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Environmental And Genetic Factors Affecting Antibiotic Resistance Of Extended Spectrum Î?-Lactamase Bacteria From The Rio Grande River In El Paso, Tx And Cd. Juarez, Mexico

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ENVIRONMENTAL AND GENETIC FACTORS AFFECTING ANTIBIOTIC RESISTANCE OF EXTENDED SPECTRUM β-LACTAMASE BACTERIA FROM THE RIO GRANDE RIVER IN EL PASO, TX AND CD. JUAREZ, MEXICO

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To *Germain*

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by

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THESIS

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Abstract

Background: The Rio Grande River provides a major source of potable and agricultural water for the population of the Texas/Mexico border region. Cattle farming and ranching are the most prevalent activities, which may contribute to the microbial burden of pharmaceuticals into our state's water resources. Antibiotics, presumably released into the environment by discharges originating from waste-water treatment plants, septic disposal systems, animal feeding operations and urban runoff have a definite impact on the ecosystem and may contribute to an increase in antibiotic resistance. We hypothesized that waters of the Rio Grande River contained Multi Drug Resistant Organisms (MDRO) and mobile genetic elements. This could represent a serious public health concern for residents in the El Paso/Juarez, Mexico border. Human health consequences as a result of antimicrobial resistant bacteria may include: increased number of infections, increased frequency of treatment failure, increased severity of infections and increase health cost. *Purpose*: The main objective of this research study was to determine the presence of selected genetic antimicrobial biomarkers such as Extended Spectrum Beta Lactamase genes (ESBL), mobile genetic elements, integrons (class I and class II), and to screen for Carbapenem Resistant (CRE) Enterobacteria in a 26 km segment of the Rio Grande River. *Methods*: Water and sediment samples were obtained from the Rio Grande River. DNA was extracted from both isolated bacteria and directly from water and sediment. Amplification of selected resistant biomarkers (TEM, SHV and CTX genes) and integrons (class I and II), was accomplished by polymerase chain reaction (PCR). Screening for CRE bacteria was done by culture on CHROMagar Chromogenic Media. *Results*: Analysis of water samples (15) collected at five different time periods throughout the year showed that 73.3% contained ESBL genes and integrons. Among these, 60% were TEM and/or 46.7% were CTX-M. Integrons were identified

in 11 water samples (73.3%). Also, a total of 310 bacterial isolates were recovered, from which 142 bacterial isolates were characterized as 18 different genera of bacteria. From these, 91 (64%) were resistant to at least 2 or more combinations of antibiotics and 105 (74%) were multi-resistant to more than 4 different antibiotics. 28 isolates were identified as Gram negative bacteria with multiple drug resistant patterns and therefore included in this study for the molecular analysis of ESBL genes. From these, 22 (78.6%) were positive for the presence of one or more ESBL and 11 (39.3%) were positive for CRE. TEM was identified as the most prevalent ESBL gene of resistance in both, water and bacterial isolates samples. *Conclusions*: To our knowledge, characterization of genes responsible for antibiotic resistance in the El Paso-Cd. Juarez region had never been performed previously. These findings may contribute to improve preventive measures and policies in the management of infectious diseases and our environment natural resources.

Statement of the Problem

According to the Center for Disease Control and Prevention (CDC, 2017), around 2 million people in the United States become infected with a bacteria strain that is resistant to antibiotics; and, around 23,000 people die due to complications of multidrug-resistant bacterial infections. The increase in infections caused by multi-drug resistance organisms (MDRO's) currently represents an important threat to global health as it increases morbidity, mortality and health care costs (Davies & Davies, 2010). Also, the majority of antibiotic residues are excreted unchanged into the environment possibly contributing to the problem of resistance (Berkner, Konradi, & Schonfeld, 2014) (Fletcher, 2015). The presence of these pharmaceutical residues along MDROs have been found in hospital wastewaters, animal production wastewaters, sewage, groundwater, wastewater treatment plants and even drinking water (Zhang, Zhang, & Fang, 2009).

The Rio Grande River is the 5th longest river in the United States and a major source of potable water to the community of El Paso, TX and Cd. Juarez, Mexico. Possible contaminants associated with agricultural activities and urbanization along the river, such as the mentioned above could represent a problem of public health with the increasing global trend of antibiotic resistance.

To manage the emergence of this public health crisis, it is important to understand the extent of antibiotic resistant bacteria strains in the environment, including our water resources, the different mechanisms of bacterial spreading and their effects on human health so researchers and public health professionals can offer solutions, prevention programs or changes in health policies to this global problem of antibiotic resistance.

Literature Review

The Global Problem of Antibiotic Resistance in Public Health

In less than 80 years that antibiotics were first discovered by Sir Alexander Fleming in the early 1930s (Flemming, 1929) and introduced as therapeutic agents to treat human infections, the golden era of "antibiotic revolution" is facing a new threat. According to the Center for Disease Control and Prevention (CDC, 2017), around 2 million people in the United States become infected with a bacteria that is resistant to the antibiotics currently available and around 23,000 people die due to complications of multidrug-resistant bacterial infections. By the year 2050, deaths by complications of infectious diseases have been expected to increase as many as 10 million per year (O'Neill J. , 2014).

Among the infections that are considered to drive resistance patterns include: otitis media, sinusitis, pharyngitis, bronchitis and pneumonitis. These infections collectively account for 70% to 80% of all prescriptions for antibiotics in outpatients (Barlett, 2006). When patients can't respond to antibiotics, they face the challenge of complications requiring hospitalization, more expensive medications or the exposure to more toxic medication resulting in higher medical costs and mortality rates. Hospitalized patients requiring antibiotic use for bacterial infections might be unable to respond effectively to the treatment options current available. According to Mainous et al., antibiotic-resistance infections are becoming increasingly commonplace in hospitalizations in the U.S., with a steady upward trend seen between a ten-year period of 1997 and 2006. Also, resistant infections are showing an increasing trend in younger patients and without health insurance (Mainous, Diaz, & Matheson, 2011).

Limited work has been done in environmental sites compared to health care centers. To manage the emergence of this public health crisis, it is important to understand the extent of antibiotic resistant bacterial populations in the environment including mobile genetic elements, the different mechanisms of bacterial spreading in the environment and their effects on human health in order for researchers and public health professionals offer solutions, prevention programs or changes in health policies to this global threat of antibiotic resistance.

Factors Involved in the Development of Resistance

Antibiotic resistance is naturally present in many bacteria within the environment (Berkner, Konradi, & Schonfeld, 2014); therefore, all environments will have a base level of resistance capable of being selected for by antibiotic residues and also heavy metals (Amos, 2015). Although it has been largely attributed to the overuse and misuse of antibiotics, there are many other factors involved in the antibiotic resistance crisis (Penchovsky R, 2015) (Davies & Davies, 2010).

Antimicrobial resistance can be intrinsic or extrinsic. Intrinsic resistance refers to a resistance mechanism that is innate (such as *Pseudomonas aeruginosa*). Extrinsic mechanism or acquired occurs when a bacterium that was previously sensitive to an antibiotic acquires a mutation or exogenous genetic material that allows it to resist the activity of that specific antibiotic (Hauser, 2007). Some driving factors leading to extrinsic antibiotic resistance bacteria are the following: long-term antibiotic exposure, prolonged ICU stay, diagnostic proceedings, nursing home residency (S Nathisuwan, 2001), self medication (Knobler, Lemon, & M, 2003), the globalization of commercial food, international traveling (Vila, 2015), disposal of toxic waste, and residues of manufacturing processes into the biosphere (Davies & Davies, 2010).

Table 1 summarizes most common factors promoting resistance. Failure of health care workers to practice strict hygiene control measures at work is considered to be a leading contributor to the spread of hospital infections (Knobler, Lemon, & M, 2003). Also, there is a lack of new antibiotic drug development by the pharmaceutical industry due to reduced economic incentives and challenging regulatory requirements (Ventola C. , 2015).

Besides factors associated to healthcare, there are other environmental factors that can be identified in agriculture and aquaculture that are also considered to be contributing to the problem of antibiotic resistance. These include the use of antibiotics as a preventive measure in animals with commercial purposes. As a main source of food for humans, food animals (poultry, cattle, swine and more) commonly receive antibiotics not only to treat infections but also as growth promoters and to prevent and control common disease events as well as to promote feed utilization and production (Roca I, 2015) (Done, Venkatesan, & Halden, 2015). Studies have found drug-resistant bacterial strains in agricultural facilities, whether originating in the meat itself or in the surrounding environment. Among the antimicrobial agents being used in food animals, which are also used to treat infections in humans, include: penicillins and cephalosporins, tetracyclines, polypeptides, macrolides, lincosamides and sulfonamides.

The control of drug residues and microbial contamination in commercial food is under the strict surveillance of the U.S. Department of Agriculture, including the Food Safety and Inspection Service. Other agencies collaborating to protect the public from residues and other hazards that could represent a threat to the public include the Agricultural Marketing Service (AMS), the Food and Drug Administration (FDA), and the U.S. Environmental Protection Agency (EPA). On the other side, the FDA's Center for Veterinary Medicine (CVM), is responsible for ensuring that drugs are safe and effective for use in animals, and that food

derived from animals is safe for human consumption. Table 1 summarizes some factors related to mechanisms of resistance.

Table 1: Important Factors Involved in Development of Global Antibiotic Resistance

Factors	**Reference**
Long-term antibiotic exposure	(S Nathisuwan, 2001)
Prolonged ICU stay	(S Nathisuwan, 2001)
Diagnostic procedures	(S Nathisuwan, 2001)
Nursing home residency	(S Nathisuwan, 2001)
Self medication	(Knobler, Lemon, & M, 2003)
Globalization of commercial food	(Vila, 2015)
Disposal of toxic waste	(Davies & Davies, 2010)
International traveling	(Vila, 2015)
Failure of strict hygiene control among health workers	(Knobler, Lemon, & M, 2003)
Use of antibiotic in commercial farm animals	(Roca I, 2015)
Waste-water treatment plants	(Amos, 2015)
Agricultural pollution and industrial detergents	(Amos, 2015)

Other important factors contributing to the rapid dissemination of antibiotic resistance is the ability of resistance genes and mobile genetic elements to transfer easily into other bacteria by a variety of genetic mechanisms (plasmids and horizontal gene transfer) within the environment. Some anthropogenic features involved in this dissemination include wastewater-treatment plant (WWTP) effluent, which increases prevalence of clinically important resistant bacteria and resistance genes, agricultural pollution where antibiotic-resistant bacteria reach the environment via animal feces, and by detergents found in industrial effluent that co-select for Class 1 integrons (Amos, 2015).

Rio Grande River and Water Resources for El Paso, TX

The Rio Grande River (Fig. 1) is the 5th longest river in the United States and extends from Alamosa, Colorado to the Gulf of Mexico. It runs about 1,255 miles along the international boundary with Mexico. The Rio Grande River serves as a natural border between the U.S. state

of Texas and the Mexican states of Chihuahua, Coahuila, Nuevo Leon and Tamaulipas. In Texas, it has marked the boundary between the sister cities of El Paso and Cd. Juarez, Mexico.

Figure 1: Rio Grande River along the U.S. and Mexico is the 5th longest river in the U.S. and the main source of potable water to the community of El Paso, TX and Cd. Juarez, Mexico.

Under a semi-arid environment, the Rio Grande River provides the major source of potable and agricultural water to the Texas – Mexico border region receiving an average of 17.7 cm of rain per year.

Water quality is constantly monitored under the Clean Water Act to help local water resource manager be aware if problems of contamination or pollution exist. In El Paso, TX the International Boundary and Water Commission / Clean Rivers Project (USIBWC/CRP) monitors water quality issues unique to an international boundary. It is also responsible for collecting water quality data throughout the portion of the Rio Grande Basin that lays within the state of Texas. The Rio Grande Basin is divided into four major sub-basins. The Upper-Sub Basin (Fig. 2) extends from the New Mexico/Texas state line downstream to International Amistad reservoir located by the Val Verde County in Texas and Coahuila on the Mexican side.

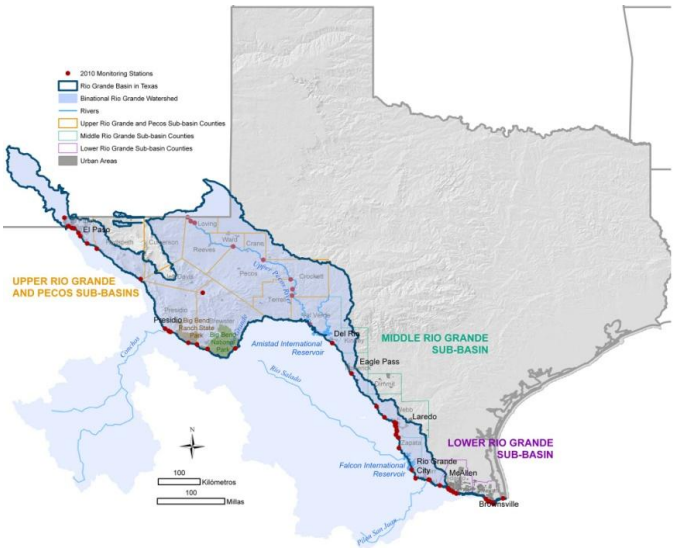

Figure 2: The Upper Rio Grande and Pecos Sub-basin extends from the New Mexico/Texas state line downstream to the International Amistad reservoir located by the Val Verde County in Texas (source: US IBWC). Water quality is constantly monitored under the Clean Water Act.

The main source of water for El Paso relies on both surface water (from the Rio Grande) and ground water (from the Mesilla Bolson and Hueco Bolson) for municipal water supply. Groundwater occurs in unconsolidated fluvial, alluvial and lacustrine sediments playing an essential role in the recharge and discharge of both basins mentioned above. Based on current capacities of wells and surface water plants, total available municipal supply in El Paso Water Utilities is around 131,000 AF/yr. Because the limitation that surface water is only available during the irrigation season, El Paso Water uses both surface water and ground water to satisfy increasing demands.

Monitoring of water quality of El Paso and data analysis by the IBWC includes physical, chemical, biological, habitat, metrics and indexes data. This data collection follows the Storage and Retrieval database (STORET) created by the Environmental Protection Agency (EPA). El Paso Water, which serves people at El Paso, Canutillo, Westway and Hudspeth counties consistently, treats surface water to 0.1 Nephelometric Turbidity Units (NTUs) measured immediately after the water has passed through different filters which is better than the required

0.3 NTUs. Results of latest annual report for drinking water analysis in El Paso (El Paso Water, 2016) can be found in Figure 3 (Appendix) and chemical analysis in Fig 4 and 5 (Appendix) according to sources to the city's areas.

Factors that may be contributing to the microbial burden and the release of antimicrobial agents and pharmaceuticals into our state's water resources include: cattle farming and ranching, waste-water treatment plants, septic disposal systems, animal feeding operations and urbanization. Consequences for human health of antimicrobial resistance in bacteria and antimicrobial residues in the Rio Grande River are not known, but may include: increased number of infections, increased frequency of treatment failure, increased severity of infections, and allergies or alteration in intestinal flora.

Antibiotic Resistance Along the United Sates El Paso, TX and Cd. Juarez, Mexico Border

In the U.S.-Mexico border the problem of antibiotic resistance increases as some population living in the U.S. without access to medical insurance seeks medical services in Mexico. On the Mexican side of the border, physician consultations and medications are available at lower costs (Benoit SR, 2014) and clerks at pharmacies often offer customers recommendations of antibiotic usage without having an educational background in a health-related field (Homedes & Ugalde, 2012). According to the Secretary of Health in Mexico, up to 70-80% of antibiotic recommendations for respiratory diseases and acute diarrheas given by pharmacy employees to clients are inaccurate in regards to dosage, time frame and type of antibiotic needed. Because antibiotics were the second most common type of medications sold in the pharmacies nationally contributing to the problem of self medication, beginning August,

2010, it is now required to bring a prescription provided by a physician to the pharmacy in order to have access to an antibiotic and other controlled substances (Secretaria de Salud, 2010).

There have been previous cases and outbreaks related to antibiotic resistant organisms reported in El Paso, TX. Also, there have been reported cases of *Methicillin-resistant Staphylococcus aureus* (MRSA) infections at hospitals (O'Brien, et al., 2005) and cases of Carbapenem-resistant bacteria (CRE) infections (Texas Department of State Health Services, 2016); yet, the prevalence of ESBL-resistant *Enterobacteriaceae* in Texas or along the US-Mexico border remains unknown.

Infections by Gram Negative Bacteria

Infections by Gram negative bacteria are becoming increasingly prevalent and constitute a global problem of public health. This problem is due mainly to the rapid spread of resistance to the antibiotics currently available making infections difficult to treat. Extended Spectrum Beta Lactamase producing organisms found in Gram negative bacteria are the leading cause of infections including: nosocomial infections (NI), pneumonia, urinary tract infections (UTIs), ventilator-associated pneumonia (VAP), intra-abdominal infections (IAIs), pediatric bacterial meningitis, septicemia, neutropenia, community-acquired infections (CAIs) and pelvic inflammatory diseases (Salabi, Walsh, & Chouchani, 2013).

Among the Gram negative pathogens that are mainly responsible, up to 70%, for the development of these infections in the United States are *Escherichia coli, Klebsiella pneumoniae* and *Pseudoma aeruginosa. E coli* is mainly responsible for causing Urinary Tract Infections (UTIs) and the second most common pathogen for causing health care associated infections overall. *Klebsiella pneumoniae* is the most common causing central line-associated bloodstream

infections. Lastly, *P. aeruginosa* is an important pathogen causing ventilator associated pneumonia.

According to the Texas Department of State Health Services cases and rates report of 2016, El Paso's most common gastrointestinal infectious (GI) diseases included: amebiasis, campylobacteriosis, carbapenem-resistant Enterobacteriaceae (CRE), cryptosporidiosis, cyclosporiasis, *Escherichia coli* shiga-toxin producing (STEC) dysentery, GI by multidrug-resistant acinetobacter (MDR-A) salmonellosis and shigellosis, many of which are potentially related to water contamination (Texas Department of State Health Services, 2016) .

The presence of these multidrug resistance organisms (MDROs), mobile genetic elements and pharmaceutical residues in our ecosystem could lead to a serious public health problem for nearly three million people living along the El Paso – Cd. Juarez, Mexico border region.

Antibiotics Used in the Management of Infections Caused by Gram Negative Bacteria and Mechanisms of Resistance

β-Lactam antibiotics were first introduced as a source to treat infections against Gram positive bacteria in 1940s (Salabi, Walsh, & Chouchani, 2013). The mold first identified by Sir Alexander Fleming was of the genus *Penicillium* and he named the antibacterial substance penicillin. The essential core of a penicillin molecule is a four-member ring called a β-Lactam ring and modifications of this structure has led to the development of a wide variety of useful antibacterial compounds with very unique characteristics. In general, β-Lactam antibiotics can be viewed as inhibitors of cell wall synthesis that normally assemble the peptidoglycan layer surrounding the bacteria. Disruption of this peptidoglycan layer leads to lysis of the bacteria.

Among the β-Lactam antibiotics, Penicillins, Cephalosporins and Carbapenems are used to treat infections caused by Gram negative bacteria.

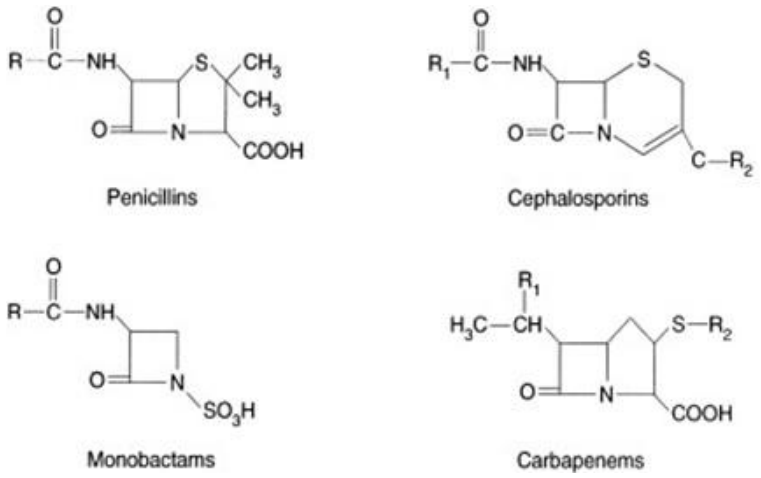

Figure 6: Molecular structure of β-Lactam antibiotics including: penicillins, cephalosporins, monobactams and carbapenems.

