YTTGSQATM
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Figure 9b: the amino sequence alignment of 3 *E. coli* isolates for the TEM gene. This alignment after the DNA sequences have been translated to the amino acid sequences on the BioEdit for further analysis using the Lahey Clinic webpage.

OXA Nucleic Acid Sequence Alignment

06-012014 TTATGGCATT TGATGCGGAA TAATAGATCA GAAAACCATA TTCAAATGGG ATAAAACCCC
CAAAGGAATG
06-012015 TTATGGCATT TGATGCGGAA TAATAGATCA GAAAACCATA TTCAAATGGG ATAAAACCCC
CAAAGGAATG
06-012019 TTATGGCATT TGATGCGGAA TAATAGATCA GAAAACCATA TTCAAATGGG ATAAAACCCC
CAAAGGAATG

06-012014 GAGATCTGGA ACAGCAATCA TACACCAAAG ACGTGGATGC AATTTTCTGT TGTTTGGGTT
TCGCAAGAAA
06-012015 GAGATCTGGA ACAGCAATCA TACACCAAAG ACGTGGATGC AATTTTCTGT TGTTTGGGTT
TCGCAAGAAA
06-012019 GAGATCTGGA ACAGCAATCA TACACCAAAG ACGTGGATGC AATTTTCTGT TGTTTGGGTT
TCGCAAGAAA

06-012014 TAACCCAAAA AATTGGATTA AATAAAATCA AGAATTATCT CAAAGATTTT GATTATGGAA
ATCAAGACTT
06-012015 TAACCCAAAA AATTGGATTA AATAAAATCA AGAATTATCT CAAAGATTTT GATTATGGAA
ATCAAGACTT
06-012019 TAACCCAAAA AATTGGATTA AATAAAATCA AGAATTATCT CAAAGATTTT GATTATGGAA
ATCAAGACTT

06-012014 CTCTGGAGAT AAAGAAAAGAA ACAACGGATT AACAGAAGCA TGGCTCGAAA GTAGCTTAAA
AATTCACCA
06-012015 CTCTGGAGAT AAAGAAAAGAA ACAACGGATT AACAGAAGCA TGGCTCGAAA GTAGCTTAAA
AATTCACCA
06-012019 CTCTGGAGAT AAAGAAAAGAA ACAACGGATT AACAGAAGCA TGGCTCGAAA GTAGCTTAAA
AATTCACCA

06-012014 GAAGAACAAA TTCAATTCCT GCGTAAAATT ATTAATCACA ATCTCCAGT TAAAACTCA
GCCATAGAAA
06-012015 GAAGAACAAA TTCAATTCCT GCGTAAAATT ATTAATCACA ATCTCCAGT TAAAACTCA
GCCATAGAAA
06-012019 GAAGAACAAA TTCAATTCCT GCGTAAAATT ATTAATCACA ATCTCCAGT TAAAACTCA
GCCATAGAAA

06-012014 ACACCATAGA GAACATGTAT CTACAAGATC TGGATAATAG TACAAAACCTG TATGGGAAAA
CTGGTGCAGG
06-012015 ACACCATAGA GAACATGTAT CTACAAGATC TGGATAATAG TACAAAACCTG TATGGGAAAA
CTGGTGCAGG
06-012019 ACACCATAGA GAACATGTAT CTACAAGATC TGGATAATAG TACAAAACCTG TATGGGAAAA
CTGGTGCAGG

06-012014 ATTCACAGCA AATAGAACCT TACAAAACGG ATGGTTTGAA GGGTTTATTA TAAGCAAATC
AGGACATAAA
06-012015 ATTCACAGCA AATAGAACCT TACAAAACGG ATGGTTTGAA GGGTTTATTA TAAGCAAATC
AGGACATAAA
06-012019 ATTCACAGCA AATAGAACCT TACAAAACGG ATGGTTTGAA GGGTTTATTA TAAGCAAATC
AGGACATAAA

06-012014 TATGTTTTTG TGCCGCACT TACAGGAA
06-012015 TATGTTTTTG TGCCGCACT tACAGGAA
06-012019 TATGTTTTTG TGCCGCACT TACAGGAA

Figure 10a: the DNA sequence alignment of 3 *E. coli* isolates for the OXA gene. This alignment after the sequences have been imported from BioNumerics to BioEdit for DNA and amino acid sequence alignment.