The first antibiotic resistance identified was *Staphylococcus* to penicillin by the 1940s after treating bacterial infections among soldiers during World War II, and in response beta-lactam antibiotics were developed (Ventola C. L., 2015). Also, the first case of MRSA was identified in 1962 in the UK and 1968 in US. Vancomycin was then introduced to treat MRSA infections in 1972. (Ventola C. L., 2015)

There are currently several mechanisms described by which a bacteria can develop resistance to antibiotics but four are the general mechanisms by which they become resistant (Fig. 7). These resistance mechanisms include: 1) *Inhibition of drug uptake:* antibiotics do not penetrate adequately into the intracellular compartment of bacterial cells; therefore, bacteria are not exposed to the effect of the administered antibiotic. 2) *Activation of Efflux pumps*- Some bacteria produce pumps that have the ability to send back antibiotics that have entered the periplasmic space and avoid accumulation of the antibiotic at sufficient concentrations to

produce a negative effect on the bacteria. 3*) Alteration of drug target:* PBPs- These are Penicillin Binding Proteins that have been modified so that they do not bind efficiently to β-Lactams making them ineffective. 4) *Inactivation of drugs by enzyme:* Penicillinases- These are the β-Lactamase enzymes that degrade β-Lactam ring of the antibiotics inactivating the drug.

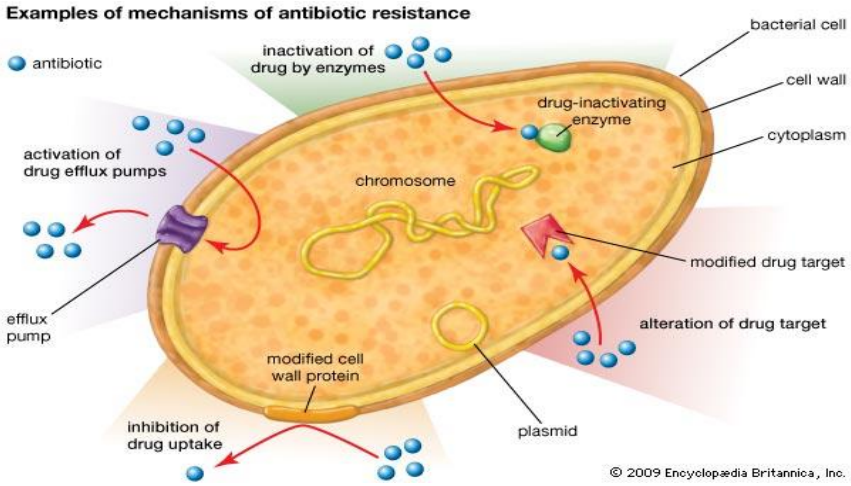

Figure 7: Primary mechanisms of resistance for antibiotics: 1) inhibition of drug uptake, 2) activation of efflux pumps, 3) alteration of drug target, and 4) inactivation of drugs by enzyme. (Source: Encyclopedia Britannica)

Mobile genetic elements from Gram negative bacteria play a key role in spreading ESBL-resistance genes carried in plasmids, integrons and transposons, which are easily found in the environment. Acquired antibiotic resistant genes are contained within mobile DNA, which can be loosely defined as any segment of DNA that is capable of translocation between genomes. The major players in horizontal gene transfer (HGT) are the conjugative and the mobilizable elements. Conjugative elements contain all the genetic information necessary to transfer from one bacteria to another. Mobilizable elements include plasmids and transposons that can transfer from host to host. There are also elements which are capable of translocation to new sites in the genome but are not capable themselves to transfer to a new host, such as transposons and mobile

introns. Bacteria capable of acquiring DNA and mobile genetic elements from the environment are referred to as "competent" bacteria.

Gene transfer is more likely in environments where bacteria are in close and continuous proximity to each other and in relatively high density areas such as waste water treatment plants. It is essential to understand how these genetic mobile elements disseminate in the river in order to control the spread of antibiotic resistance of bacteria within our region.

Extended Spectrum β-Lactamases

Extended-Spectrum-β-Lactamases (ESBLs) are hydrolytic enzymes which cleave the β-Lactam ring to inactivate antibiotics and are commonly produced by the Enterobacteriaceae of Gram negative organisms (Falagas & Karageorgopoulus, 2009) (Dhillon & Clark, 2012). Random mutations of ESBL genes have evolved as a defense mechanism through a natural process of Darwinian selection conferring resistance to penicillins, first-, second- and third-cephalosporins and aztreonam (Paterson & Bonomo, 2005). These enzymes can be carried on bacterial chromosomes or may be plasmid-mediated with the potential to move between bacterial populations. Most ESBL-producing bacteria include: *K. pneumonia*, among other *Klebsiella* species, and *E. coli*. They are also produced by non-fermentative Gram-negative organisms, such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa*.

There are 2 types classification for β-Lactamases. According to Ambler's molecular classification, there are four different groups (A-D) which orders enzymes according to protein homology and not on phenotype characteristics (Paterson & Bonomo, 2005). The majority of ESBLs belong to Group A, in which enzymes act through a serine-based mechanism (Ambler, 1980). See Table 2 below. These β-Lactamases may also be defined as plasmid-mediated

enzymes that inactivate oxyimino, or third-generation, cephalosporins, penicillins and monobactams; but, they can also be inhibited in vitro by clavulanic acid, tazobactam and sulbactam (Dhillon & Clark, 2012) (Pitout, Nordmann, Laupland, & Poirel, 2005).

Table 2: Structural Classification of β-Lactamases (Ambler, 1980)

Ambler Class	**Bush-Jacoby Medeiros group**	**Active site**	**Enzyme type**	**Host organisms**	**Substrates**
A	2b, 2be, 2br, 2c, 2e, 2f	Serine	Broad-spectrum β-lactamases (TEM, SHV) ESBL (TEM, SHV, CTX-M)	Enterobacteriaceae and nonfermenters	Ampicillin, cephalotin, Penicillins, 3rd-generation cephalosporins
			Carbapenases (KPC, GES, SME)		All β-lactams
B	3	Zinc-binding thiol group	Carbapenases (VIM, IMP)	Enterobacteriaceae and nonfermenters	All β-lactams
C	1	Serine	AmpC cephamycinases (AmpC)	*Enterobacter* species *Citrobacter* species	Cephamycins ,3rd-generation cephalosporins
			AmpC cephamycinases (CMY, DHA, MOX FOX, ACC)	Enterobacteriaceae	Cephamycins ,3rd-generation cephalosporins
D	2d	Serine	Broad-spectrum β-lactamases (OXA) ESBL (OXA) Carbapenases (OXA)	Enterobacteriaceae and nonfermenters	Oxacillin, ampicillin, cephalotin Penicillins, 3rd-generation cephalosporins All β-lactams

The other is a functional classification, which takes into account substrate and inhibitor profiles in order to group the enzymes in ways that can be correlated to a phenotype (Bush & Jacoby, 2010). Although a structural approach is easier, Bush's classification is able to provide the opportunity to relate to their clinical role and resistance patterns to different classes of B-lactam antibiotics. Group 1 includes enzymes that not inhibited by clavulanic acid, group 2 are inhibited by clavulanic acid and group 3 are inhibited by metallo-β-lactamases, such as carbapenems (Dhillon & Clark, 2012). Most ESBLs are classified into the subgroup 2be, which can hydrolyze penicillins, cephalosporins and monobactams besides being inhibited by clavulanic acid. See Table 3.

ESBL-producing organisms represent a global problem to public health since they are often resistant to many classes of current antibiotics available limiting physicians with few or none treatment options that could have a harmful impact on the clinical outcomes of patients (S Nathisuwan, 2001). They were first identified in the early 1980s in Europe. In France, there was reported the first large outbreak of ESBL resistant *K. pneumoniae* in 1986 where 54 patients were infected in an intensive care unit spreading the infections to other sections of the hospital (Paterson & Bonomo, 2005). By early 1990s, 35% of all nosocomial infections by *K. pneumoniae* in all France were already ESBLs (Paterson & Bonomo, 2005). Outbreaks by ESBL bacteria are now being reported all over Europe. In North America, the first reports of ESBL-producing bacteria were in 1988. ESBL infections most commonly identified in outbreaks within the United States have been infections with TEM and CTX-M in *K. pneumoniae* isolates (Paterson & Bonomo, 2005).

ESBLs are transferred to other microorganisms through plasmids or HGT (Davies & Davies, 2010) (Carattoli, 2013). HGT has played a fundamental role in the distribution of

multidrug-resistant bacteria in both community and hospital infections (Davies & Davies, 2010) (van Hoek, Mevius, Guerra, Mullany, Roberts, & Aarts, 2011) or environmental exposure (Njage & Buys, 2017).

Table 3: Functional Classification of β-Lactamases (Bush & Jacoby, 2010)

Enzyme family	Functional group or subgroup	No. of enzymes	Representative enzymes
CMY	1, 1e	50	CMY-1 to CMY-50
TEM	2b, 2be, 2br, 2ber	172	
	2b	12	TEM-1, TEM-2, TEM-13
	2be	79	TEM-3, TEM-10, TEM-26
	2br	36	TEM-30 (IRT-2), TEM-31 (IRT-1), TEM-163
	2ber	9	TEM-50(CMT-1), TEM-158 (CMT-9)
SHV	2b, 2be, 2br	127	
	2b	30	SHV-1, SHV-11, SHV-89
	2be	37	SHV-2, SHV-3, SHV-115
	2br	5	SHV-10, SHV-72
CTX-M	2be	90	CTX-M-1, CTX-M-44 (Toho-1) to CTX-M-92
PER	2be	5	PER-1 to PER-5
VEB	2be	7	VEB-1 to VEB-7
GES	2f	15	GES-2 to GES-7 (IBC-1) to GES-15
KPC	2f	9	KPC-2 to KPC-10
SME	2f	3	SME-1, SME-2, SME-3
OXA	2d, 2de, 2df	158	
	2d	5	OXA-1, OXA-2 OXA-10
	2de	9	OXA-11, OXA-14, OXA-15
	2df	48	OXA-23 (ARI-1), OXA-51, OXA-58
IMP	3a	26	IMP-1 to IMP-26
VIM	3a	23	VIM-1 to VIM-23
IND	3a	8	IND-1, IND-2, IND-2a,

Today, resistance to fluroquinolones, one of the most widely used antibiotic to treat Urinary Tract and Bloodstream infections caused by *E. coli*, is widespread in many parts of the world. Also to carbapenems, used to treat infections caused by *K. pneumonia*, which represent a major problem to hospital-acquired infections such as pneumonia, bloodstream infections, and urinary tract infections, and a threat to newborns and ICU patients. (World Health Organization, 2014) The high proportion of resistance to 3rd generation cephalosporins reported for *E. coli* and *K. pneumonia* means that treatment of severe infections by these bacteria would eventually rely

solely on carbapenems, the last resort to treat severe community and hospital acquired infections by such bacteria. (World Health Organization, 2014) This means limiting the current available oral treatment for conditions which are common in the community.

TEM, SHV and CTX-M Enzymes

There are various genotypes of ESBLs. The most common types are Class A and include the following: TEM, SHV and CTX-M (Dhillon & Clark, 2012). The majority of ESBLs are derivatives of TEM or SHV enzymes (Bradford, 2001) (S Nathisuwan, 2001) which have been recognized across the world with over 100 mutations being reported as offering resistance mainly to cephalosporins (Dhillon & Clark, 2012); and more than 340 β-Lactamase enzymes have been detected to date (Shah A, 2004).

TEM enzymes are derivative of TEM-1 and TEM-2. TEM-1, is the most common plasmid-mediated β-Lactamase associated with up to 90% of ampicillin resistance in Gram negative bacteria (Paterson & Bonomo, 2005). It is also associated with resistance in *E. coli, K pneumoniae, Haemophilus influenza and Neisseria gonorrhea*. TEM enzymes were first discovered in *E coli* bacteria isolate in Greece in 1965 and spread worldwide within a time frame of 10 years. The name of the patient was Temoneira, hence the designation TEM (Paterson & Bonomo, 2005). There are 140 TEM-enzymes currently identified and the most common in the United States are TEM-10, TEM-12 and TEM-26.

The enzyme SHV-1 β-Lactamase, which refers to a *sulfhydryl* variable, is generally found in *Klebsiella pneumonia* but it can also be found in *E coli, Pseudomonas aeruginosa* and *Acinetobacter spp.* strains. ESBL SHV shares a similar structure and 68% of its amino acids with

TEM. Currently, more than 60 SHV varieties are known being SHV-5 and SHV-12 the most prevalent and with up to 20% resistance patterns to ampicillin.

CTX-M is another group of β-Lactamase enzymes present in Gram negative bacteria which was first discovered in *Kluyvera* strains in the 1990s. This group shares ~40% identity with TEM and SHV enzymes (Monstein, 2007) and its denomination comes from particular activity against cefotaxime and ceftriaxone (Casellas, 2011). Environmental CTX-M has proved to be the most successful disseminating and the most prevalent worldwide (Marco D'Andrea, 2013) showing a great ability to cause outbreaks (Dhillon & Clark, 2012). This can be attributed to the spread of CTX-M genes among bacterial species by plasmids or other mobile genetic elements. CTX-M is found most commonly in *Salmonella enterica*, in Latin America (Casellas, 2011), *E. coli* and *K. pneumoniae*. There are over 50 variants of CTX-M to date and associated with numerous outbreaks of infection in hospitals and the community. CTX-M-15 is currently the most widespread type *E coli* in the community and associated with urinary infections (Dhillon & Clark, 2012). Particular attention should be placed to the increasing prevalence rate of ESBL production in our El Paso, TX- Cd. Juarez, Mexico border region, as they represent a global threat for public health (Davies & Davies, 2010).

Integrons (INT-1 & INT-2)

Among mobile genetic elements that play an important role in spreading antibiotic-resistance genes, integrons are suspected to be of most importance since that can be easily found in the environment, including waste water treatment plants.

Integrons consist of three key elements: a tyrosine-recombinase gene or integrase (*intI*), a primary recombination site (*attI*) and one or two promoters (Pc). Variations in amino acid

sequence of *intI* gene and a wide variety of gene cassettes in *attI* give rise to five different classes of integrons from which Class 1, Class 2 and Class 3 are the most common and conferring resistance to aminoglycoside and β-Lactam antibiotics. Fig. 6.

Classic 1 integrons are genetic elements that routinely contain mobile antibiotic and biocide-resistance genes that can be found on polluted environments such as sewage-sludge-amended soil. They are considered to be directly linked with the Tn3 transposon family. Although class 1 integrons are not self-movable, such as plasmids, they are capable of capturing and integrating gene cassettes into a variable region to specific integration sites (*attI*) through the activity of an integrase. Class 1 integrons has been studied in various microorganisms associated with distribution and spreading of β-Lactam antibiotic resistance with a prevalence ranging from 22 to 59% and identified in clinical Gram-negative bacteria including *Acinetobacter, Aeromonas, Alcaligenes, Burkholderia, Campylobacter, Citrobacter, Enterobacter, Escherichia, Klebsiella, Mycobacterium, providencia, Pseudomonas, Salmonallea, Serratia, Shigella, Stenotrophomonas,* and *Vibrio* (Deng, 2015).

Class 2 integrons are similar in structure to class 1 integrons and share identical gene cassettes which confer resistance to several antibiotics including streptothricin, erythromycin and trimethoprim. They are also associated with the Tn7 transposon family which helps mediate the mobility of class 2 integrons via a preferential insertion into a unique site within bacterial chromosomes (Deng, 2015). Class 2 integrons are considered to be a major contributor to the wide spread and distribution of antibiotic resistance in microorganisms. They have been reported in some species of Gram negative bacteria including *Acinetobacter* and *Enterobacteriaceae* such as *Salmonella* and *Pseudomonas* in a lower prevalence to class 1 integrons. Currently there are

over 130 gene cassettes conferring a diverse antibiotic-resistance phenotype (Amos, 2015). Class 1 and 2 integrons are included for analysis in this study.

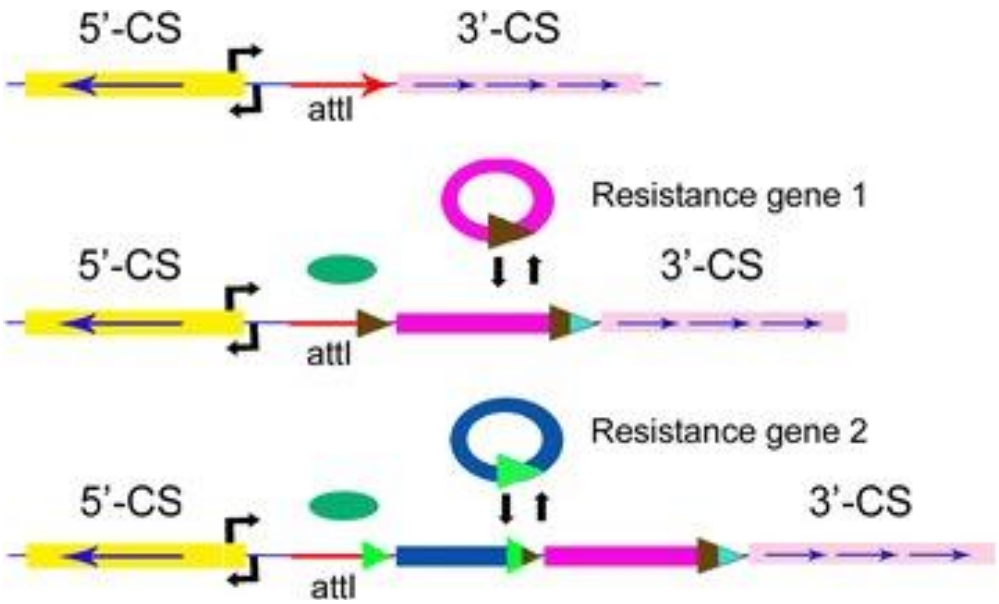

Figure 8: General organization of an integron and gene cassette recombination mechanism. (Source: Deng, 2015)

Carbapenem Resistant (CRE) Enterobacteriaceae

Carbapenem resistance, mainly among Gram-negative bacteria such as *Klebsiella* species and *E. coli* are becoming an increasing global health problem as it is spreading rapidly causing serious outbreaks with limited options of treatment as CRE bacteria are becoming resistant to most available antibiotics as ESBLs. Currently, CRE cause 9,000-drug resistant infections per year and 600 deaths. Almost half of hospitalized patients who get bloodstream infections from CRE die from the infection. The Center for Disease Control published a report outlining the top 18 drug-resistant threats to the United Sates classifying Carbapenem-Resistant Enterobacteriaceae as an "Urgent Threat". These threats may be not currently widespread but

have the potential to become so and require urgent public health attention to identify infections and limit transmission.

The purpose of this study is to isolate, identify and determine the presence of genes SHV, TEM and CTX for Extended Spectrum Beta-Lactamase (ESBL) producing bacteria along the Rio Grande River. Characterization of genes responsible for the multi antibiotic resistance in the region can lead to improved measures and policies in the management of infectious diseases and offer preventive measures in the antibiotic crisis we are facing worldwide.

Goals

To assess the extent of antimicrobial resistant bacteria populations through the identification of genes for Extended Spectrum Beta-Lactamase (ESBL) bacteria in surface water of the Rio Grande River in the El Paso, Texas/Cd. Juarez, Mexico border region (Courchesne, Anapra and Riverbend).

Main Objectives

1. To determine the presence of Extended Spectrum Beta-Lactamases (ESBL) genetic markers (SHV, TEM and CTX-M) in water along a 26 km segment of the Rio Grande River.
2. To determine the presence of integrons I and 2 in water samples from the Rio Grande River.
3. To screen for Carbapenem Resistant (CRE) Enterobacteria from bacterial isolates recovered from water samples from the Rio Grande River.

Hypothesis

We hypothesize that waters of the Rio Grande River carry antibiotic resistant bacteria (ESBL and CRE) and mobile genetic elements (Class 1 and 2 integrons) that could contribute to public health and environmental problems of antibiotic resistance to the community of El Paso, TX.

Methods

Funding and IBC Approval

This project has been approved by the Institutional Biosafety Commission (IBC) and financial support was received through the Edward N. and Margaret Marsh foundation. A copy of approval by the IBC of the current research proposal is attached (Appendix).

Human Interactions and/or Potential Risks

There were no human interactions in this study. Risk Group 2 level organisms were Part of this research project handled by the researchers only. An Institutional Biosafety Committee Appendix Form 2 is attached (Appendix).

Study Design

This is a descriptive type of study that aimed to assess the extent of antimicrobial resistant bacterial populations through the identification of genetic markers such as Integrons and ESBL β-Lactamase genes in water of the Rio Grande River. Through a multidisciplinary collaboration with the UTEP Department of Chemistry and EPCC Department of Biology we identified the presence of pharmaceutical residues in the river, obtained phenotypic characterization of isolates, susceptibility patterns and geno amplification of selected ESBLs and screened for Carbapenem Resistant (CRE) Enterobacteria.

Methodology Overview and Sampling

Collection of water samples was performed by undergraduate and graduate students from EPCC and UTEP from three different sites along a 26 km segment of the Rio Grande River within the El Paso, TX-Juarez, Mexico border. A total of 15 samples were collected during five different time periods throughout the year 2017: February, April, June, September and December.

Following water collection, samples were sent to EPCC to evaluate the presence of bacteria and identification of coliforms (bacteria present in the digestive tract of animals and humans found in waste material, plants and soil) through the IDEXX Colilert and Microscan panels for bacterial identification. As a graduate student of the Master's program in Public Health of UTEP I performed molecular analysis of the samples including: PCR for resistance biomarkers (ESBL genes) and mobile genetic elements Integrons (Int-1 and Int-2) in water samples. In addition, bacterial isolates that showed multidrug resistance patterns to Beta Lactam antibiotics were be examined by PCR. CHROMagar chromogenic media was used for screening of Carbapenem Resistant (CRE) Enterobacteria among the recovered isolates tested for ESBLs.

DNA extraction was obtained using the rapid water DNA isolation kit. Identification of ESBL genes was obtained following Monstein's PCR protocol (Monstein, 2007). ESBL genes tested, selected based on their worldwide increased prevalence, were: TEM, SHV and CTX-M genes. Identification of integrons Intl-1 and Intl-2 was done obtained following Dr. Barrantes PCR protocol (Barrantes, Chacon, Solano, & Achi, 2014).

Water Sampling

Water and sediment samples (15) were taken by undergraduate and graduate students from both El Paso Community College and the University of Texas at El Paso. The collection of water was obtained from the following sites along a 26 km segment of the Rio Grande River on the border of El Paso, Texas and Juarez, Mexico:

Site 1: Racetrack Bridge, 50 meters downstream to Sunland Park, NM Wastewater treatment plant (WWTP) (Fig. 4).

Figure 9: Sunland Waste Water Treatment Plant (Site 1 for Water Sampling)

The first site for water sampling from the Rio Grande river is located 50 meters downstream to Sunland Park WWTP belonging to the Camino Real Regional Utility Authority (CRRUA) department which is responsible for the management and maintenance of the City of Sunland Park and Santa Teresa, NM water and waste water system. Their service population is projected to increase from over 18,000 to 45,000 by 2034. While the North Sunland Park WWTP has failed and pending to be replaced due to health-harming levels of arsenic, it is still used mostly for pretreatment and the South WWTP completes the water treatment.

Site 2: Fifty meters downstream from Courchesne Bridge, El Paso, TX and 1 kilometer downstream from the Montoya Drain.

Figure 10: Courchesne area (Site 2 for Water Sampling)

The second location for Rio Grande water sampling is located fifty meters downstream from Courchesne bridge and 1 kilometer from the Montoya Drain. The Montoya Drain is a canal belonging to the County of El Paso, TX and 2.08 miles far from Montoya. Many agriculture activities that take place around this area include dairy, cattle, cotton and farm products such as pecans, onions and peppers. Also many horse famrs and racing stables can be identified close to this area.

Site 3: At the River Bend across from The University of Texas at El Paso and near a public park on the Mexican side of the river (Anapra).

Figure 11: Anapra area across UTEP (Site 3 for Water Sampling)

The third location for water sampling within the 26-km section of the Rio Grande river is located across The University of Texas at El Paso in between the border of El Paso, TX and Anapra, which is located on Cd. Juarez on Mexican side and we required permission from the U.S. Customs and Border Protection to have access. Anapra is a neighborhood in Cd. Juarez, Mexico and one of the poorest communities within the city. Still, this section was selected for high recreational activities of families and children on the river.

Samples were received during five different timeframe periods throughout the year in which the environment could play an important factor on the dissemination of MDRO's and residues along the River. The dates of sample collection were on February 23rd, April 5th, June 5th, September 5th and December 5th of the year 2017.

A total volume of 1,500 ml of water and sediment will be collected from each of the geographical areas and separated into 3 different groups of 500ml each. Samples will be then taken to EPCC for chemical analysis (antimicrobial resistance patterns) and to UTEP for DNA

extraction and genetic analysis (ESBLs and other mobile genetic elements), and identification of antibiotic residues.

DNA Extraction from Water Samples from the Rio Grande River

Filtration of water and DNA extraction will be performed using materials and protocol provided by Rapid Water DNA Isolation kit (QIAGEN). Appendix.

DNA Extraction from Bacterial Isolates

Identification and isolation of bacteria was performed through Microscan™ and measurement of *E. coli* was performed through IDEXX Colilert. This process was done by the Department of Biology at El Paso Community College. Once bacteria isolates were phenotypically characterized and susceptibility patterns identified, they were sent to UTEP College Health Sciences Dominuez Lab 471/475 for molecular analysis of MDRO's and identification of Extended Spectrum Lactamase genes (SHV, TEM and CTX-M) and integrons (Intl-1 and Intl-2).

Resistant bacteria selected was cultured in Luria Agar Base plates with LB broth and incubated at 35˚C for 24 hours. After a 48-hour bacterial growth culture (Table 9), DNA extraction was obtained using a DNeasy Tissue Kit (Qiagen Inc., Valencia, CA) following the boiling method protocol for Gram negative bacteria (Chacon) (Table 10) with an extension in centrifuge time from 5 minutes to 10 minutes and lower RPMs (10,000 instead of 14,000). The integrity of the genomic DNA will be assessed by electrophoresis in a 1.5% agarose gel in Tris-acetate-EDTA (TAE) buffer.

Table 4: Protocol for Bacteria Growth in Agar Plates

Procedure
1. Weigh 30.5g of Luria Agar Base 2. Dissolve powder in 1 L of purified water and mix thoroughly 3. Heat with frequent agitation and boil for 1 minute (until completely dissolved) 4. Autoclave at 121˚C for 15 minutes 5. Cool to 45 – 50˚C 6. Pour into plates 7. Incubator 18-24h

Table 5: Protocol for DNA Extraction from Bacteria

Procedure
1. Grow one well isolated colony in LB broth O/N, 37C, 200 rpm 2. After 24h centrifuge at 14,000 rpm 5 min 3. Resuspend in 300 ul distilled sterile water 4. Centrifuge 14,000 rpm- 5 min 5. Resuspend in 300 ul distilled sterile water 6. Boil for 10 min 7. Centrifuge at 14,000 rpm for 10 min 8. Take 250 ul of supernatant and save at -70C until analysis

Multiplex PCR

PCR was performed according to Monstein's Multiplex PCR protocol (Monstein, 2007). PCR samples were run in 1.5% agarose gel Tris-acetate-EDTA (TAE) buffer for 90 minutes at 75 Volts (V) to identify the presence of SHV, TEM and CTX genes in *E. coli* strains. Primers used included the following: bla-SHV.SE, bla-SHV.AS, TEM-165 AS, TEM-164.SE, CTX-M-U1 and CTX-M-U2 (SIGMA). (Figure X). Promega 200bp DNA Step ladder (1 µl of Orange dye mixed per 5 µl of 200 ruler) will be used to asses genome size of controls and *E. coli* samples.

Multiplex and Singleplex PCR Amplification for ESBL Genes

Multiplex PCR amplification for ESBL genes was performed following Monstein's PCR protocol (Monstein, 2007). ESBL genes tested were TEM, SHV and CTX-M due to their high prevalence associated with community and hospital-acquired infections. PCR multiplex reactions were carried out using: 1 µl of DNA solution (*E. coli* strains), 12µl of Qiagen HotStar Taq Master Mix, 6 µl of molecular grade (mol) H_2O and 1 µl of each F/R gene-specific primer (SHV, TEM and CTX) in a final volume of 25 µl. For controls, PCR singleplex were carried out using: 1 µl of DNA solution (*E. coli* strains or each SHV, TEM, CTX gene), 12µl of Qiagen HotStar Taq Master Mix, 10 µl of mol H_2O and 1 µl of corresponding F/R gene-specific primer (SHV, TEM and CTX) in a final volume of 25 µl. PCR amplification conditions for both multiplex and singleplex controls were as follows: initial denaturation step at 95˚C for 15 min; 30 cycles of denaturation at 95˚C for 30 s, annealing at 55˚C for 30 s, extension at 72˚C for 2 min, followed by a final extension step at 72˚C for 5 minutes.

Chromagar Analysis for CRE Screening

CromagarKPC is a chromogenic medium for overnight detection of gram negative bacteria with reduced sensitivity to all carbapenem agents. It was be applied to all bacteria isolates sent by EPCC that were also analyzed for ESBL genes.

Molecular Analysis of Integrons in Water Samples from the Rio Grande River

Singleplex PCR for Integrons

Singleplex PCR for Integrons intl-1 and intl-2 was performed following Kotlarska's (Kotlarska, 2014) protocol. Original concentration of intl-1 (F & R) and intl-2 (F & R) was (1µG/µL). Aliquots were made using filtered (Filter Device, Puradisc) mol H_2O to a diluted concentration as indicated by the technical datasheet (Sigma-Aldrich).

Singleplex PCR reactions were carried out using 3µl DNA solution, 10 µl Qiagen HotStar Taq Master Mix (Qiagen Nr. 203445) and 3µl of each integron (forward and reverse) specific primer in a final volume of 25 ml.

PCR amplification conditions were as follows (Kotlarska, Luczkiewicz, Pisowacka, & Burzynski, 2015): initial denaturation step at 94˚C for 9 min; followed by 30 cycles of denaturation at 94˚C for 1 minute, annealing at temperature indicate in table for 30s and extension at 72˚C for 1 min. Final extension step at 72˚C for 10 min.

Statistical Analysis

SPSS software was used for descriptive analysis of data to determine frequency distribution of ESBL genes, Integrons and CRE bacteria as well as frequency distribution according to geographical sampling sites.

Results

I. Molecular Analysis of Water Samples from the Rio Grande

Identification of Resistance Biomarkers (ESBL Genes: SHV, TEM CTX-M) by Polymerase Chain Reaction (PCR) in Water Samples

A total of 15 water samples were collected from the previously mentioned sites and labeled as the following: Site 1, Site 2 and Site 3. Selected biomarkers of resistance included the Extended Spectrum Beta-Lactamase (ESBL) genes TEM, CTX-M and SHV and mobile genetic elements Integron 1 and Integron II. DNA was extracted from water and then genes were detected by PCR (Figs 12 and 13).

Table 6: Detection of ESBL (TEM, CTX-M and SHV) Genes directly From Water Samples

	2 /23/17	**4/5/17**	**6/ 5/2017**	**9/5/2017**	**12/5/2017**
Site 1	Positive for TEM	Positive for TEM	Positive for TEM	Positive for TEM	Positive for TEM,CTX-M
Site 2	Positive for TEM, CTX-M	Positive for TEM	Positive for TEM	Positive for TEM	Positive for TEM, CTX-M
Site 3	Positive for TEM, CTX-M	Positive for TEM	Positive for TEM	Positive for TEM, CTX-M	Positive for TEM, CTX-M

Among 15 water samples collected through the months of February (3), April (3), July (3), September (3) and December (3), a total of 11 tested positive (73.3%) and 4 negative (26.7%) for the presence of ESBL (SHV, TEM and CTX-M) genetic markers. From these, 7 (46.7%) were positive for CTX-M gene and 9 samples (60%) were positive for the presence of TEM genes. SHV was not identified in any of the water samples. In summary, the presence of ESBL genes was successfully identified in water samples taken from the Rio Grande river throughout the year being ESBL genes TEM and CTX-M the most prevalent (Tables 7-10)

Table 7: Frequency Distribution and Percentages of all ESBL Genes in Water Samples

	Frequency	Percent	Valid Percent %
Positive	11	73.3	73.3
Negative	4	26.7	26.7
Total (*N*= 15)	15	100.0	100.0

Table 8: Frequency Distribution and Percentages of ESBL TEM in Water Samples

	Frequency	Percent	Valid Percent
Positive	9	60.0	60.0
Negative	6	40.0	40.0
Total (*N*=15)	15	100.0	100.0

Table 9: Frequency Distribution and Percentages of ESBL CTX-M in Water Samples

	Frequency	Percent	Valid Percent
Positive	7	46.7	46.7
Negative	8	53.3	53.3
Total (*N*=15)	15	100.0	100.0

Table 10: Frequency Distribution and Percentages of ESBL SHV in Water Samples

	Frequency	Percent	Valid Percent
Positive	0	0.0	0.0
Negative	15	100.0	100.00
Total (*N*=15)	15	100.0	100.00

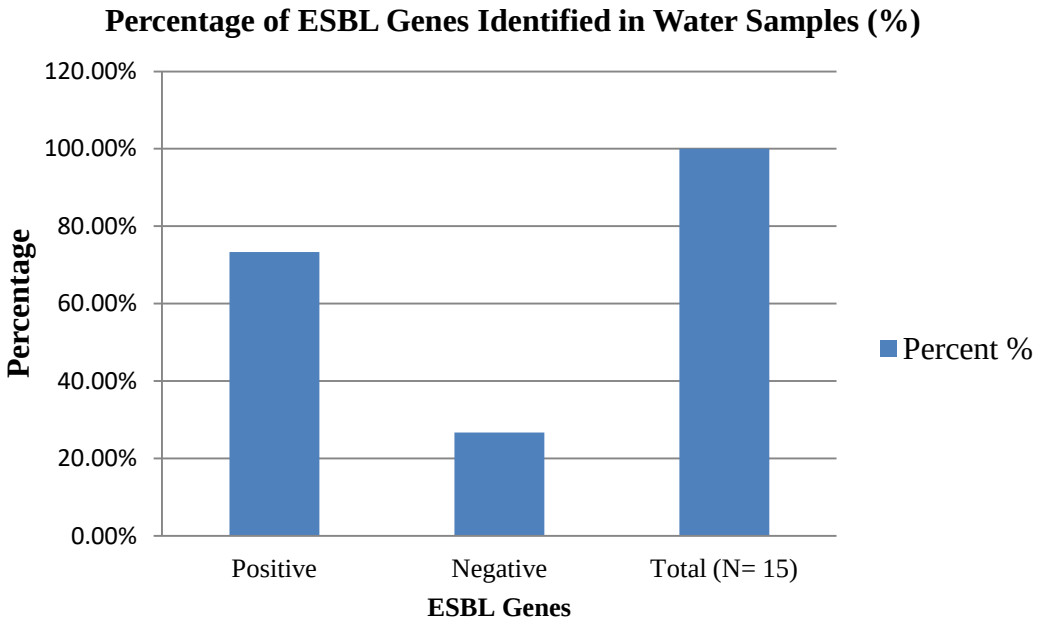

Figure 12: Percentage of total ESBL genes identified in water samples from the Rio Grande River throughout the year (2017).

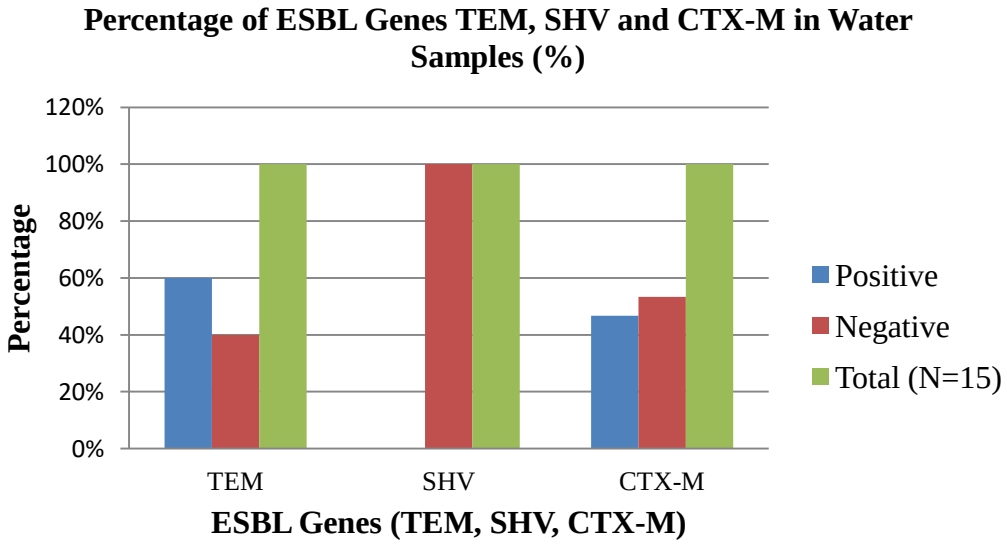

Figure 13: Percentage of positive ESBL genes TEM (60%), SHV (0%) and CTX-M (46.7%) identified in all water samples (15) from the Rio Grande River.

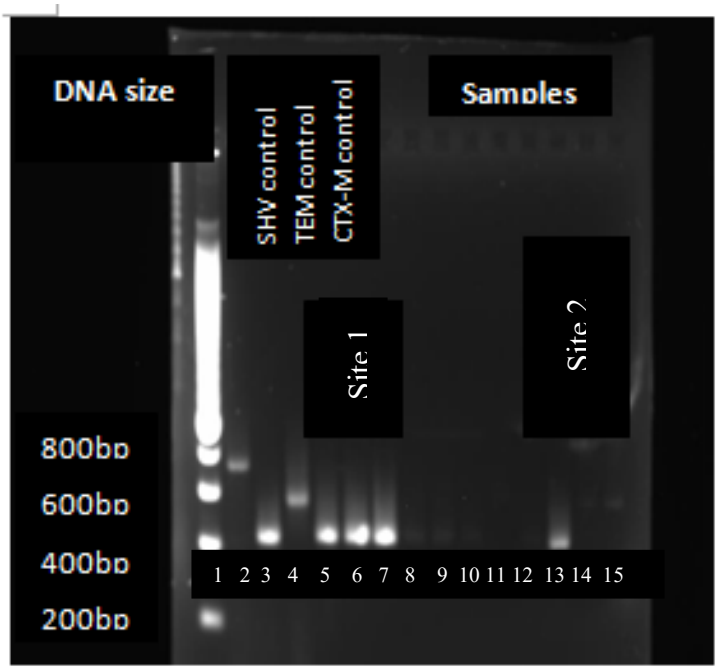

Figure 14: Gel electrophoresis showing positive for the identification of ESBL gene TEM in water samples from Site 1 and Site 2 locations.

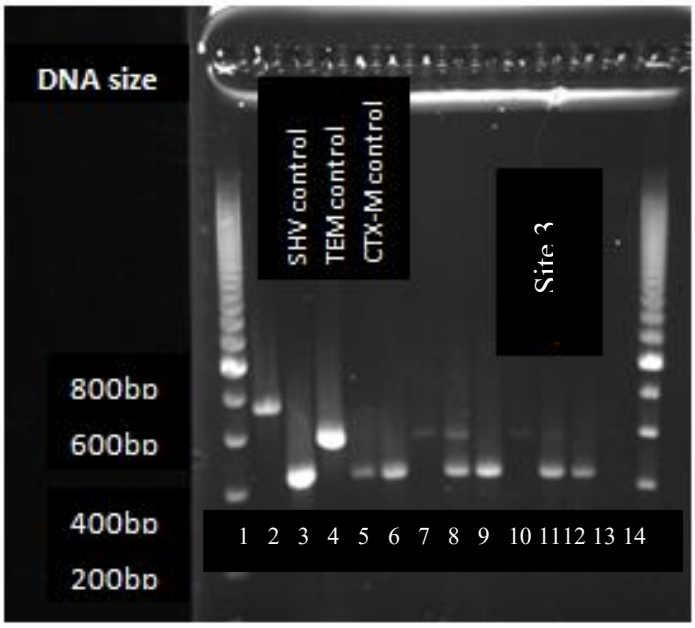

Figure 15: Gel electrophoresis showing positive for the identification of ESBL genes TEM and CTX-M in water sample from Site 3 area.

Identification of Mobile Genetic Elements (Integrons Intl-1 & Intl-2) by Polymerase Chain Reaction (PCR) in Water Samples

Table 11: Detection of Mobile Genetic Elements (Integrons I and II) from Water Samples

	2 /23/17	4/5/17	6/ 5/2017	9/5/2017	12/5/2017
Site 1	Positive Int I	Negative	Positive Int I	Positive Int I	Positive Int II
Site 2	Positive Int I	Negative	Negative	Positive Int I	Positive Int I
Site 3	Positive Int I, Int II	Positive Int I	Negative	Positive Int II	Positive Int I

Among 15 water samples collected through the months of February (3), April (3), July (3), September (3) and December (3) (Table 11), a total of 11 (73.3%) were positive and 4 were negative (26.7%) for the identification of integrons Int-1 and Int-2 (Table 12). In summary, identification of mobile genetic elements integrons (intl-1 and intl-2) were positively found in water samples from all collection sites throughout the year (Table 12 and Fig 16).