OXA Amino Acid Sequence Alignment

06-012014 YGI*CGIIDQ KTIFKWDKTP KGMEIWNSNH TPKTWMQFSV VWVSQEITQK IGLNKIKNYL KDFDYGNQDF
06-012015 YGI*CGIIDQ KTIFKWDKTP KGMEIWNSNH TPKTWMQFSV VWVSQEITQK IGLNKIKNYL KDFDYGNQDF
06-012019 YGI*CGIIDQ KTIFKWDKTP KGMEIWNSNH TPKTWMQFSV VWVSQEITQK IGLNKIKNYL KDFDYGNQDF

06-012014 SGDKERNNGL TEAWLESSLK ISPEEQIQLF RKIINHNLV KNSAIENTIE NMYLQDLDNS TKLYGKTGAG
06-012015 SGDKERNNGL TEAWLESSLK ISPEEQIQLF RKIINHNLV KNSAIENTIE NMYLQDLDNS TKLYGKTGAG
06-012019 SGDKERNNGL TEAWLESSLK ISPEEQIQLF RKIINHNLV KNSAIENTIE NMYLQDLDNS TKLYGKTGAG

06-012014 FTANRTLQNG WFEGFIISKS GHKYVFVSAL TG
06-012015 FTANRTLQNG WFEGFIISKS GHKYVFVSAL TG
06-012019 FTANRTLQNG WFEGFIISKS GHKYVFVSAL TG

Figure 10b: the amino sequence alignment of 3 *E. coli* isolates for the OXA gene. This alignment after the DNA sequences have been translated to the amino acid sequences on the BioEdit for further analysis using the Lahey Clinic webpage.

SHV Nucleic Acid Sequence Alignment

06-012056 CGCTTTCCCA TGATGAGCAC CTTTAAAGTA GTGCTCTGCG GCGCAGTGCT GGCGCGGGTG
GATGCCGGTG
06-012055 CGCTTTCCCA TGATGAGCAC CTTTAAAGTA GTGCTCTGCG GCGCAGTGCT GGCGCGGGTG
GATGCCGGTG
06-012057 CGCTTTCCCA TGATGAGCAC CTTTAAAGTA GTGCTCTGCG GCGCAGTGCT GGCGCGGGTG
GATGCCGGTG

06-012056 ACGAACAGCT GGAGCGAAAG ATCCACTATC GCCAGCAGGA TCTGGTGGAC TACTCGCCGG
TCAGCGAAAA
06-012055 ACGAACAGCT GGAGCGAAAG ATCCACTATC GCCAGCAGGA TCTGGTGGAC TACTCGCCGG
TCAGCGAAAA
06-012057 ACGAACAGCT GGAGCGAAAG ATCCACTATC GCCAGCAGGA TCTGGTGGAC TACTCGCCGG
TCAGCGAAAA

06-012056 ACATCTTGCC GACGGCATGA CGGTCGGCGA ACTCTGCGCC GCCGCCATTA CCATGAGCGA
TAACAGCGCC
06-012055 ACACCTTGCC GACGGCATGA CGGTCGGCGA ACTCTGTGCC GCCGCCATTA CCATGAGCGA
TAACAGCGCC
06-012057 ACACCTTGCC GACGGCATGA CGGTCGGCGA ACTCTGCGCC GCCGCCATTA CCATGAGCGA
TAACAGCGCC

06-012056 GCCAATCTGC TGCTGGCCAC CGTCGGCGGC CCCGCAGGAT TGA CTGCCTT TTTGCGCCAG
ATCGGCGACA
06-012055 GCCAATCTGC TGCTGGCCAC CGTCGGCGGC CCCGCAGGAT TGA CTGCCTT TTTGCGCCAG
ATCGGCGACA
06-012057 GCCAATCTGC TGCTGGCCAC CGTCGGCGGC CCCGCAGGAT TGA CTGCCTT TTTGCGCCAG
ATCGGCGACA