Table 12: Frequency Distribution and Percentages of Integrons in Water Samples

	Frequency	Percent	Valid Percent
Positive	11	73.3	73.3
Negative	4	26.7	26.7
Total (*N*=)	15	100.0	100.0

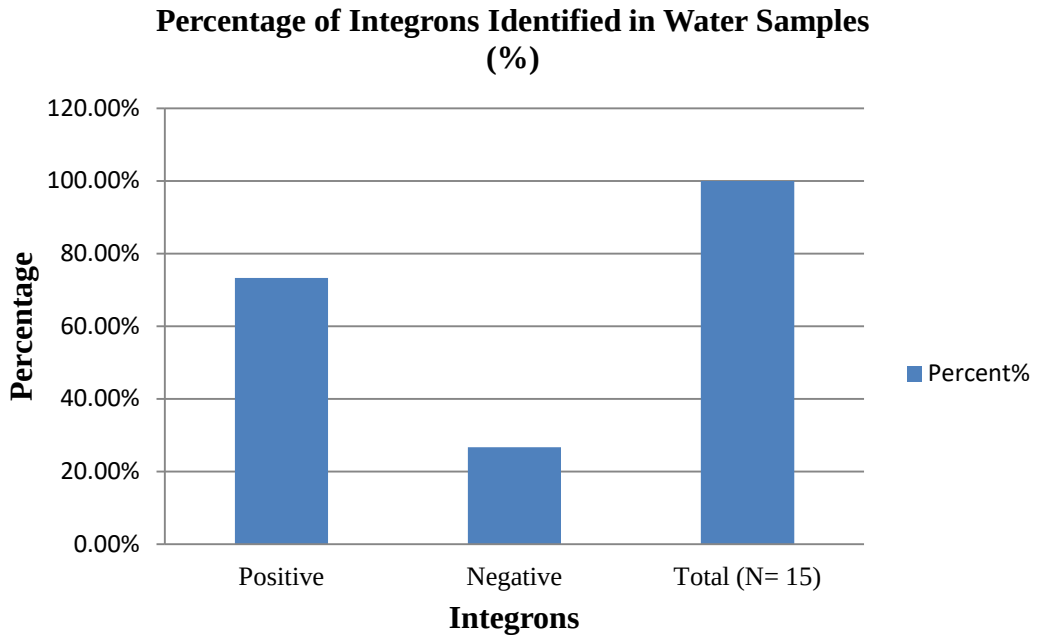

Figure 16: Percentage of integrons identified directly from water (15) samples collected during five different time periods throughout the year (2017) from water of the Rio Grande river.

Class 1 and Class 2 integrons were both identified in water samples. Class 1 AFAR (46.7%) and Class 1 BFBR (40%) (Tables 13-14) were easier to identify and therefore, more prevalent in water samples than Class 2 AFAR (6.7%) and Class 2 BFBR (20%) (Tables 13-16)

Table 13: Frequency Distribution and Percentages of Integron-1AFAR in Water Samples

	Frequency	Percent	Valid Percent %
Positive	7	46.7	46.7
Negative	8	53.3	53.3
Total (*N*=15)	15	100.0	100.0

Table 14: Frequency Distribution and Percentages of Integron-1BFBR in Water Samples

	Frequency	Percent	Valid Percent %
Positive	6	40.0	40.0
Negative	9	60.0	60.0
Total (*N*=15)	15	100.0	100.0

Table 15: Frequency Distribution and Percentages of Integron-2 AFAR in Water Samples

	Frequency	Percent	Valid Percent %
Positive	1	6.7	6.7
Negative	14	93.3	93.3
Total (*N*= 15)	15	100.0	100.0

Table 16: Frequency Distribution and Percentages of Integron-2 BFBR in Water Samples

	Frequency	Percent	Valid Percent %
Positive	3	20.0	20.0
Negative	12	80.0	80.0
Total (*N*=15)	15	100.0	100.0

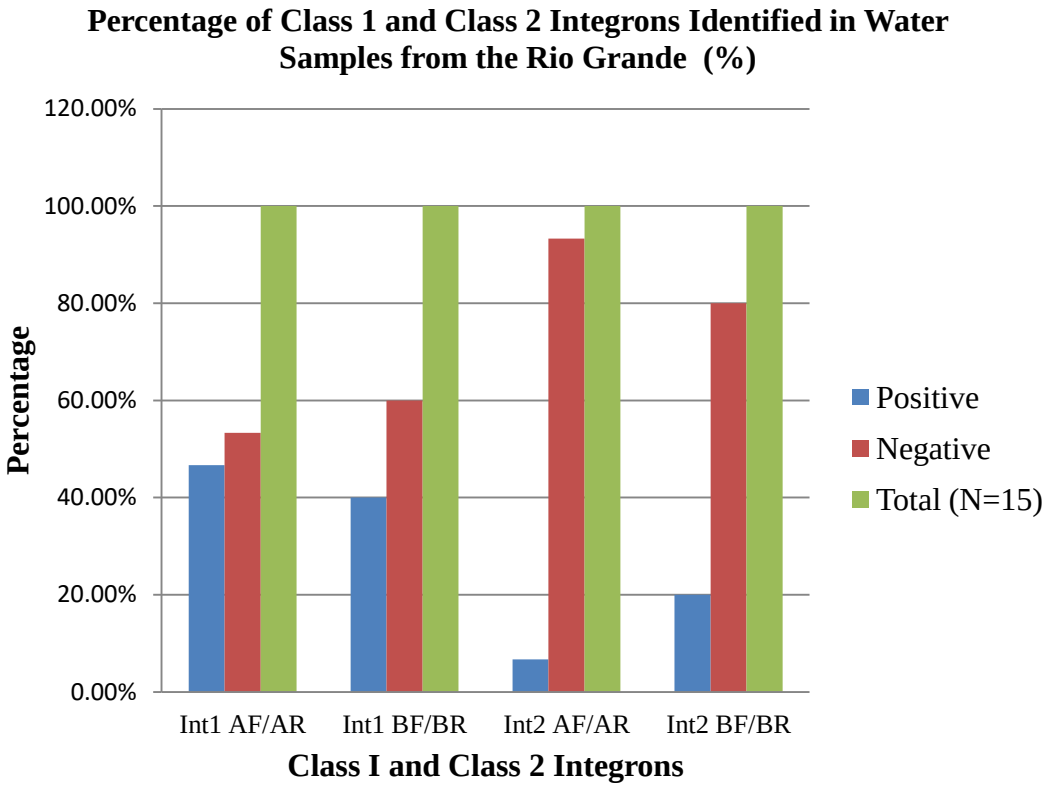

Figure 17: Percentage of Integrons Class 1 and Class 2 identified in water samples from different time periods throughout the year (2017) from the Rio Grande River.

II. Analysis of Bacterial Isolates Recovered from the Rio Grande River Water Samples

Description of Recovered Bacterial Isolates

Identification of bacterial isolates was performed at El Paso Community College Department of Biology. Water samples were collected in one-quart Cubitainer using the grab method. The samples were filtered and cultured for bacterial growth. Bacterial isolates were characterized with the Microscan Negative Breakpoint Combo 34 (NBP34) panel for Gram negative isolates, and the Positive Breakpoint Combo 20 (PBPC 20) panel for Gram positive bacteria. The autoSCAN4 system of >99.99% was used to determine the Minimum Inhibitory Concentration to antibiotics by the all bacterial isolates recovered from the water samples.

February Isolates

In the month of February, 17 out of 39 isolates were identified with a probability of correct ID of 99.99% as determined by the Microscan autoSCAN 4 system. Two isolates displayed resistance or intermediate resistance to 2 or more synergistic combinations including Trimethoprim/Sulfamethoazole, Amoxicillin/K Clavulanate, and Piperacillin/Tazobactam.

April isolates

In the month of April, 56 isolates were processed and 27 isolates were identified with a probability of correct ID of 97% or above. Of these, 1 *E. coli* isolate was flagged as ESBL by the Microscan AutoSCAN system, 13 isolates showed resistance and two intermediate resistance to two or more synergistic combinations including Amox/K Clav, Amp/Sulbactam, Pip/Tazo, Trimeth/Sulfa and Ticar/K Clav.

July Isolates

In the month of June, Out of 60 isolates, 18 were processed with a probability of correct identification of 94% or above. Of these, 9 (Five *E. coli* and 4 *Klebsiella*) isolates were flagged as ESBL by the Microscan Autoscan system, while a total of 15 had resistance to two or more synergistic combinations including Amox/K Clav , Amp/Sulbactam, Pip/Tazo, Trimeth/Sulfa and Ticar/K Clav.

September Isolates

In the month of September, out of 79 isolates, 37 isolates were identified with a probability of correct identification of 94% or better. There were no isolates flagged as ESBL, and 26 isolates were resistant to 2 or more synergistic combinations including Amox/K Clav, Amp/Sulbactam, and Trimeth/Sulfa.

December Isolates

In the month of December, out of 76 isolates, 43 were identified with a probability of correct identification of 92% or better. One *Klebsiella pneumoniae* isolate was flagged as ESBL. There were 33 isolates resistant to two or more synergistic combinations including Amox/K Clav , Amp/Sulbactam, Pip/Tazo, Trimeth/Sulfa and Ticar/K Clav.

In summary, out of a total of 310 isolates processed by the MicroScan AutoScan 4 system, 142 were identified with a probability of correct identification varying from 92-99.99%. A total of 91 isolates had resistance to at least two or more synergistic combinations including Amox/K Clav, Amp/Sulbactam, Pip/Tazo, Ticar/K Clav, and Trimeth/Sulfa, and 105 isolates were resistant to more than four individual antibiotics. Eleven *E. coli* and *Klebsiella* isolates

were identified as ESBLs by the Microscan Autoscan 4 system and were also resistant to more than 20 individual antibiotics. Most of the *Staphylococcus* isolates were also resistant to multiple antibiotics.

A total of 28 Gram negative bacteria identified as ESBLs by the Microscan Autoscan 4 system and these were selected for molecular analysis for ESBL genes (SHV, TEM and CTX-M), integrons (Intl 1 and Intl 2) and KPC screening.

*Bacterial isolates identified and susceptibilities are in the Appendix

IDEXX Colilert Most Probable Number (MPN)

Water samples were sent to El Paso Community College Department of Biology to analyze the Most Probable Number (MPN), which is used as a fecal indicator for surface water samples. It is a statistical number, which gives the most probable number of *E.coli* in 100ml of water. The standard is 126 MPN/100 ml for this segment of the river which is set by the Water Quality Standards Team at Texas Commission on Environmental Quality (TCEQ).

In the months of February, April, and September all samples were above the standard set by the TCEQ. In the month of December and possibly due to the lower temperatures, MPN values were much lower and Sites 2 and 3 were within the standard.

Table 17: Total Values of E. coli MPN (per ml) Identified Throughout the Year (2017)

	MPN				
	2 /23/17	**4/5/17**	**7/19/2017**	**9/ 5/2017**	**12/5/2017**
Site 1	1203.3	1732.87	648.8	829.7	378.4
Site 2	196.8	983.5	616.7	162.4	123.4
Site 3	980.4	770.1	1732.87	913.9	0

**Normal value: 126/100ml*

Table 18: Frequency Distribution of E. coli MNP in Water Samples

N	Valid	15
Mean		751.5
Median		770.1
Std. Deviation		537.9
Skewness		.481
Minimum		.00
Maximum		1732.8
Percentiles	25	196.8
	50	770.1
	75	983.5

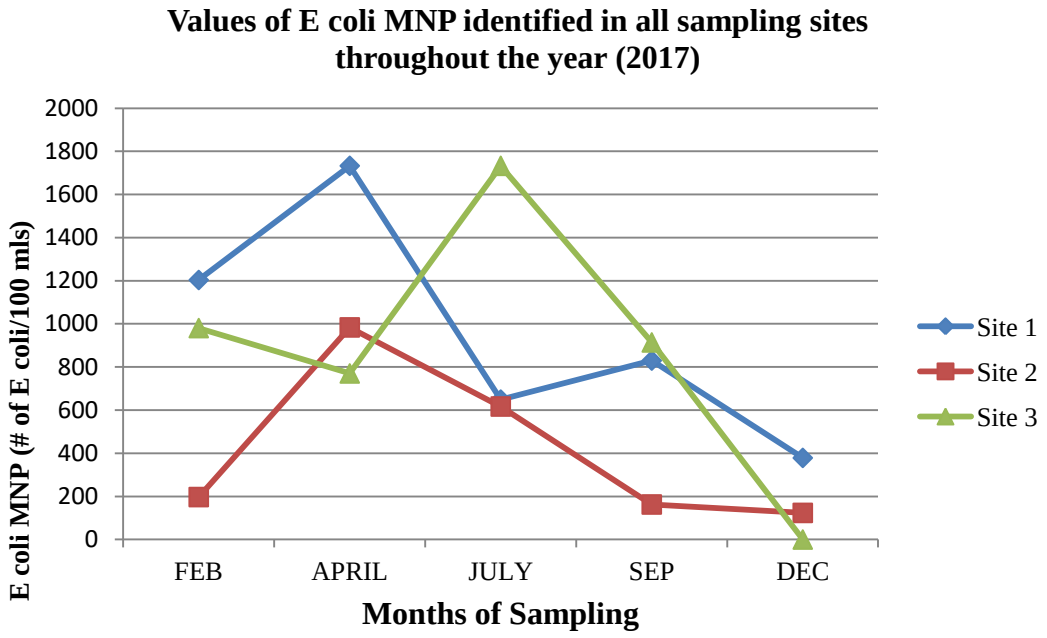

Figure 18: Values of E coli (per ml) identified from water samples from the Rio Grande River during the year 2017

Among the 15 water samples collected through February, April, July, September and December of 2017, the five point summary for MNP is 0.00, 196.80, 983.5 and 1732.87. Throughout the year the fecal indicator for water appear to differ according to the season, which could be related to irrigation season which lasts from April to September aimed to preserve groundwater from the Mesilla and Hueco aquifers. The graph shown above (Fig. 18) shows the MNP variability throughout the year reaching a highest peak during the month of April and lowest during the month of December (non irrigation season).

One sample T-test was selected to compare the mean of all water samples with the standard value of 126/100ml. The mean MNP values (751.5) is different from the TCEQ standard of 126 per 100 ml for this segment of the Rio Grande River (p=0.001). (Table 15 and 16 below for One-sample T-test). In summary, the average of *E. coli* identified in all samples

throughout the year is higher than expected by the Water Quality Standards Team at Texas Commission on Environmental Quality (TCEQ).

Table 19: One Sample Statistics for *E. coli* MNP Values During the Year 2017

	N	Mean	Std. Deviation
MNP	15	751.5*	537.9

**Normal value= 126ml/100ml*

Table 20: One Sample Test Value (126) for E. coli MNP

					95% Confidence Interval of the Difference	
	T	Df	Sig. (2-tailed)	Mean Difference	Lower	Upper
MNP	4.504	14	.000	625.5	327.6	923.4

Normal value= 126ml/100ml

III. Molecular Analysis and Identification of Resistance Biomarkers ESBL (SHV, TEM, CTX-M) Genes by PCR from Bacterial Isolates Recovered from the Rio Grande River Water Samples

Frequency Distribution of ESBL Genes among Bacterial Isolates

A total of 310 bacterial isolates were recovered from water samples from the Rio Grande River. In the present study, 142 (45.8%) bacterial isolates were identified belonging to 18 genera of bacteria. From these, 28 isolates (9.03%) were identified as Gram negative microorganisms with multiple drug resistant (MDR) patterns, either to two or more antibiotics and/or to antibiotic combinations, and were analyzed for ESBL genes through PCR.

Among 28 bacterial isolates analyzed, 4 (14.3%) were identified as SHV positive and 13 (46.4%) as TEM positive; 4 (14.3%) were positive for both SHV and TEM and only 1 (3.6%) was identified positive for both TEM and CTX-M. Only 6 (21.4%) were not identified as being ESBL positive through PCR on this study. (Table 17)

According to the literature, it was mentioned that TEM is strongly associated with up to 90% ampicillin resistance patterns in *E coli, Klebsiella pneumoniae, H influenza* and *N. gonorrhoeae* and SHV up to 20% resistance also to ampicillin in *E. coli* and *Pseudomonas aeruginosa*. Since both genes share as much as 68% of their molecular structure it supports the findings of this study. SHV and TEM were the most common ESBL genes identified in bacterial isolates from the Rio Grande water samples.

The bacteria isolates that were positive for SHV gene were all identified as *K. pneumonia.* Bacteria strains that were positive for TEM gene were more diverse and included the following: *Vibrio fluvialis, Aeromonas hydrophilia, E. coli, Cedecea davisae, Citrobacter freundii complex, Klebsiella oxytoca and Klebsiella pneumonia*. For CTX-M gene the only strain that was positive was *Klebsiella oxytoca*.

Table 21: Frequency Distribution and Percentages of ESBL genes in Bacterial Isolates (N=28)

	Frequency	Percent	Valid Percent %
Non-ESBL	6	21.4	21.4
SHV	4	14.3	14.3
SHV,TEM	4	14.3	14.3
TEM	13	46.4	46.4
TEM,CTX-M	1	3.6	3.6
CTX-M	0	0.0	0.0
Total (*N*=28)	28	100.0	100.0

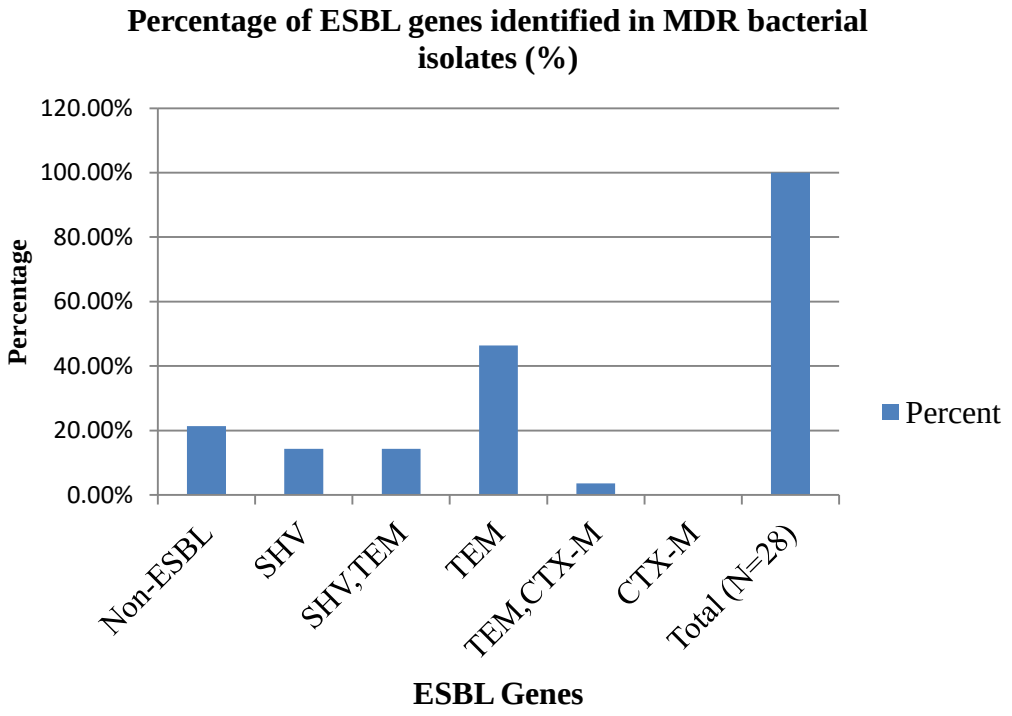

Fig. 19: Percentages of ESBL genes identified, alone or in combination, among bacterial isolates (N=28) from water samples collected from the Rio Grande River during the year 2017

ESBL and antibiotic resistance profile:

The bacteria *K. oxytoca* identified as the only strain to be positive for ESBL CTX-M gene was shown to be resistant for all (100%) the cephalosporin class of antibiotics. The cephalosporins tested through Microscan and showing varying degrees of resistance included: cefazolin, cefepime, cefotaxime, cefotetan cefoxitin, ceftazidime, ceftriaxone, cefuroxime. Also ESBL-a and ESBL-b were positive through Microscan. This CTX-M positive bacteria was collected during the sample collection of June, 2017.

The occurrence of bacteria strains that were positive for ESBL SHV genes showed the same resistance patterns against all (100%) the cephalosporins mentioned above, and also to ESBL-a, ESBL-b. These bacteria were from water samples collected during the month of June,

2017. The same occurrence rate and characteristics of cephalosporin resistance was identified for those strains that were positive for more than one ESBL gene.

Bacteria strains that were positive for ESBL TEM genes showed a diverse resistance occurrence rate among the cephalosporin group of antibiotics and to ESBLa, ESBLb.

Frequency Distribution of Resistant Bacterial Isolates According to Location

Among the 28 multidrug resistant isolates that were recover and underwent molecular analysis for ESBL genes, they were most commonly found in the following sample collection sites from the Rio Grande River: Anapra (57.1%), Courchesne (21.4%) and Sunland park area (21.4%). Anapra, which is in close proximity to the University of Texas at El Paso, was the sampling site with the highest identification of MDR bacterial isolates with positive identification of ESBL genes. See table 22 below**.**

Table 22: Frequency Distribution and Percentages of ESBL Genes in Bacterial Isolates According to Location.

	Frequency	Percent	Valid Percent %
Anapra	16	57.1	57.1
Courchesne	6	21.4	21.4
Sunland	6	21.4	21.4
Total (*N*=28)	28	100.0	100.0

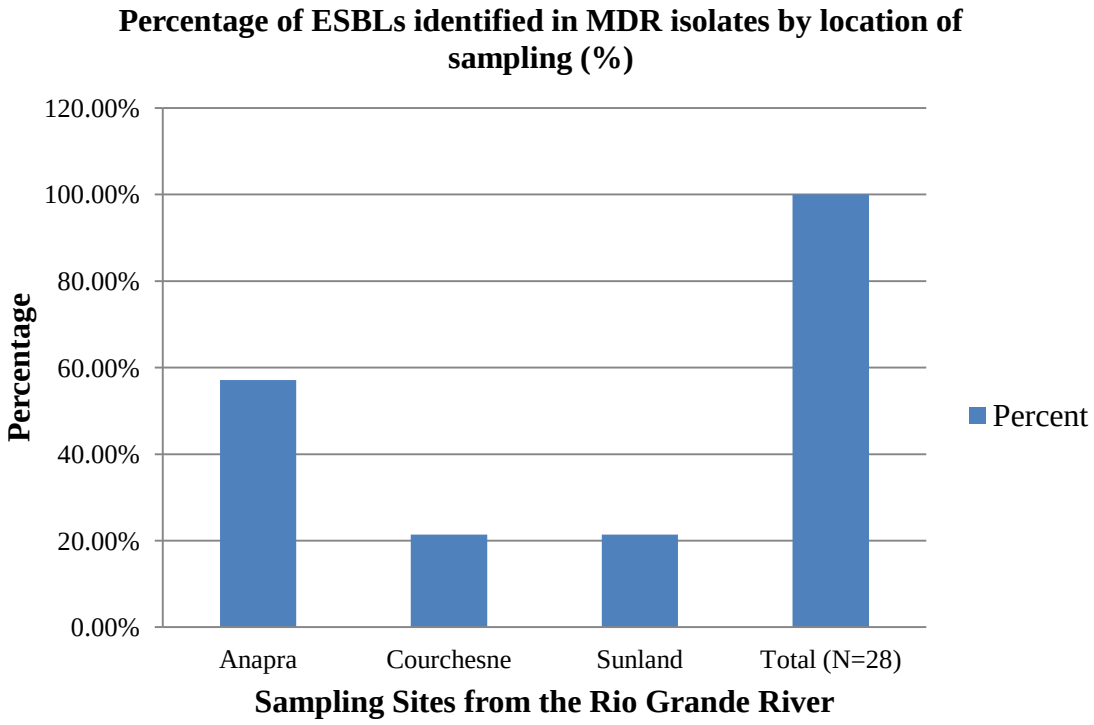

Figure 20: Percentages of MDR bacterial isolates (28) identified from water samples from the Rio Grande River according to location: Anapra, Courchesne and Sunland Park WWTP (2017)

Frequency Distribution of Resistant Bacterial Isolates According to Classification and Type of Microorganism

A total of 28 MDR isolates were included and analyzed for ESBL genes in this study. These were selected according to their multiple resistance antibiotic patters identified through MICROSCAN and also for being identified as Gram negative and possible carriers for ESBLs. These isolates were also identified as being resistant for 2 or more antibiotics.

Bacterial isolates analyzed for ESBL were identified as belonging to following genera: *A. hydrophilia* (3.6%), *C. davisae* (3.6%), *C. freundii* (3.6%), *E. coli* (39.3%), *K. oxytoca* (7.1%), *K. pneumoniae* (32.1%), *Leminorella species* (3.6%), *V. fluvialis* (3.6%) and *V. parahaemolyticus* (3.6%). In summary, the most common Gram negative bacteria analyzed for the presence of ESBL genes in this study were *E. coli* and *Klebsiella sp*. (Table 19).

Table 23: Frequency Distribution and Percentages of MDR Bacterial Isolates by Genera

	Frequency	Percent	Valid Percent %
A. hydrophilia	1	3.6	3.6
C. davisae	1	3.6	3.6
C. freundii	1	3.6	3.6
E. coli	11	39.3	39.3
K. oxytoca	2	7.1	7.1
K. pneumoniae	9	32.1	32.1
Leminorella. sp.	1	3.6	3.6
V. fluvialis	1	3.6	3.6
V. parahaemolyticus	1	3.6	3.6
Total (*N*=28)	28	100.0	100.0

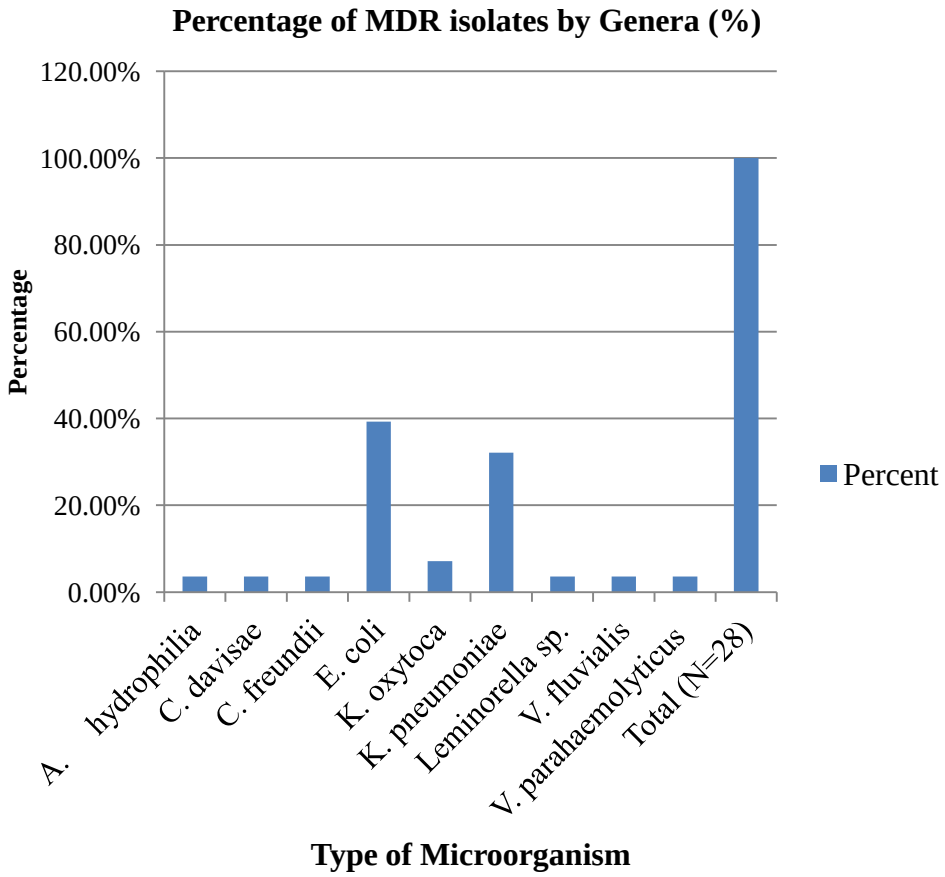

Figure 21: Percentages of MDR bacterial Isolates by Genera

Frequency Distribution of ESBL (SHC, TEM and CTX-M) Genes According to β-Lactam Antibiotics (Penicillins, Cephalosporins and Carbapenems)

Antibiotic Resistance Patterns

A total of 28 Gram negative bacterial isolates, mostly *E. coli* and *K. pneumoniae*, were analyzed for resistant/sensitive patterns to different antibiotics and their association to ESBL genes. Antibiotic analysis includes only B-Lactam derivatives used to treat Gram negative infections: Penicillins, Cephalosporins and Carbapenems.

Penicillins

Among the 28 isolates analyzed, resistance to penicillin derivatives was identified in the following antibiotics and antibiotic combinations: 26 isolates to Amoxicillin/Clavulanate (92.9%), all 28 isolates to Ampicillin/Sulbactam (100%), 27 isolates to Ampicillin alone (96.4%), 23 isolates to the combination of Piperacillin/Tazobactam (82.1%), 21 isolates to Piperacillin (75%) and 22 isolates to Ticarcillin/Clavulanate (78.6%). Based on these results, amoxicillin and ampicillin, alone or in combination with other inhibitors, were the most common to show resistance among the Gram-negative isolates (Tables 24-29)

Tables 24: Frequency Distribution of Amoxicillin/Clavulanate among MDR Bacterial Isolates

	Frequency	Percent	Valid Percent %
Resistant	26	92.9	92.9
Sensitive	6	7.1	7.1
Total (*N*=28)	28	100.0	100.0

Tables 25: Frequency Distribution of Ampicillin/Sulbactam among MDR Bacterial Isolates

	Frequency	Percent	Valid Percent %
Resistant	28	100.0	100.0
Sensitive	0	0.0	0.0
Total (*N*=28)	28	100.0	100.0

Tables 26: Frequency Distribution of Ampicillin among MDR Bacterial Isolates

	Frequency	Percent	Valid Percent %
Resistant	27	96.4	96.4
Sensitive	1	3.6	3.6
Total (*N*=28)	28	100.0	100.0

Tables 27: Frequency Distribution of Piperacillin/Tazobactam among MDR Bacterial Isolates

	Frequency	Percent	Valid Percent %
Resistant	23	82.1	82.1
Sensitive	5	17.9	17.9
Total (*N*=28)	28	100.0	100.0

Tables 28: Frequency Distribution of Piperacillin among MDR Bacterial Isolates

	Frequency	Percent	Valid Percent %
Resistant	21	75.0	75.0
Sensitive	7	25.0	25.0
Total (*N*=28)	28	100.0	100.0

Tables 29: Frequency Distribution of Ticarcillin/Clavulanate among MDR Bacterial Isolates

	Frequency	Percent	Valid Percent %
Resistant	22	78.6	78.6
Sensitive	3	10.7	10.7
None Reported	3	10.7	10.7
Total (*N*=28)	28	100.0	100.0

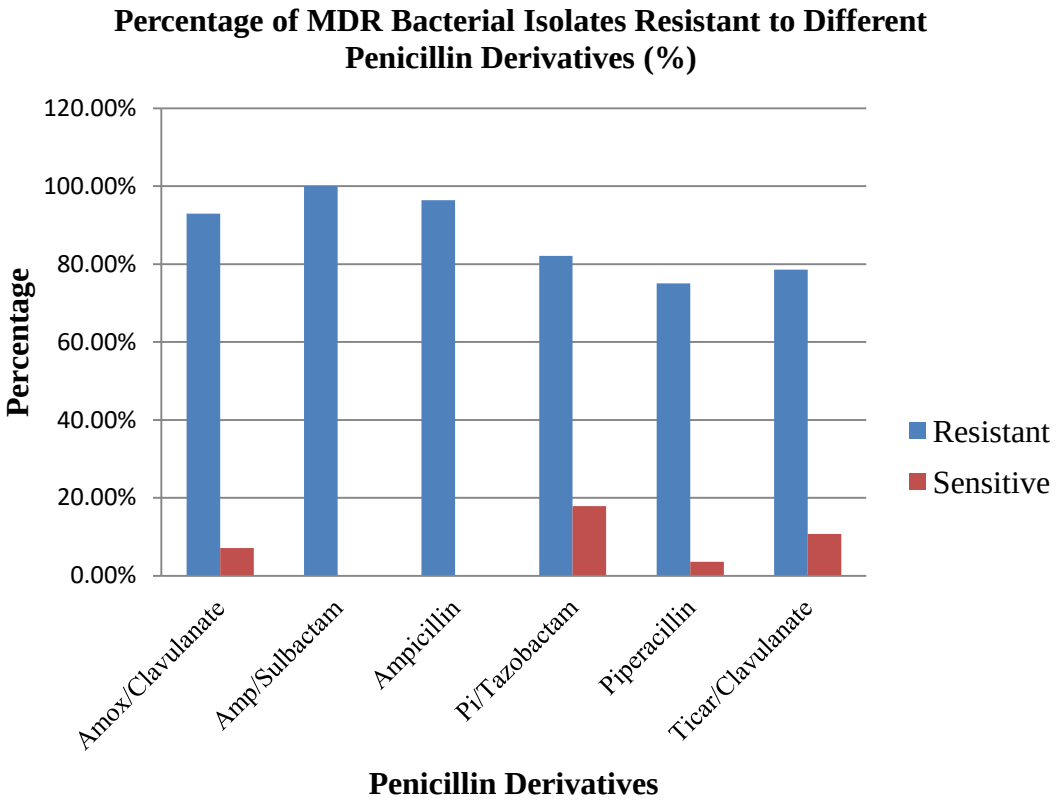

Figure 22: Percentage MDR bacterial isolates patterns of resistance to different penicillin derivatives

After analyzing the frequency distribution of antibiotic resistance to penicillins among the recovered Gram-negative isolates with MDR patterns, this study now analyzed the frequency and association of each ESBL gene (TEM, SHV, CTX-M or a combination of them) in relation to each penicillin derivative.

ESBLs and Amoxicillin/Clavulanate

Among the 28 MDR bacteria samples, the frequency of ESBL genes identified with resistance to Amoxicillin and Clavulanate was the following: SHV (15.4%), both SHV/TEM

(15.4%), TEM (42.3%) and both TEM/CTX-M (3.8%). ESBL gene TEM was the most common gene identified to be resistant to the combination of Amoxicillin and Clavulanate.

Table 30: Frequency Distribution and Percentages of ESBLs in MDR isolates by Resistance Patterns to Amoxicillin/Clavulanate

ESBL gene	Resistant (%)	Sensitive (%)	Total
Non-ESBL	6 (23.1%)	0 (0.0%)	6
SHV	4 (15.4%)	0 (0.0%)	4
SHV,TEM	4 (15.4%)	0 (0.0%)	4
TEM	11 (42.3%)	2 (100%)	13
TEM,CTX-M	1 (3.8%)	0 (0.0%)	1
CTX-M	0 (0.0%)	0 (0.0%)	0
Total	26	2	28

Total (*N*=28) MDR organisms may have tested to one or more ESBL genes.

ESBLs and Ampicillin

Among the 28 MDR bacteria samples, the frequency of ESBL genes identified with resistance to Ampicillin was the following: SHV (14.8%), both SHV/TEM (14.8%), TEM (44.4%) and both TEM/CTX-M (3.7%). ESBL gene TEM was the most common gene identified

Table 31: Frequency Distribution and Percentages of ESBLs in MDR isolates by Resistance Patterns to Ampicillin

ESBL gene	Resistant (%)	Sensitive (%)	Total
Non-ESBL	6 (22.2%)	0 (0.0%)	6
SHV	4 (14.8%)	0 (0.0%)	4
SHV,TEM	4 (14.8%)	0 (0.0%)	4
TEM	12 (44.4%)	1 (100%)	13
TEM,CTX-M	1 (3.7%)	0 (0.0%)	1
CTX-M	0 (0.0%)	0 (0.0%)	0
Total	26	2	28

Total (*N*=28) MDR organisms may have tested to one or more ESBL genes.

ESBLs and Ampicillin/Sulbactam

Among the 28 MDR bacteria samples, the frequency of ESBL genes identified with resistance to Ampicillin and Sulbactam was the following: SHV (14.3.4%), both SHV/TEM (14.3%), TEM (46.4%) and both TEM/CTX-M (3.6%). ESBL gene TEM was the most common gene identified to be resistant to the combination of Ampicillin and Sulbactam.

Table 32: Frequency Distribution and Percentages of ESBLs in MDR isolates by Resistance Patterns to Ampicillin/Sulbactam

ESBL gene	Resistant (%)	Sensitive (%)	Total
Non-ESBL	6 (21.4%)	0 (0.0%)	6
SHV	4 (14.3%)	0 (0.0%)	4
SHV,TEM	4 (14.3%)	0 (0.0%)	4
TEM	13 (46.4%)	0 (0.0%)	13
TEM,CTX-M	1 (3.6%)	0 (0.0%)	1
CTX-M	0 (0.0%)	0 (0.0%)	0
Total	28	0	28

Total (*N*=28) MDR organisms may have tested to one or more ESBL genes.

ESBLs and Piperacillin

Among the 28 MDR bacteria samples, the frequency of ESBL genes identified with resistance to Piperacillin was the following: SHV (19%), SHV/TEM (19%), TEM (42.9%) and both TEM/CTX-M (4.8%). ESBL gene TEM was the most common gene identified to be resistant to the penicillin derivative Piperacillin.

Table 33: Frequency Distribution and Percentages of ESBLs in MDR isolates by Resistance Patterns to Piperacillin

ESBL gene	Resistant (%)	Sensitive (%)	NR (%)	Total
Non-ESBL	3 (14.3%)	0 (0.0%)	3 (50.0%)	3
SHV	4 (19.0%)	0 (0.0%)	0 (0.0%)	4
SHV,TEM	4 (19.0%)	0 (0.0%)	0 (0.0%)	4
TEM	9 (42.9%)	1 (100%)	3 (50.0%)	9
TEM,CTX-M	1 (4.8%)	0 (0.0%)	0 (0.0%)	1
CTX-M	0 (0.0%)	0 (0.0%)	0 (0.0%)	0
Total	21	1	6	28

Total (*N*=28) MDR organisms may have tested to one or more ESBL genes.

ESBLs and Ticarcillin/Clavulanate

Among the 28 MDR bacteria samples, the frequency of ESBL genes identified with resistance to Ticarcillin and Clavulanate was the following: SHV (18.2%), both SHV/TEM (18.2%), TEM (36.4%) and both TEM/CTX-M (4.5%). ESBL gene TEM was the most common gene identified to be resistant to the combination of Ticarcillin and Clavulanate.

Tables 34: Frequency Distribution and Percentages of ESBLs in MDR isolates by Resistance Patterns to Ticarcillin/Clavulanate

ESBL gene	Resistant (%)	Sensitive (%)	NR (%)	Total
Non-ESBL	5 (22.7%)	0 (0.0%)	1 (33.3%)	6
SHV	4 (18.2%)	0 (0.0%)	0 (0.0%)	4
SHV,TEM	4 (18.2%)	0 (0.0%)	0 (0.0%)	4
TEM	8 (36.4%)	3 (100%)	2 (66.7%)	13
TEM,CTX-M	1 (4.5%)	0 (0.0%)	0 (0.0%)	1
CTX-M	0 (0.0%)	0 (0.0%)	0 (0.0%)	0
Total	22	3	3	28

Total (*N*=28) MDR organisms may have tested to one or more ESBL genes.

ESBLs and Piperacillin/Tazobactam

Among the 28 MDR bacteria samples, the frequency of ESBL genes identified with resistance to Amoxicillin and Clavulanate was the following: SHV (17.4%), both SHV/TEM

(17.4%), TEM (34.8%) and both TEM/CTX-M (4.3%). ESBL gene TEM was the most common gene identified to be resistant to the combination of Amoxicillin and Clavulanate.

Table 35: Frequency Distribution and Percentages of ESBLs in MDR isolates by Resistance Patterns to Piperacillin/Tazobactam

ESBL gene	Resistant (%)	Sensitive (%)	Total
Non-ESBL	6 (26.1%)	0 (0.0%)	6
SHV	4 (17.4%)	0 (0.0%)	4
SHV,TEM	4 (17.4%)	0 (0.0%)	4
TEM	8 (34.8%)	5 (100%)	8
TEM,CTX-M	1 (4.3%)	0 (0.0%)	1
CTX-M	0 (0.0%)	0 (0.0%)	0
Total	23	5	28

Total (*N*=28) MDR organisms may have tested to one or more ESBL genes.