06-012056 ACGTCACCCG CTTGACCGC TGGGAAACGG AACTGAATGA GGCGTTCCC GGCGACGCC
GCGACACCAC
06-012055 ACGTCACCCG CTTGACCGC TGGGAAACGG AACTGAATGA GGCGTTCCC GGCGACGCC
GCGACACCAC
06-012057 ACGTCACCCG CTTGACCGC TGGGAAACGG AACTGAATGA GGCGTTCCC GGCGACGCC
GCGACACCAC

06-012056 TACCCCGGCC AGCATGGCCG CGACCCTGCG CAAGCTGCTG ACCAGCCAGC GTCTGAGCGC
CCGTTGCAA
06-012055 TACCCCGGCC AGCATGGCCG CGACCCTGCG CAAGCTGCTG ACCAGCCAGC GTCTGAGCGC
CCGTTGCAA
06-012057 TACCCCGGCC AGCATGGCCG CGACCCTGCG CAAGCTGCTG ACCAGCCAGC GTCTGAGCGC
CCGTTGCAA

06-012056 CGGCAGCTGC TGCAGTGGAT GGTGGACGAT CGGGTCGCCG GACCGTTGAT CCGCTCCGTG
CTGCCGGCGG
06-012055 CGGCAGCTGC TGCAGTGGAT GGTGGACGAT CGGGTCGCCG GACCGTTGAT CCGCTCCGTG
CTGCCGGCGG
06-012057 CGGCAGCTGC TGCAGTGGAT GGTGGACGAT CGGGTCGCCG GACCGTTGAT CCGCTCCGTG
CTGCCGGCGG

06-012056 GCTGGTTTAT CGCCGATAAG ACCGGAGCTG GCGAACGGGG TGCGCGCGGG ATTGTCGCC
TGCTTGGCC
06-012055 GCTGGTTTAT CGCCGATAAG ACCGGAGCTG GAAAGCCAGC TGTgCGcGGC CGCGTAGGCC
TGATAGAAAT
06-012057 GCTGGTTTAT CGCCGATAAG ACCGGAGCTA GCAAGCGGGG TGCGCGCGGG ATTGTCGCC
TGCTTGGCC

06-012056 GAATAACAAA GCAGAGCGC
06-012055 GGATaaCTGG CCAGCGGCC
06-012057 GAATAACAAA GCAGAGCGC

Figure 11a: the DNA sequence alignment of 3 *K. pneumoniae* isolates for the SHV gene. This alignment after the sequences have been imported from BioNumerics to BioEdit for DNA and amino acid sequence alignment.

SHV Amino Acid Sequence Alignment

06-012056 RFPMMSTFKV VLCGAVLARV DAGDEQLERK IHYRQQDLVD YSPVSEKHLA DGMTVGELCA AAITMSDNSA
06-012055 RFPMMSTFKV VLCGAVLARV DAGDEQLERK IHYRQQDLVD YSPVSEKHLA DGMTVGELCA AAITMSDNSA
06-012057 RFPMMSTFKV VLCGAVLARV DAGDEQLERK IHYRQQDLVD YSPVSEKHLA DGMTVGELCA AAITMSDNSA

06-012056 ANLLLATVGG PAGLTAFLRQ IGDNVTRLDR WETELNEALP GDARDTTTPA SMAATLRKLL TSQRLSARSQ
06-012055 ANLLLATVGG PAGLTAFLRQ IGDNVTRLDR WETELNEALP GDARDTTTPA SMAATLRKLL TSQRLSARSQ
06-012057 ANLLLATVGG PAGLTAFLRQ IGDNVTRLDR WETELNEALP GDARDTTTPA SMAATLRKLL TSQRLSARSQ

06-012056 RQLLQWMVDD RVAGPLIRSV LPAGWFIADK TGAGERGARG IVALLGPNNK AER
06-012055 RQLLQWMVDD RVAGPLIRSV LSAGWFIADK TGAGKPAVRG RVGLIEMDNW PAA
06-012057 RQLLQWMVDD RVAGPLIRSV LPAGWFIADK TGASKRGARG IVALLGPNNK AER

Figure 11b: the amino sequence alignment of 3 *K. pneumoniae* isolates for the OXA gene. This alignment after the DNA sequences have been translated to the amino acid sequences on the BioEdit for further analysis using the Lahey Clinic webpage.

AR Profile and DNA Sequencing Analysis

Isolate #	PCR Gel Working #	Antibiotic Resistance	E-Test Resistance Pattern	ESBL	Bla _{CTX-M}	Bla _{TEM}	Bla _{OXA}	Bla _{SHV}
<i>E. coli</i> Isolates								
06-012011	-1	SXT, TE, CIP, GM, AM, NA, CF	CE	negative	X	X	X	X
06-012012	1	CRO, TE, CIP, AM, NA, CF, CPD, CTX	CE and TX	positive	X	X	OXA-1	X
06-012013	2	CRO, SXT, TE, CIP, GM, AM, NA, FEP, ATM, SAM, CF, CPD, CAZ, CTX	CE and TX	positive	X	X	OXA-1	X
06-012014	3	CRO, AN, SXT, TE, CIP, GM, AM, NA, FEP, ATM, SAM, CF, CPD, CAZ, CTX	CE, TX, TZ, PM	positive	CTX-M-15	X	OXA-1	X
06-012015	4	CRO, SXT, TE, CIP, GM, AM, NA, FEP, ATM, SAM, CF, CPD, CAZ, CTX	CE, TX, TZ, PM	positive	CTX-M-15	TEM-1	OXA-1	X
06-012016	5	CRO, TE, CIP, AM, NA, ATM, CF, CPD, CAZ	CE, TX, TZ	positive	CTX-M-15	X	X	X
06-012017	6	C, SXT, TE, CIP, GM, AM, NA, FEP, ATM, CF, CPD, CAZ, CTX	CE, TX, TZ, PM	positive	CTX-M-15	X	X	X
06-012018	7	SXT, TE, CIP, NA	None	Negative	CTX-M-15	X	X	X

06-012019	8	CRO, SXT, TE, CIP, GM, AM, NA, ATM, SAM, CF, CPD, CTX	CE, TX, PM	positive	CTX-M-15	X	OXA-1	X
06-012020	-2	SXT, TE, CIP, AM, NA, SAM, CF	TX	Negative	X	X	X	X
06-012021	-3	SXT, TE, CIP, AM, NA, SAM, CF	CE	Negative	X	X	X	X
06-012022	9	CRO, SXT, TE, CIP, GM, AM, NA, ATM, SAM, CF, TIM, CPD, CTX	CE and TX	positive	CTX-M-15	X	OXA-1	X
06-012023	10	CRO, SXT, TE, CIP, GM, AM, NA, ATM, SAM, CF, CPD, CTX	CE, TX, PM	positive	CTX-M-15	TEM-1	OXA-1	X
06-012024	11	CRO, SXT, AM, NA, ATM, CF, CPD, CTX	CE and TX	positive	CTX-M-15	TEM-1	OXA-1	X
06-012025	12	CRO, SXT, TE, CIP, GM, AM, NA, FEP, ATM, SAM, CF, CPD, CTX	CE and TX	positive	CTX-M-15	X	OXA-1	X
06-012026	13	CRO, TE, GM, AM, NA, ATM, CF, TIM, CPD, CTX	CE and TX	positive	CTX-M-15	TEM-1	OXA-1	X
06-012027	14	CRO, TE, GM, AM, NA, ATM, SAM, CF, TIM, CPD, CTX	CE and TX	positive	CTX-M-15	TEM-1	OXA-1	X
06-012028	15	CRO, SXT, TE,	CE, TX,	positive	CTX-M-15	X	OXA-1	X