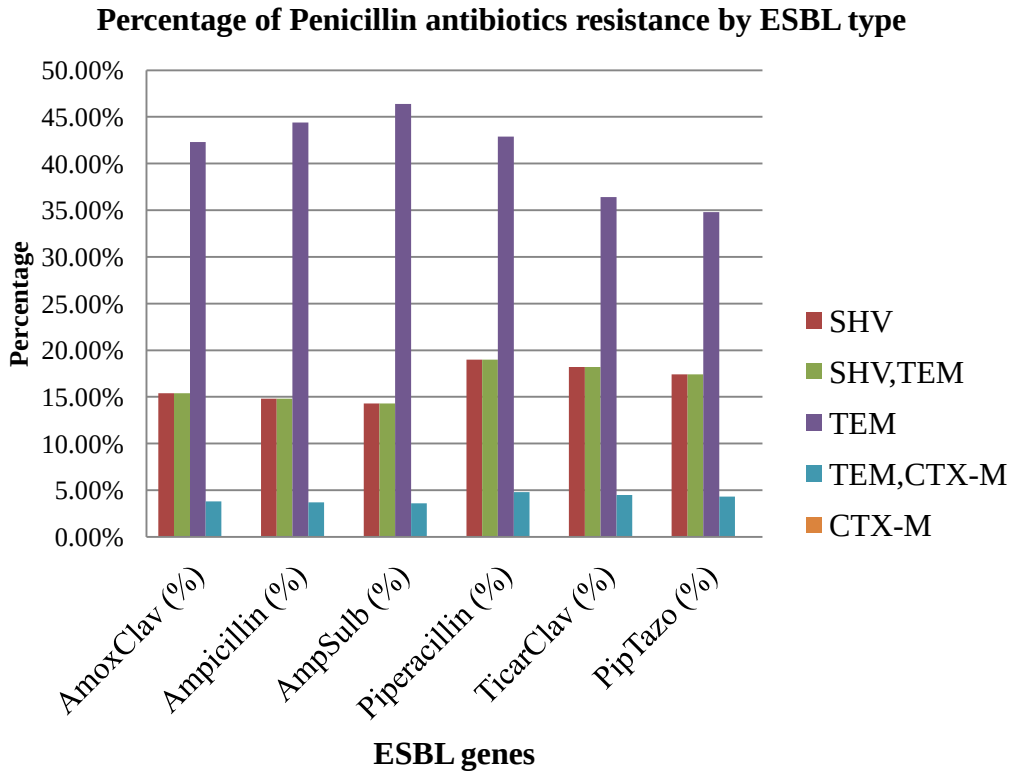

Figure 23: Percentage of different penicillin derivatives and their resistance patterns according to the ESBL gene identified in the MDR isolates

Cephalosporins

Among the 28 isolates analyzed, resistance to cephalosporin derivatives was identified in the following antibiotics and antibiotic combinations: 25 isolates were resistant to Cefazolin (89.3%), 23 isolates to Cefepime (82.1%), 23 isolates (ESBL identified plus general resistance patterns) to Cefotaxime (82.2%), 23 isolates to the combination of Cefotetan (82.1%), 25 isolates to Cefoxitine (89.3%), 23 isolates (ESBL identified plus general resistance patterns) to Ceftazidime (82.2%), 22 isolates (ESBL identified plus general resistance patterns) to Ceftriaxone (78.6%) and 24 isolates to Cefuroxime (85.7%). Based on these results, all cephalosporin derivatives were similar in high resistance among the Gram-negative isolates. The following tables 36-41 show the frequency distribution and resistance patterns found in the MDR bacterial isolates included in this study.

Tables 36: Frequency Distribution and Percentages of Cefazolin Resistance in MDR Isolates

	Frequency	Percent	Valid Percent %
Resistant	25	89.3	89.3
Sensitive	3	10.7	10.7
Total (*N*=28)	28	100.0	100.0

Tables 37: Frequency Distribution and Percentages of Cefepime Resistance in MDR Isolates

	Frequency	Percent	Valid Percent %
Resistant	23	82.1	82.1
Sensitive	5	17.9	17.9
Total (*N*=28)	28	100.0	100.0

Tables 38: Frequency Distribution and Percentages of Cefotaxime Resistance in MDR Isolates

	Frequency	Percent	Valid Percent %
Resistant	23	75.0	75.0
Sensitive	5	25.0	25.0
Total (*N*=28)	28	100.0	100.0

Tables 39: Frequency Distribution and Percentages of Cefoxitine Resistance in MDR Isolates

	Frequency	Percent	Valid Percent %
Resistant	25	89.3	89.3
Sensitive	3	10.7	10.7
Total (*N*=28)	28	100.0	100.0

Tables 40: Frequency Distribution and Percentages of Ceftazidime Resistance in MDR Isolates

	Frequency	Percent	Valid Percent %
Resistant	23	75.0	75.0
Sensitive	5	25.0	25.0
Total (*N*=28)	28	100.0	100.0

Tables 41: Frequency Distribution and Percentages of Ceftriaxone Resistance in MDR Isolates

	Frequency	Percent	Valid Percent %
Resistant	22	78.6	78.6
Sensitive	4	14.3	14.3
NR	2	7.1	7.1
Total (*N*=28)	28	100.0	100.0

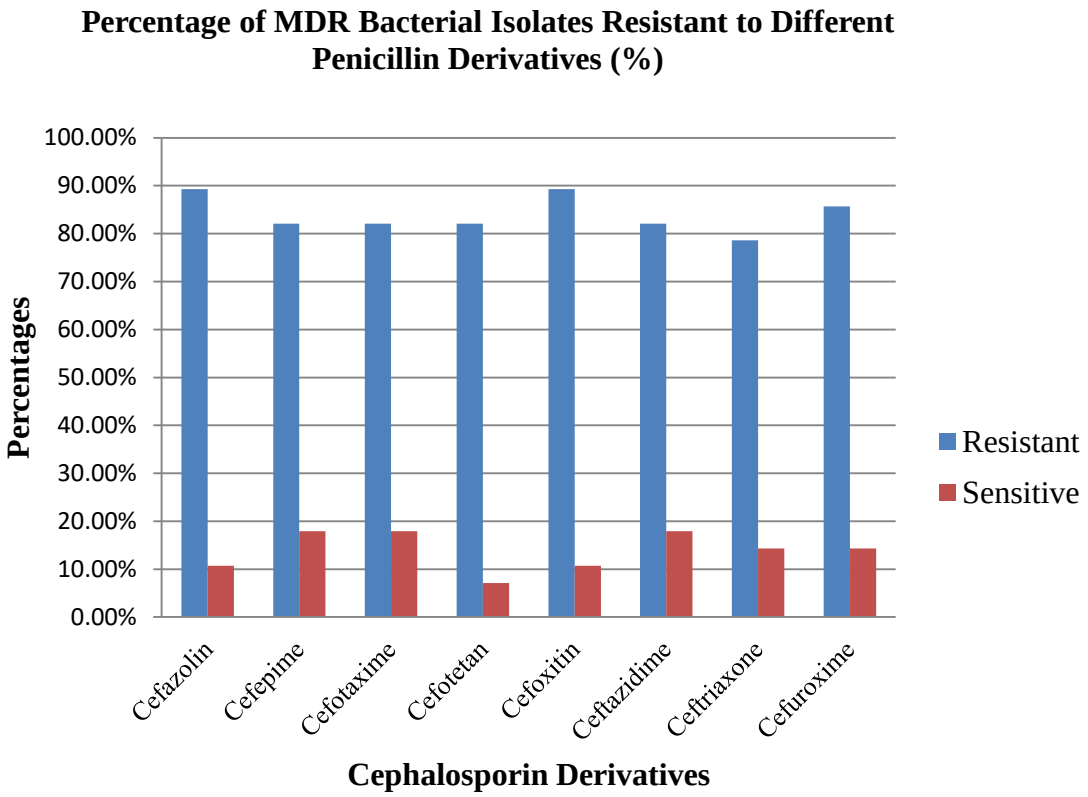

Figure 24: Percentages of antibiotic resistance to different cephalosporin derivatives among Gram negative bacterial isolates identified as MDROs

After analyzing the frequency distribution of antibiotic resistance to cephalosporins among the recovered Gram-negative isolates with MDR patterns, this study now analyzed the frequency and association of each ESBL gene (TEM, SHV, CTX-M or a combination of them) in relation to each cephalosporin derivative.

ESBLs and Cefazolin

Among the 28 MDR bacteria samples, the frequency of ESBL genes identified with resistance to Cefazolin, a 1st generation cephalosporin, was the following: SHV (16%), both

SHV/TEM (16%), TEM (10%) and both TEM/CTX-M (4%). Based on these results, SHV was most commonly identified in association with resistance to Cefazolin.

Table 42: Frequency Distribution and Percentages of ESBLs in MDR isolates by Resistance Patterns to Cefazolin

ESBL gene	Resistant (%)	Sensitive (%)	Total
Non-ESBL	6 (24.0%)	0 (0.0%)	6
SHV	4 (16.0%)	0 (0.0%)	4
SHV,TEM	4 (16.0%)	0 (0.0%)	4
TEM	10 (40.0%)	3 (100.0%)	13
TEM,CTX-M	1 (4.0%)	0 (0.0%)	1
CTX-M	0 (0.0%)	0 (0.0%)	0
Total	25	3	28

Total (*N*=28) MDR organisms may have tested to one or more ESBL genes.

ESBLs and Cefepime

Among the 28 MDR bacteria samples, the frequency of ESBL genes identified with resistance to Cefepime, a 4th generation cephalosporin, was the following: SHV (17.4%), both SHV/TEM (17.4%), TEM (34.8%) and both TEM/CTX-M (4.3%). Based on these results, TEM was most commonly identified in association with resistance to Cefepime.

Table 43: Frequency Distribution and Percentages of ESBLs in MDR isolates by Resistance Patterns to Cefepime

ESBL gene	Resistant (%)	Sensitive (%)	Total
Non-ESBL	6 (26.1%)	0 (0.0%)	6
SHV	4 (17.4%)	0 (0.0%)	4
SHV,TEM	4 (17.4%)	0 (0.0%)	4
TEM	8 (34.8%)	5 (100.0%)	13
TEM,CTX-M	1 (4.3%)	0 (0.0%)	1
CTX-M	0 (0.0%)	0 (0.0%)	0
Total	23	5	28

Total (*N*=28) MDR organisms may have tested to one or more ESBL genes.

ESBLs and Cefotaxime

Among the 28 MDR bacteria samples, the frequency of ESBL genes identified with resistance to Cefotaxime, a 3rd generation cephalosporin, was the following: SHV (22.2%), both SHV/TEM (22.2%), TEM (38.9%) and both TEM/CTX-M (5.6%). ESBL gene TEM was the most common gene identified to be resistant to Cefotaxime.

Table 44: Frequency Distribution and Percentages of ESBLs in MDR isolates by Resistance Patterns to Cefotaxime

ESBL gene	Resistant (%)	Sensitive (%)	Total
Non-ESBL	6 (91.1%)	0 (0.0%)	6
SHV	4 (22.2%)	0 (0.0%)	4
SHV,TEM	4 (22.2%)	0 (0.0%)	4
TEM	8 (58.9%)	5 (100.0%)	13
TEM,CTX-M	1 (5.6%)	0 (0.0%)	1
CTX-M	0 (0.0%)	0 (0.0%)	0
Total	23	5	28

Total (*N*=28) MDR organisms may have tested to one or more ESBL genes.

ESBLs and Cefotetan

Among the 28 MDR bacteria samples, the frequency of ESBL genes identified with resistance to Cefotetan, was the following: SHV (17.4%), both SHV/TEM (17.4%), TEM (39.1%) and both TEM/CTX-M (4.3%). Based on these results, TEM was most commonly identified in association with resistance to Cefotetan.

Table 45: Frequency Distribution and Percentages of ESBLs in MDR isolates by Resistance Patterns to Cefotetan

ESBL gene	Resistant (%)	Sensitive (%)	NR (%)	Total
Non-ESBL	5 (21.7%)	0 (0.0%)	1 (33.3%)	6
SHV	4 (17.4%)	0 (0.0%)	0 (0.0%)	4
SHV,TEM	4 (17.4%)	0 (0.0%)	0 (0.0%)	4
TEM	9 (39.1%)	2 (100.0%)	2 (66.7%)	13
TEM,CTX-M	1 (4.3%)	0 (0.0%)	0 (0.0%)	1
CTX-M	0 (0.0%)	0 (0.0%)	0 (0.0%)	0
Total	23	2	3	28

Total (*N*=28) MDR organisms may have tested to one or more ESBL genes.

ESBLs and Cefoxitin

Among the 28 MDR bacteria samples, the frequency of ESBL genes identified with resistance to Cefoxitin, a 2nd generation cephalosporin, was the following: SHV (16%), both SHV/TEM (16%), TEM (40%) and both TEM/CTX-M (4%). Based on these results, TEM was most commonly identified in association with resistance to Cefoxitin.

Table 46: Frequency Distribution and Percentages of ESBLs in MDR isolates by Resistance Patterns to Cefotaxime

ESBL gene	Resistant (%)	Sensitive (%)	Total
Non-ESBL	6 (24.0%)	0 (0.0%)	6
SHV	4 (16.0%)	0 (0.0%)	4
SHV,TEM	4 (16.0%)	0 (0.0%)	4
TEM	10 (40.0%)	3 (100.0%)	13
TEM,CTX-M	1 (4.0%)	0 (0.0%)	1
CTX-M	0 (0.0%)	0 0.0%)	0
Total	25	3	28

Total (*N*=28) MDR organisms may have tested to one or more ESBL genes.

ESBLs and Ceftazidime

Among the 28 MDR bacteria samples, the frequency of ESBL genes identified with resistance to Ceftazidime, a 3rd generation cephalosporin, was the following: SHV (22.2%), both SHV/TEM (22.2%), TEM (38.9%) and both TEM/CTX-M (5.6%). ESBL gene TEM was the most common gene identified to be resistant to Cefazolin. Based on these results, TEM was most commonly identified in association with resistance to Ceftazidime.

Table 47: Frequency Distribution and Percentages of ESBLs in MDR isolates by Resistance Patterns to Ceftazidime

ESBL gene	Resistant (%)	Sensitive (%)	Total
Non-ESBL	6 (91.1%)	0 (0.0%)	6
SHV	4 (22.2%)	0 (0.0%)	4
SHV,TEM	4 (22.2%)	0 (0.0%)	4
TEM	8 (58.9%)	5 (100.0%)	13
TEM,CTX-M	1 (5.6%)	0 (0.0%)	1
CTX-M	0 (0.0%)	0 (0.0%)	0
Total	23	5	28

Total (*N*=28) MDR organisms may have tested to one or more ESBL genes.

ESBLs and Ceftriaxone

Among the 28 MDR bacteria samples, the frequency of ESBL genes identified with resistance to Ceftriaxone, a 3rd generation cephalosporin, was the following: SHV (22.2%), both SHV/TEM (22.2%), TEM (38.9%) and both TEM/CTX-M (5.6%). ESBL gene TEM was the most common gene identified to be resistant to Ceftriaxone.

Table 48: Frequency Distribution and Percentages of ESBLs in MDR isolates by Resistance Patterns to Ceftriaxone

ESBL gene	Resistant (%)	Sensitive (%)	NR (%)	Total
Non-ESBL	5 (86.1%)	0 (0.0%)	1 (50.0%)	6
SHV	4 (22.2%)	0 (0.0%)	0 (0.0%)	4
SHV,TEM	4 (22.2%)	0 (0.0%)	0 (0.0%)	4
TEM	8 (63.9%)	4 (100.0%)	1 (50.0%)	13
TEM,CTX-M	1 (5.6%)	0 (0.0%)	0 (0.0%)	1
CTX-M	0 (0.0%)	0 (0.0%)	0 (0.0%)	0
Total	22	4	2	28

Total (*N*=28) MDR organisms may have tested to one or more ESBL genes.

ESBLs and Cefuroxime

Among the 28 MDR bacteria samples, the frequency of ESBL genes identified with resistance to Cefuroxime, a 2nd generation cephalosporin, was the following: SHV (16.7%), both SHV/TEM (16.7%), TEM (37.5%) and both TEM/CTX-M (4.2%). Based on these results, TEM was most commonly identified in association with resistance to Cefuroxime.

Table 49: Frequency Distribution and Percentages of ESBLs in MDR isolates by Resistance Patterns to Cefuroxime

ESBL gene	Resistant (%)	Sensitive (%)	Total
Non-ESBL	6 (25.0%)	0 (0.0%)	6
SHV	4 (16.7%)	0 (0.0%)	4
SHV,TEM	4 (16.7%)	0 (0.0%)	4
TEM	9 (37.5%)	4 (100.0%)	13
TEM,CTX-M	1 (4.2%)	0 (0.0%)	1
CTX-M	0 (0.0%)	0 (0.0%)	0
Total	24	4	28

Total (*N*=28) MDR organisms may have tested to one or more ESBL genes.

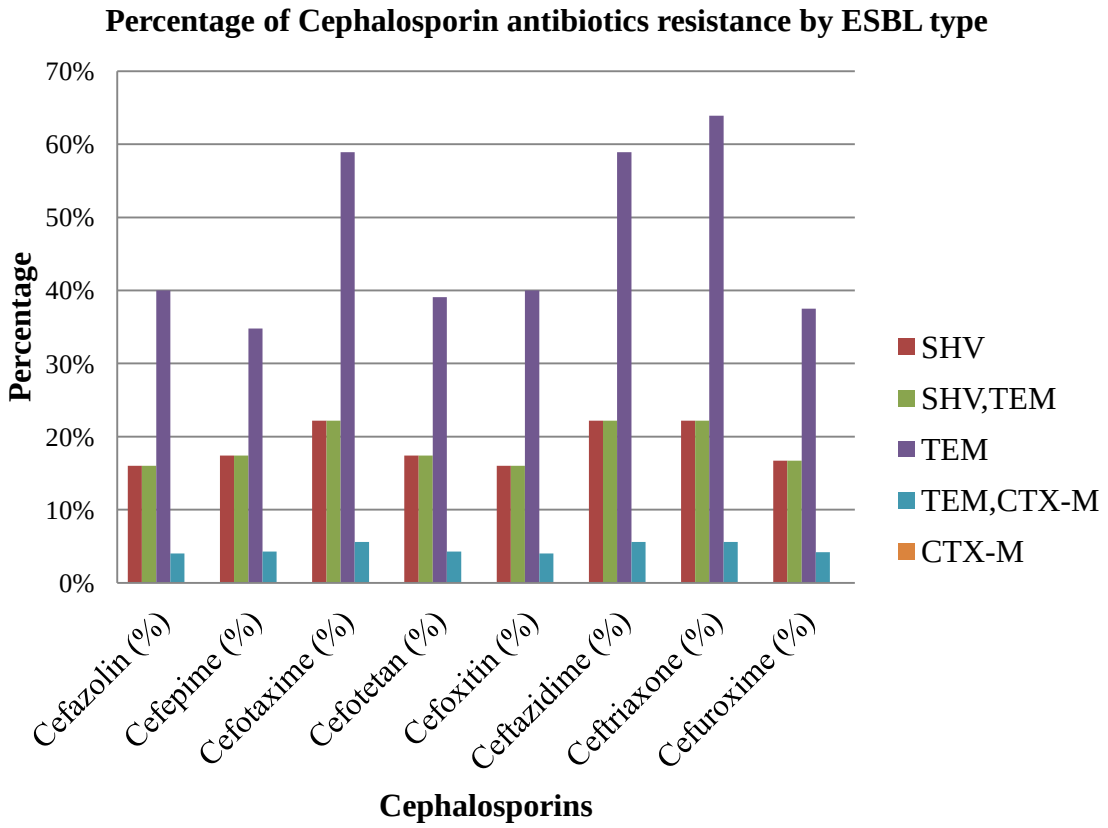

Figure 25: Percentages of different Cephalosporin antibiotics among bacterial isolates by ESBLs

Carbapenems

Among the 28 isolates analyzed, resistance to carbapenem derivatives was identified in the following antibiotics and antibiotic combinations: 23 isolates were resistant to Meropenem (82.1%), 25 isolates to Imipenem (89.3%) and 4 isolates to Ertapenem (14.3%). Based on these results, Meropenem and Imipenem were the carbapenem antibiotics with higher resistance among the MDR Gram-negative isolates. The following tables show the frequency distribution and resistance patterns of bacterial isolates to different Carbapenems.

Tables 50: Frequency Distribution and Percentages to Meropenem in MDR Isolates

	Frequency	Percent	Valid Percent %
Resistant	23	82.1	82.1
Sensitive	5	17.9	17.9
Total (*N*=28)	28	100.0	100.0

Tables 51: Frequency Distribution and Percentages to Imipenem in MDR Isolates

	Frequency	Percent	Valid Percent %
Resistant	25	89.3	89.3
Sensitive	3	10.7	10.7
Total (*N*=28)	28	100.0	100.0

Tables 52: Frequency Distribution and Percentages to Ertapenem in MDR Isolates

	Frequency	Percent	Valid Percent %
Resistant	4	14.3	14.3
Sensitive	1	3.6	3.6
NR	23	82.1	82.1
Total (*N*=28)	28	100.0	100.0

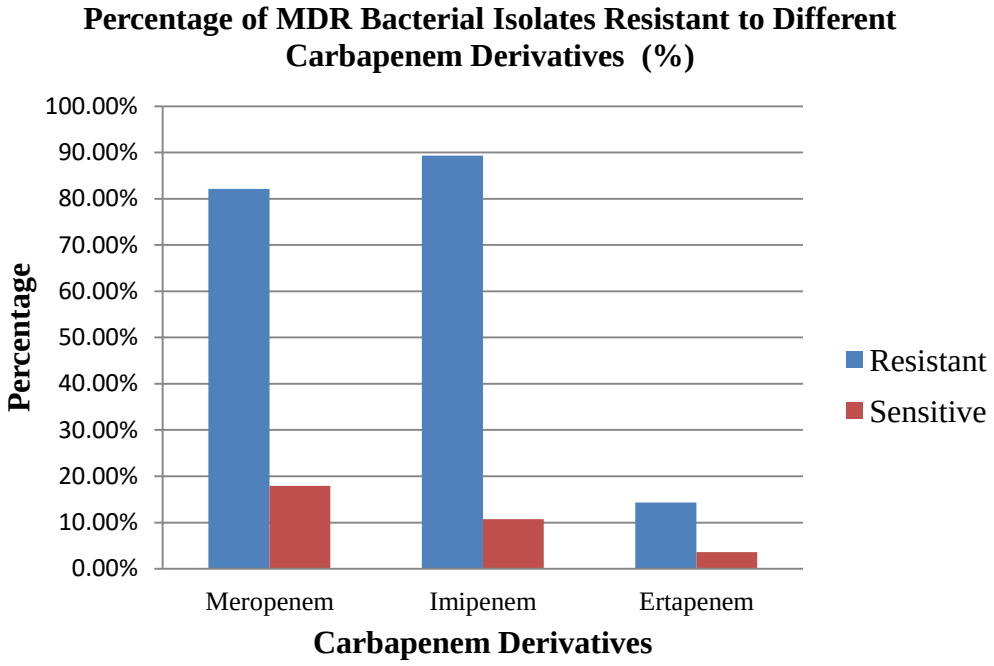

Figure 26: Percentages of antibiotic resistance to different carbapenem MDR isolates

ESBLs and Ertapenem

After analyzing the frequency distribution of antibiotic resistance to carbapenems among the recovered Gram-negative isolates with MDR patterns, this study now analyzed the frequency and association of each ESBL gene (TEM, SHV, CTX-M or a combination of them) in relation to each carbapenem derivative.