		CIP, AM, NA, FEP, ATM, SAM, CF, CPD, CTX	PM					
06-012029	16	CRO, TE, CIP, AM, NA, CF, TIM, CPD, CTX	CE and TX	positive	X	X	X	X
06-012030	17	CRO, SXT, TE, CIP, GM, AM, NA, CF, CPD, CAZ, CTX	CE and TX	positive	X	TEM-1	OXA-1	X
06-012031	18	CRO, TE, CIP, AM, NA, ATM, CF, TIM	CE and TX	positive	CTX-M-15	X	OXA-1	X
06-012032	19	TE, CIP, GM, AM, NA, ATM, CF, CPD	CE	positive	X	X	X	X
06-012033	-4	NA & CF	None	Negative	X	X	X	X
06-012034	20	C, CRO, SXT, TE, CIP, GM, AM, NA, FEP, ATM, SAM, CF, CPD, CAZ, CTX	CE, TX, TZ, PM	positive	CTX-M-15	TEM-1	OXA-1	X
06-012035	21	C, CRO, SXT, CIP, GM, AM, NA, ATM, CF, TIM, CPD, CTX	CE and TX	positive	CTX-M-15	TEM-1	OXA-1	X
06-012036	-5	SXT, TE, CIP, AM, NA	None	Negative	X	X	X	X
06-012037	22	CRO, SXT, TE, GM, AM, FEP, ATM, SAM, CF, CPD, CAZ, CTX	CE and TX	positive	CTX-M-15	TEM-1	OXA-1	X
06-012038	-6	CIP	None	Negative	X	X	X	X

06-012039	23	CRO, TE, GM, AM, FEP, ATM, CF, CPD, CAZ, CTX	CE, TX, TZ, PM	positive	X	TEM-1	X	X
06-012040	24	CRO, CIP, AM, NA, FEP, SAM, CF, CPD, CAZ, CTX	CE and TX	positive	X	TEM-1	X	X
06-012041	25	C, CRO, SXT, TE, AM, FEP, ATM, SAM, CF, CPD, CTX	CE and TX	positive	CTX-M-15	TEM-1	X	X
06-012042	26	CRO, AN, SXT, TE, CIP, GM, AM, NA, FEP, ATM, SAM, CF, TIM, CPD, CAZ, CTX	CE, TX, TZ, PM	positive	CTX-M-15	TEM-1	OXA-1	X
06-012043	-7	CIP & CF	CE	Negative	X	X	X	X
06-012044	27	CRO, SXT, TE, CIP, AM, NA, CF, CPD	CE and TX	Positive	X	TEM-1	X	X
06-012045	28	C, CRO, SXT, TE, AM, NA, ATM, CF, CPD, CTX	CE and TX	Positive	CTX-M-15	TEM-1	X	X
06-012046	29	CRO, SXT, TE, AM, CF, TIM, CPD, CTX	CE and TX	Positive	CTX-M-15	TEM-1	X	X
<i>Klebsiella</i> Isolates								
06-012047 <i>K. oxytoca</i>	30	CRO, AM, NA, ATM, CF, CPD	CE, TX, PM	positive	CTX-M-15	X	X	X
06-012048	-8	AM, NA, ATM, CF, CPD	CE	Negative	X	X	X	X

06-012049	-9	AM & CF	CE and TX	Negative	X	X	X	X
06-012050	31	C, CRO, SXT, TE, CIP, GM, AM, NA, ATM, SAM, CF CPD, CTX	CE and TX	positive	CTX-M-15	TEM-1	OXA-1	SHV-1
06-012051	32	CRO, TE, CIP, AM, NA, FEP, CF, CPD, CAZ	CE, TX, TZ	positive	CTX-M-15	TEM-1	OXA-1	SHV-11
06-012052	33	SXT, TE, CIP, AM, SAM, CF, CPD, CTX	CE and TX	positive	CTX-M-15	TEM-1	OXA-1	SHV-11
06-012053	34	CRO, GM, AM, FEP, ATM, SAM, CF, CPD, CAZ, CTX	CE and TX	positive	X	TEM-1	OXA-1	SHV-1
06-012054	35	AN, TE, CIP, GM, AM, NA, ATM, CPD, CAZ	CE and TZ	positive	X	TEM-1	X	X
06-012055	36	CRO, GM, AM, FEP, ATM, SAM, CF, CPD, CAZ, CTX	CE and TX	positive	CTX-M-15	TEM-1	X	SHV-12
06-012056	37	CRO, SXT, CIP, AM, NA, FEP, ATM, CF, CPD, CAZ, CTX	CE, TX, TZ	positive	CTX-M-15	X	X	SHV-11
06-012057	38	C, CRO, SXT, TE, GM, AM, FEP, ATM, SAM, CF, CPD, CTX	CE, TX, TZ	positive	CTX-M-15	X	X	SHV-12
06-012058	39	CRO, AM, NA,	CE and TZ	positive	X	X	X	SHV-12

		ATM, CF, CPD, CAZ, CTX						
06-012059	40	C, CRO, TE, CIP, AM, NA, FEP, ATM, CF, CPD, CAZ, CTX	CE and TX	positive	CTX-M-15	X	X	SHV-11
06-012060	41	TE, CIP, AM, ATM, CF, CPD, CAZ	CE	positive	X	X	X	SHV-12

Table 5: table showing all the AR and sequencing data compiled. The gray shaded rows are the ESBL positive isolates, while the non-shaded rows represent the ESBL negative isolates. The second column from the left refers to the working number by which the isolates are labeled in the previous PCR gels. The yellow highlighted row refers to isolate 06-012018 that is ESBL negative but was positive for CTX-M-15.

E. coli PFGE Dendrogram

Dice (Opt:1.50%) (Tol 1.5%-1.5%) (H>0.0% S>0.0%) [0.0%-98.3%]
PFGE-XbaI PFGE-XbaI

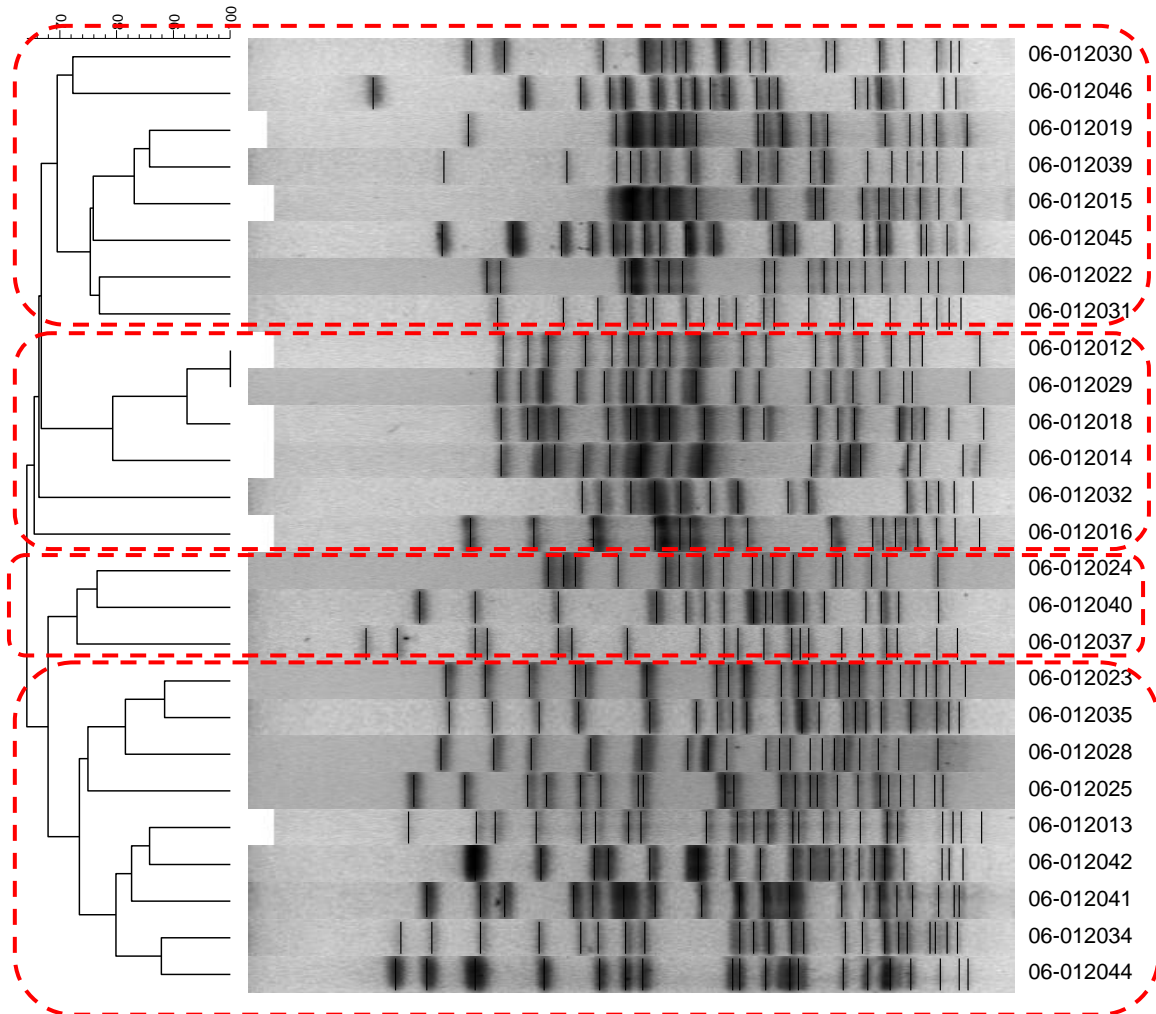