Among the 28 MDR bacteria samples, the frequency of ESBL genes identified with resistance to Ertapenem was the following: SHV (17.4%), both SHV/TEM (17.4%), TEM (47.7%) and both TEM/CTX-M (4.3%). Based on these results, TEM was most commonly identified in association with resistance to Ertapenem.

Table 53: Frequency Distribution and Percentages of ESBLs in MDR isolates by Resistance Patterns to Ertapenem

ESBL gene	Resistant (%)	Sensitive (%)	Total
Non-ESBL	6 (88.0%)	0 (0.0%)	6
SHV	4 (17.4%)	0 (0.0%)	4
SHV,TEM	4 (17.4%)	0 (0.0%)	4
TEM	12 (72.8%)	1 (100.0%)	13
TEM,CTX-M	1 (4.3%)	0 (0.0%)	1
CTX-M	0 (0.0%)	0 (0.0%)	0
Total	27	1	28

Total (*N*=28) MDR organisms may have tested to one or more ESBL genes.

ESBLs and Imipenem

Among the 28 MDR bacteria samples, the frequency of ESBL genes identified with resistance to Imipenem was the following: SHV (16%), both SHV/TEM (16%), TEM (40%) and both TEM/CTX-M (4%). Based on these results, TEM was most commonly identified in association with resistance to Imipenem.

Table 54: Frequency Distribution and Percentages of ESBLs in MDR isolates by Resistance Patterns to Imipenem

ESBL gene	Resistant (%)	Sensitive (%)	Total
Non-ESBL	6 (24.0%)	0 (0.0%)	6
SHV	4 (16.0%)	0 (0.0%)	4
SHV,TEM	4 (16.0%)	0 (0.0%)	4
TEM	10 (40.0%)	3 (100.0%)	13
TEM,CTX-M	1 (4.0%)	0 (0.0%)	1
CTX-M	0 (0.0%)	0 (0.0%)	0
Total	25	3	28

Total (*N*=28) MDR organisms may have tested to one or more ESBL genes.

ESBLs and Meropenem

Among the 28 MDR bacteria samples, the frequency of ESBL genes identified with resistance to Meropenem was the following: SHV (17.4%), both SHV/TEM (17.4%), TEM (34.8%) and both TEM/CTX-M (4.3%). Based on these results, TEM was most commonly identified in association with resistance to Meropenem.

Table 55: Frequency Distribution and Percentages of ESBLs in MDR isolates by Resistance Patterns to Meropenem

ESBL gene	Resistant (%)	Sensitive (%)	Total
None	6 (26.1%)	0 (0.0%)	6
SHV	4 (17.4%)	0 (0.0%)	4
SHV,TEM	4 (17.4%)	0 (0.0%)	4
TEM	8 (34.8%)	5 (100.0%)	13
TEM,CTX-M	1 (4.3%)	0 (0.0%)	1
CTX-M	0 (0.0%)	0 (0.0%)	0
Total	23	5	28

Total (*N*=28) MDR organisms may have tested to one or more ESBL genes.

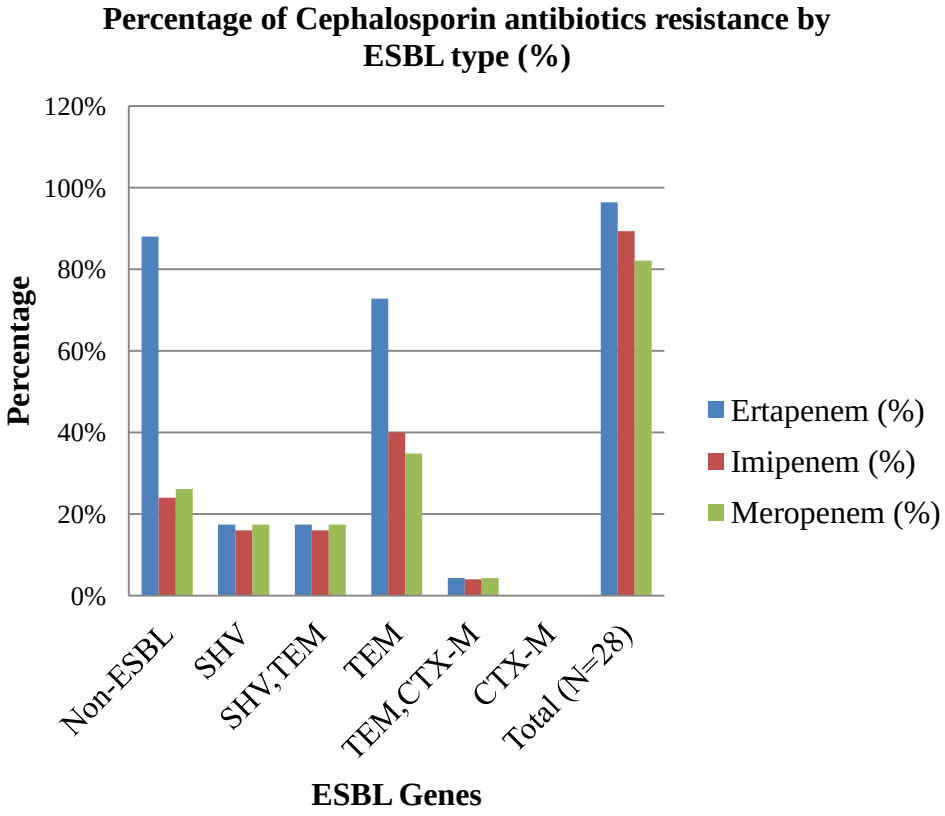

Figure 27: Percentage of ESBL genes among bacterial isolates with resistance to Carbapenems.

IV. Screening for Carbapenem Resistant (CRE) Enterobacteriaceae by Chromagar Culture of Bacterial Isolates Recovered from the Rio Grande River Water Samples

Frequency Distribution of CRE Identified in Bacterial Isolates from the Rio Grande River Water Samples

A total of 28 MDR bacteria samples were cultured with KPC Chromagar media to screen for the presence of CRE in the Rio Grande river. Among the 28 recovered isolates, the frequency of positive CRE containing bacteria was 11 (39.3%). Further molecular analysis with PCR to corroborate the presence of CRE genes is suggested to understand the impact of having CRE bacteria among water from the Rio Grande.

Table 56: Frequency Distribution and Percentages of CRE in MDR Bacterial Isolates

KPC	Frequency	Percemt	Valid Percent %
Negative	17	60.7	60.7%
Positive	11	39.3	39.3%
Total	28	100.0	

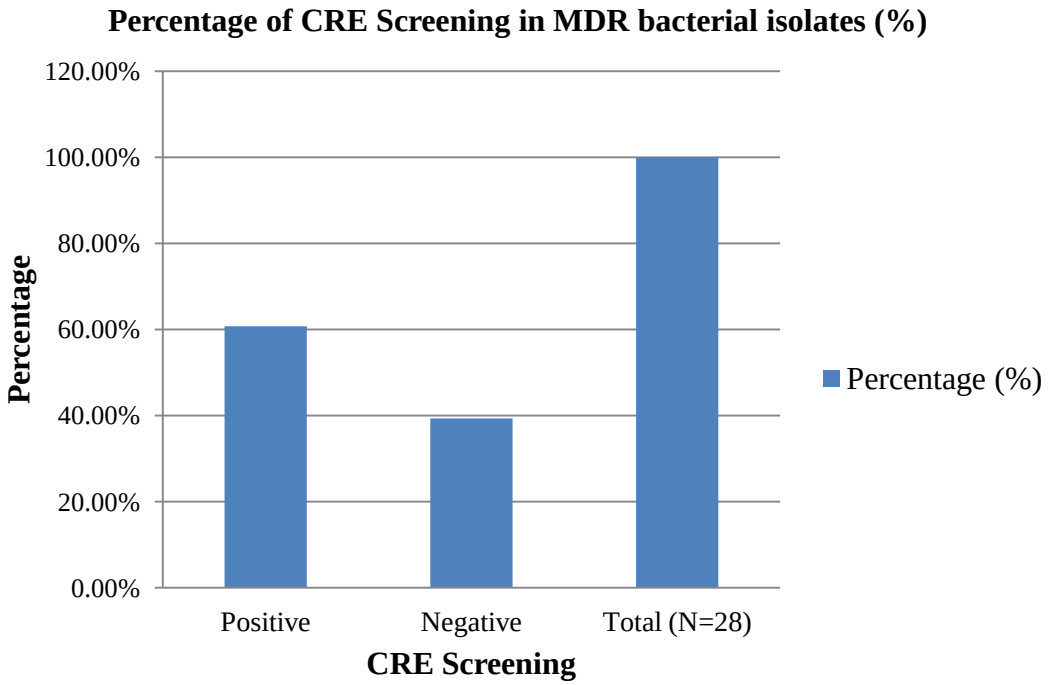

Figure 28: Percentages of Carbapenem Resistant (CRE) Enterobacteriaceae identified among Gram negative bacterial isolates identified as MDROs

Among the 28 recovered isolates, CRE was positively identified in the following strains: *Citrobacter* (100%), *Klebsiella oxytoca* (100%) and *Klebsiella pneumoniae* (88.9%) (Table 57)

Table 57: Frequency Distribution of CRE in Bacterial Isolates According to Genera

MDRO	Positive	Negative	Total (N=28)
A. hydrophilia	0%	100%	3.60%
C. davisae	0%	100%	3.60%
C. freundii	100%	0%	3.60%
E. coli	0%	100%	39.30%
K. oxytoca	100.00%	0.00%	7.10%
K. pneumoniae	88.90%	11.10%	32.10%
Leminorella sp.	0%	100%	3.60%
V. fluvialis	0%	100%	3.60%
V. parahaemolyticus	0%	100%	3.60%

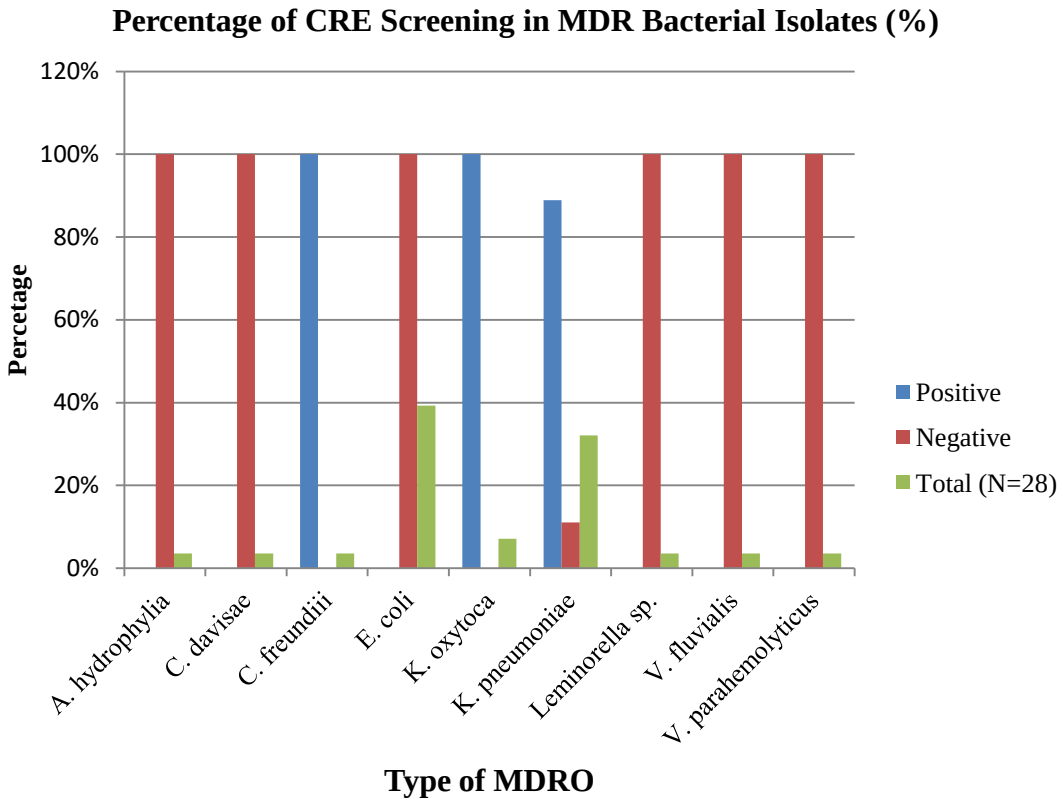

Figure 29: Percentages of Carbapenem Resistant (CRE) Enterobacteriaceae identified as positive among each type of bacterial isolates identified as MDROs

Among the 28 recovered isolates in which the screening for CRE was performed, CRE was positively identified in the MDR bacterial isolates found in the following water sampling locations from the Rio Grande river: Anapra/UTEP area (50%), Courchesne (50%) and near the Sunland Park WWTP (0%).

Table 58: Frequency Distribution and Percentages of CRE According to Sampling Locations

KPC	Anapra	Courchesne	Sunland	Total
Negative	8 (50.0%)	3 (50.0%)	6 (100.0%)	6
Positive	8 (50.0%)	3 (50.0%)	0 (0.0%)	4
Total	16	6	6	28

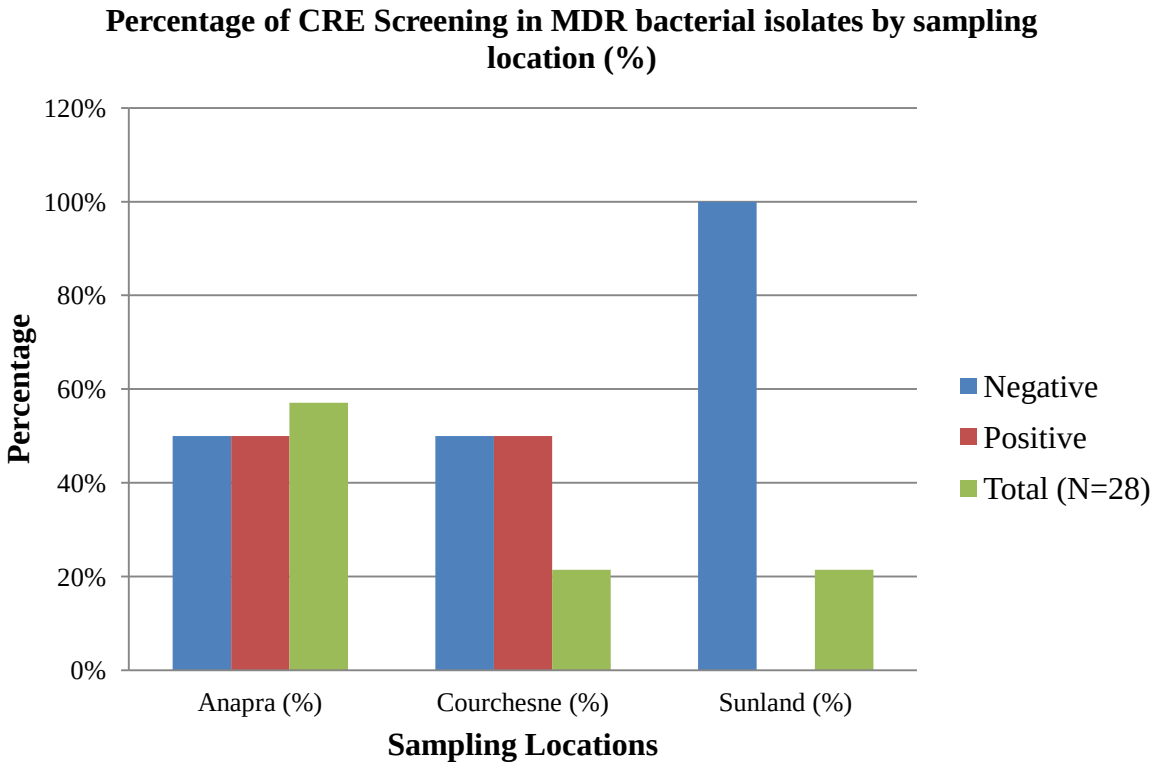

Figure 30: Frequency distribution of Carbapenem Resistant (CRE) Enterobacteriaceae identified as positive according to the location of samples from the Rio Grande River.

Discussion

Overview

The prevalence of infections caused by multidrug resistant organisms (MDROS) continue to increase and represent a serious public health threat at a global scale due to multiple factors including: overuse and misuse of antibiotics and self-medication, globalization of commercial food (cattle, poultry, swine) treated with antibiotics as growth promoters and preventive measures for safety, failure of strict hygiene control among health workers, international traveling that allows easy spread of resistant organisms, and prolonged exposure to antibiotic at hospitals, nursing home residencies and diagnostic procedures. While these factors are well known to be contributing to the problem of antibiotic resistance, there are also other anthropogenic factors that could also be contaminating our environment (water resources) with MDROs and pharmaceutical residues at a major scale causing a more rapid dissemination of resistance among bacteria that require a complete understanding and a fast approach in the management of health policies.

El Paso, TX is a city located at the far western corner of the state of Texas in the United States. It stands on the Rio Grande river across the Mexico-United States border from Cd. Juarez, Mexico and, it is also a neighbor city and state to Las Cruces, NM. El Paso, Las Cruces and Juarez are often referred to as Paso del Norte, a region close to a population of 3 million people. The Rio Grande river, being the 5th longest river in the U.S., provides the major source of water to this Texas-Mexico border region receiving an average of 17.7cm of rain per year.

Being the main source of water for the community of El Paso-Juarez area, water of the Rio Grande is constantly monitored by the International Boundary and Water Commission /

Clean Rivers Project (USIBWC/CRP). Under the Clean Water Act they are responsible to collect water quality data and help local water authorities be aware if problems of contamination or pollution exist. Among the data that they analyze includes physical, chemical, biological, metrics and habitat. In regards to the biological and possible contamination by bacteria that could represent a threat to health includes the MNP, which is the used as a fecal indicator for surface water samples and gives the most probable number of *E coli* found in 100ml of water sample.

Possible factors that have been considered to contribute to the microbial burden and release of antibiotics into the river and the source of drinking water for the region of Paso del Norte include: cattle farming and ranching activities along the river, failure in the management of waste-water treatment plants and septic disposal systems of the region, animal feeding operations and urbanization.

The purpose of this project, which was funded by the Edward N. and Margaret Marsh Foundation, was to isolate, identify and determine the presence of ESBL gene (SHV, TEM and CTX-M) producing bacteria and mobile genetic elements (integrons) in water from a 26km segment of the Rio Grande river that could be contributing to the burden of antibiotic resistance along the U.S. – Mexico border region and represent a possible contaminant to the community's water resources.

ESBLS and Integrons in the Rio Grande River Water Samples

Water samples were collected from different locations from the Rio Grande river during five different time periods throughout the year (2017). Among 15 water samples collected through the months of February (3), April (3), July (3), September (3) and December (3), a total of 11 tested positive (73.3%) for the presence of ESBL (SHV, TEM and CTX-M) resistant

genetic markers. From these, 7 (46.7%) were positive for CTX-M gene and 9 samples (60%) were positive for the presence of TEM genes identified directly from water samples. SHV was not identified in any of the water samples.

TEM is the most common plasmid-mediated β-lactamase associated with 90% of ampicillin resistance among Gram negative bacteria including *E. coli* and *Klebsiella* species, and it was also the most prevalent gene identified in all the water samples from the Rio Grande River. Although there are over 140 TEM-enzymes currently identified around the world, being TEM-10, TEM-12 and TEM 26 the most common in the United States. Still subtype phenotype classification was not included in this study, only a general identification of the ESBL genes.