Figure 12: the *E. coli* PFGE dendrogram representing the genotypic relationship among 26 *E. coli* isolates. The isolates clustered into four main clusters according to their genotypic similarities. An overall similarity of 64% among the 4 clusters has been established. Isolates 06-012012 and 06-012029 were shown to be indistinguishable (100% similarity).

K. pneumoniae PFGE Dendrogram

Dice (Opt:1.50%) (Tol 1.5%-1.5%) (H>0.0% S>0.0%) [0.0%-98.3%]
PFGE-Xbal PFGE-Xbal

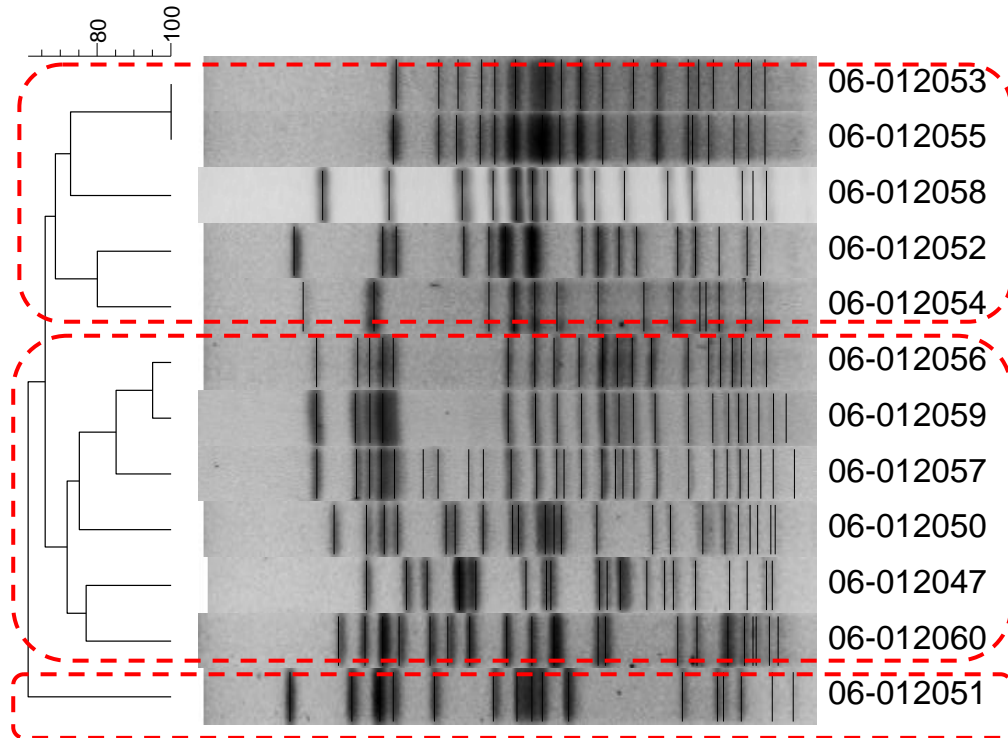


Figure 13: the *K. pneumoniae* PFGE dendrogram representing the genotypic relationship among 12 *K. pneumoniae* isolates. The isolates clustered into two main clusters according to their genotypic similarities and a single outlier. An overall similarity between the isolates ranged from 61% to 100% has been established. Isolates 06-012053 and 06-012055 were shown to be indistinguishable (100% similarity).