CTX-M was the other gene that was successfully identified in water samples from the Rio Grande. This gene has been proven to be the most successful disseminating worldwide in a shorter time and associated with *E. coli* infections among the community and a threat for public health. ESBL gene CTX-M is also the most common in Latin America. It is worth mentioning that while TEM genes were identified in water samples from all sampling sites from the Rio Grande river and all different time periods, with no difference during the irrigation seasons, the gene CTX-M was identified also on all three locations but only during the non-irrigation season months of February and December.

Mobile genetic elements play an important role in spreading resistant genes and can be easily found in the environment. As previously mentioned, Class I integrons are most commonly found in polluted environments and well established to be found in Gram negative microorganisms with a major role in the distribution of antimicrobial resistance gene cassettes. Class 1 integrons were positively identified in this environmental study. Among the water samples collected throughout the year a total of 11 samples were successfully (73.3%) identified

for the presence of both integrons Int-1 (Int-1AF/AR and Int-1BF/BR) and Int-2 (Int-2 AF/AR and Int-2 BF/BR) in all sampling sites along the Rio Grande. From these, mobile genetic element Int-1 (46.7%) was the most commonly identified in as many as half of all the samples which is associated with polluted environments and conferring resistance to β-Lactam antibiotics. Mobile genetic elements integrons Class II were less common or not as easy to identify in the environment; Int-2 AF/AR (6.7%) and Int-2 BF/BR (20%). These results are compatible to literature found in that Class I integrons are the most commonly found in polluted environments and also in Gram negative bacteria. Although this study did not perform a molecular analysis of integrons in bacterial isolates, the most common bacteria found in this research was *E. coli, K. oxytoca, K. pneumoniae, Citrobacter* and *A. hydrophila*, which were also strongly associated with the use of class 1 integrons for the spreading of resistance among them.

ESBL Genes and MNP in MDR Bacterial Isolates

Water samples collected from the Rio Grande River were also sent to El Paso Community College Department of Biology where a total of 310 isolates were recovered and processed by the MicroScan AutoScan 4 system. From these, 142 (identified with a probability of correct identification varying from 92-99.99%) belonged to 18 different genera of bacteria. A total of 91 isolates (64%) had resistance to at least two or more synergistic combinations including Amox/K Clav, Amp/Sulbactam, Pip/Tazo, Ticar/K Clav, and Trimeth/Sulfa, and 105 isolates (74%) were resistant to more than four individual antibiotics. Eleven *E. coli* and *Klebsiella* isolates were identified as ESBLs by the Microscan AutoSCAN 4 system and were also resistant to more than 20 individual antibiotics. A total of 28 Gram negative bacterial

isolates were identified as possible ESBLs and were selected for molecular analysis for ESBL genes (SHV, TEM and CTX-M) and CRE screening in this study.

Among the 28 bacterial isolates analyzed, 4 (14.3%) were identified as SHV positive and 13 (46.4%) as TEM positive; 4 (14.3%) were positive for both SHV and TEM and only 1 (3.6%) was identified positive for both TEM and CTX-M. Only 6 (21.4%) were not identified as being ESBL positive through PCR on this study. Also, Anapra (57.1%) was the most prevalent site for carrying ESBL resistant bacteria vs. Courchesne (21.4%) and Sunland park area (21.4%). This represents a public health hazard as it is a common recreational place for people living in Juarez, Mexico.

According to the literature, it was mentioned that TEM is strongly associated with up to 90% ampicillin resistance patterns in *E. coli, K. pneumoniae, H.influenza* and *N. gonorrheae* and SHV up to 20% resistance also to ampicillin in *E coli* and *Pseudomonas aeruginosa*. Since both genes share as much as 68% of their molecular structure it supports the findings of this study. SHV and TEM were the most common ESBL genes identified in bacterial isolates from the Rio Grande water samples.

The bacteria isolates that were positive for SHV gene were all identified as *K. pneumoniae.* Bacteria strains that were positive for TEM gene were more diverse and included the following: *V. fluvialis, A. hydrophilia, E. coli, C. davisae, C. freundii complex, K. oxytoca and K. pneumonia*. For CTX-M gene the only strain that was positive was *K. oxytoca*.

The El Paso Community College Department of Biology also analyzed MPN, which is used as a fecal indicator for surface water samples. It is a statistical number which gives the most probable number of *E.coli* in 100 ml of water. The standard is 126 MPN/100 ml for this

segment of the river which is set by the Water Quality Standards Team at Texas Commission on Environmental Quality (TCEQ).

During the months of February, April, and September all samples were above the standard set by the TCEQ. In the month of December and possibly due to the lower temperatures, MPN values were much lower and Sites 2 and 3 were within the standard. Throughout the year the fecal indicator for water appear to differ according to the season, which could be related to irrigation season which lasts from April to September aimed to preserve groundwater from the Mesilla and Hueco aquifers. Still, the average of *E. coli* identified in all samples throughout the year is higher than expected by the Water Quality Standards Team at Texas Commission on Environmental Quality (TCEQ) representing a concern to human health to the people that have recreational activities at the river, including children that swim during the summer.

The Rio Grande provides the major source of water to both sister cities, El Paso, TX and Juarez, Mexico. On the Anapra area, which is across the University of Texas at El Paso, as well as Courchesne water distribution is mainly supplied by the Rio Grande through the Robertson/Umbenhauer Water Treatment Plant during the months of March and September. These months are considered to be part of the irrigation season where the levels of *E. coli* MNP also appear to reach a highest peak on April. The other months (with a year round access) water is supplied by the Upper Valley Well Field. The last report by El Paso Water on this treatment plant includes physical and chemical parameters to monitor water safety and they showed to meet standards established by the Clean Water Act on all WTPs. Although this analysis is pre-treatment and there is no other data to compare results after treatment, a pre and post evaluation is suggested for a better understanding of the problem.

Antibiotic Resistance Patterns in MDR Bacterial Isolates

A total of 28 Gram negative bacterial isolates, mostly *E. coli* and *K. pneumoniae*, were analyzed for resistant/sensitive patterns to different antibiotics and their association to ESBL genes. Antibiotic analysis included a wide variety, not only β-Lactam derivatives, of antimicrobials used to treat Gram negative infections. This study includes genetic analysis of ESBL genes only to: penicillins, cephalosporins and carbapenems. TEM was the ESBL gene with a higher prevalence of resistance among all the antimicrobial groups and consistent with the literature as being the most common worldwide.

Resistance to different antibiotics was successfully identified in this study. Among the 28 isolates analyzed, resistant patterns to different penicillin derivatives commonly used in the community included: amoxicillin/clavulanate, ampicillin alone or in combination with sulbactam, ticarcillin/clavulanate and Piperacillin alone or in combination with tazobactam. Although not recommended as the primary treatment for ESBL infections, cephalosporin derivatives also showed high levels of resistance patterns among the MDR bacterial isolates included in this study. Carbapenems are considered the last antibiotic of choice against severe infections caused by ESBL producing organisms. Ertapenem was the carbapenem with higher levels of resistance. All the β-Lactam antibiotics included in this study showed TEM as the ESBL gene to be the most common associated to resistance among all the bacterial isolate and class I integron as the primary mechanism of resistance spreading. According to the classification of Bush & Jacoby, the functional classification of TEM, SHV and CTX-M belongs to class 2, which is characterized by an enzymatic inhibition to clavulanic acid, such as sulbactam and tazobactam. The β-Lactam inhibitor combinations (amoxicillin/clavulanate, ampicillin/sulbactam, piperacillin/tazobactam and ticarcillin/clavulanate) included in this study showed high resistance prevalence among the

MDR isolates with a phenotypic characterization positive of TEM and SHV with a very low response to these β-Lactam inhibitor combinations.

Strengths and Limitations

As of this study, there was no previous research registered that identified that presence of ESBL resistant organisms and mobile genetic elements in the environment along the El Paso, TX and Juarez, Mexico border region. This study establishes the importance of developing a new model in the assessment of water quality to include analysis for antibiotic resistant bacteria and reduce its possible contribution to this global public health concern that could be included as part of the USIBWC/CRP under the Clean Water Act and EPA.

Even though it was a 26-km segment of the Rio Grande river the sampling site was still small-scale and have no previous research or controls to compare. For further research studies, it is ideally advised to take samples from the beginning of the river from Alamosa, Colorado to fully understand the changes in the environment (urbanization and anthropogenic features) and its active role on antibiotic resistant bacteria and mobile genetic elements that could help develop new preventive measures and policies.

Phenotypic characterization of ESBL markers included TEM, SHV and CTX-M but not of the subtypes in the region. This characterization has clear implications regarding spread of infection and infection control.

Implications to Public Health

The findings of this research help understand the health concerns for the communities of El Paso, TX and Juarez, Mexico region, even the Sunland Park region of New Mexico. Some of

the consequences for human health of antimicrobial resistance in bacteria and residues in the Rio Grande River are not known, but may include: increased number of infections, increased frequency of treatment failure, increased severity of infections and an increase in allergies in intestinal flora.

Conclusions

Results from this study showed that water from the Rio Grande river carry antibiotic resistant bacteria and mobile genetic elements during the irrigation and non irrigation seasons indicating a polluted environment from the community's main water resource. The presence of ESBL genes was successfully identified in water from the Rio Grande throughout the year being ESBL genes TEM and CTX-M the most prevalent and Class I integrons as the most common mobile genetic elements that easily spread resistance within the environment. Results of this study are similar to published literature.

This study also identified ESBL resistance patterns among some of the most common β-Lactam antibiotics used to treat Gram negative associated infections. For Penicillins, Carbapenems and Cephalosporins, the ESBL gene TEM was the most prevalent resistant gene identified along the El Paso, TX- Juarez, Mexico border region. In regards to location, Anapra showed to be the highest in E coli concentrations and MDR patterns. Screening for CRE was also positive among bacterial isolates. Although ESBLs and CRE positive bacteria are considered to be a threat under the CDC, some of the recommendations include a report to state health authorities when identified. As of this study, there are no reports in the area of El Paso, TX-Juarez, Mexico or a surveillance system of these MDROs. Further analysis with a focus to the community and our water resources are highly recommended in order to understand the

public health implications that this could have in the near future and immediate action in policy and disease prevention among health professional is needed.

MPH Core Competencies

This study included the following core competencies of the Masters in Public Health Program from the University of Texas at El Paso:

Environmental Health Sciences

- Describe the direct and indirect human, ecological and safety effects of major environmental and occupational agents.
- Describe genetic, physiologic and psychosocial factors that affect susceptibility to adverse health outcomes following exposure to environmental hazards.
- Describe federal and state regulatory programs, guidelines and authorities that control environmental health issues.
- Specify current environmental risk assessment methods.
- Specify approaches for assessing, preventing and controlling environmental hazards that pose risks to human health and safety.
- Explain the general mechanisms of toxicity in eliciting a toxic response to various environmental exposures.

Biostatistics

- Distinguish among the different measurement scales and the implications for selection of statistical methods to be used based on these distinctions.

- Apply descriptive techniques commonly used to summarize public health data. 6. Apply common statistical methods for inference.
- Apply descriptive and inferential methodologies according to the type of study design for answering a particular research question.
- Apply basic informatics techniques with vital statistics and public health records in the description of public health characteristics and in public health research and evaluation.
- Interpret results of statistical analyses found in public health studies.
- Develop written and oral presentations based on statistical analyses for both public health professionals and educated lay audiences.

Health Policy and Management

- Identify the main components and issues of the organization, financing and delivery of health services and public health systems in the US.
- Discuss the policy process for improving the health status of populations.
- Apply the principles of program evaluation in organizational and community initiatives.
- Apply quality and performance improvement concepts to address organizational performance issues.
- Communicate health policy and management issues using appropriate channels and technologies.
- Demonstrate leadership skills for building partnerships.

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Appendix

Drinking Water Analysis

Figure 3: Drinking Water Chemical Analysis. Source: El Paso Water (2017)

DRINKING WATER ANALYSIS

Substance (Units)	Sample Year	Average Level	Minimum Level	Maximum Level	MCL	MCLG	Possible Source
Turbidity							
Turbidity (NTU)	2016	N/A	100%[1]	0.28	Treatment Technique	N/A	Soil runoff
Inorganics							
Antimony (ppb)	2016	0.09	0	0.3	6	6	Discharge from petroleum refineries, retardants, ceramics
Arsenic (ppb)	2016	4.9	0	10.7[2]	10	0	Erosion of natural deposits
Barium (ppm)	2016	0.09	0.04	0.18	2	2	Erosion of natural deposits
Chromium (ppb)	2016	2.82	1	6.60	100	100	Erosion of natural deposits
Selenium(ppb)	2016	1.90	0	5	50	50	Erosion of natural deposits
Fluoride (ppm)	2016	0.75	0.708	0.794	4	4	Erosion of natural deposits
Nitrate as Nitrogen (ppm)	2016	1.01	0.058	2.46	10	10	Runoff from fertilizer use
Gross Alpha (pCi/L)	2015	4.2	0	10.3	15	0	Erosion of natural deposits
Gross Beta (pCi/L)	2015	9.4	0	17.7	50	0	Decay of natural and man-made deposits
Combined Radium (pCi/L)	2015	0.09	0	1.2	5	0	Erosion of natural deposits
Lead and Copper							
Copper (ppm)	2016	0.12[3]	0.012	0.16	Action Level = 1.3	1.3	Corrosion of household plumbing systems
Lead (ppb)	2016	1.0[3]	0	8.0	Action Level = 15	0	Corrosion of household plumbing systems
Coliform Bacteria							
Total Coliform Bacteria	2016	N/A	0.0%	0.4%	5%	0	Naturally present in the environment
Disinfection Residual							
Chlorine (ppm)	2016	N/A[4]	N/A[4]	2.2	4[5]	4[6]	Water additive used to control microbes
Chlorine Dioxide (ppb)	2016	N/A[4]	N/A[4]	670	800[5]	800[6]	Water additive used to control microbes
Disinfection Byproducts							
Bromate (ppb)	2016	N/A[4]	N/A[4]	8.8	10	0	By-product of drinking water disinfection
Chlorite (ppm)	2016	N/A[4]	N/A[4]	0.316	1	0.8	By-product of drinking water disinfection
Total Haloacetic Acids (THAA) (ppb)	2016	12.3[8]	0	28.6	60	N/A	By-product of drinking water disinfection
Total Trihalomethanes (TTHM) (ppb)	2016	30.9[7]	0	66.5[9]	80	N/A	By-product of drinking water disinfection
Total Organic Carbon							
(Removal Ratio)	2016	2.49	2.08	3.02	Treatment Technique[10]	N/A	Naturally present in the environment
Unregulated Contaminants[11]							
Bromodichloromethane (ppb)	2016	10.52	0	21.7	N/A	0	By-product of drinking water disinfection
Chloroform (ppb)	2016	7.95	0	17.2	N/A	70	By-product of drinking water disinfection
Bromoform (ppb)	2016	4.70	0	11.8	N/A	0	By-product of drinking water disinfection
Dibromochloromethane (ppb)	2016	11.54	0	23.5	N/A	60	By-product of drinking water disinfection

Chemical Analysis of El Paso Water

CITY OF EL PASO
EL PASO WATER
CHEMICAL ANALYSIS – CITY WATER

(All results expressed in milligrams per liter, mg/l)

PARAMETER TESTED	WATER SOURCE LOCATION							MAXIMUM LEVEL PERMITTED
	UPPER VALLEY	DOWNTOWN CENTRAL	NORTHEAST	AIRPORT CENTRAL	FORT BLISS AIRPORT	LOWER VALLEY	LOWER VALLEY CENTRAL	
	UPPER VALLEY WELL FIELD SUPPLIES WELL WATER TO THE UPPER VALLEY WTP *YEAR-ROUND*	ROBERTSON/ UMBENHAUER WTP SUPPLIED BY RIO GRANDE *MAR - SEPT*	NORTHEAST WELL FIELD WELL WATER BLENDED INTO DISTRIBUTION *OCT - FEB*	AIRPORT WELL FIELD WELL WATER BLENDED INTO DISTRIBUTION *OCT - FEB*	FORT BLISS & AIRPORT WELL FIELDS SUPPLY WELL WATER TO KBH DESAL PLANT *YEAR-ROUND*	JONATHAN ROGERS WTP SUPPLIED BY RIO GRANDE *MAR - SEPT*	LOWER VALLEY & CENTRAL WELL FIELDS SUPPLY WELL WATER DIRECTLY INTO DISTRIBUTION *OCT - FEB*	
	SOURCE 1	SOURCE 2	SOURCE 3	SOURCE 4	SOURCE 5	SOURCE 6	SOURCE 7	
	AVG	*AVG*	*AVG*	*AVG*	*AVG*	*AVG*	*AVG*	
Total Dissolved Solids	630	650	470	627	454	700	862	1000 mg/l
Phenol Alkalinity as $CaCO_3$	3.8	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	Not established
Total Alkalinity as $CaCO_3$	121	130	115	110	24.5	133	125	Not established
Total Hardness as $CaCO_3$	119	235	149	168	63	230	236	Not established
Chlorides as Cl	117	107	177	195	182	133	283	300 mg/l
Sulfates as SO_4	189	191	71	104	14	182	160	300 mg/l
Fluorides as F	0.59	0.58	0.87	0.68	NA	0.62	0.55	4.0 mg/l
Silica as SiO_2	36	20	32	31	NA	20	29	Not established
Nitrates as NO_3	<0.10	0.41	1.96	1.6	NA	2.1	0.95	10 mg/l
Nitrites as NO_2	<0.05	<0.05	<0.05	<0.05	NA	<0.05	0.06	1.0 mg/l
Phosphates as PO_4	<0.05	0.55	<0.05	0.07	0.51	0.68	0.14	Not established
Calcium as Ca	42	71	43	43	14	71	78	Not established
Magnesium as Mg	5.5	15	11.3	13	3.9	15	21	Not established
Sodium as Na	168	130	105	153	109	133	217	Not established
Potassium as K	3.6	7.9	7.3	10.4	4.5	8.6	9.4	Not established
Iron as Fe	<0.020	<0.020	<0.020	<0.020	<0.03	<0.03	0.027	0.3 mg/l
Manganese as Mn	0.050	0.012	<0.010	<0.010	NA	<0.010	0.016	0.05 mg/l
pH	8.1	7.3	7.7	7.9	7.5	7.3	7.6	pH 6.5-8.5

The city is supplied by seven distinct blended sources that are interconnected in the distribution system and it is possible that the water from one source can be distributed to other parts of the city. Normally, the distribution of these sources to the city's areas is as follows: **Upper Valley and Northwest – Source 1; West – Sources 1 & 2; Downtown Central – Sources 2, 4, & 7; Airport Central – Sources 2 & 4; Northeast – Sources 3, 5, & 6; East – Sources 4, 5 & 6; Far East – Sources 3, 5, & 6; and Lower Valley – Sources 4, 6, & 7.** The areas supplied by each of these sources are as indicated by the attached distribution maps on a seasonal basis. However, the ratios of water supplied by one source or another are constantly changing and therefore intermixing of water within these areas is possible.*

Figure 4: Last Report of El Paso Water Chemical Analysis (Source: El Paso Water, 2017)

Water Distribution of El Paso Water

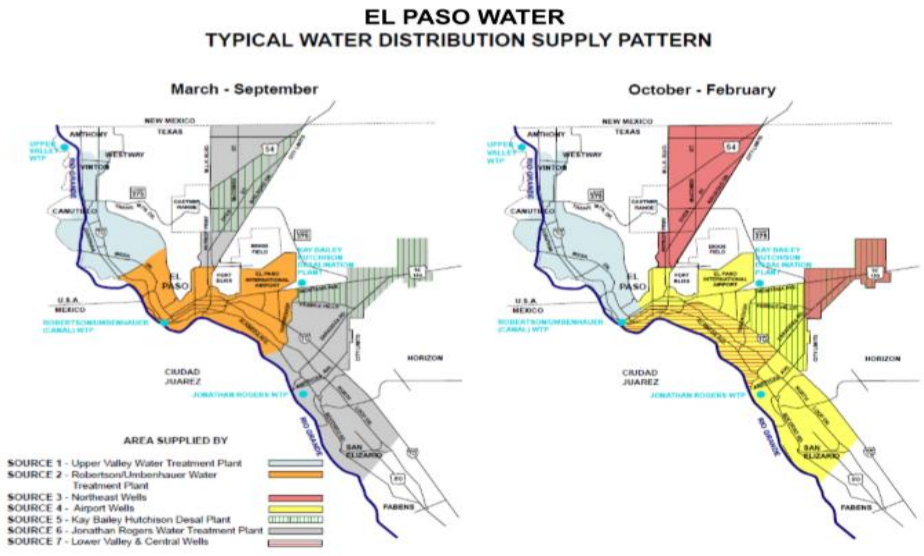

Figure 5: Water Distribution in El Paso, TX (Source: El Paso Water, 2017).

IBC Protocol

The University of Texas at El Paso
Institutional Biosafety Committee
New Protocol Form

Instructions: Forms need to be completed and submitted via IRBNet prior to the 5th of each month to be considered for review at the IBC meeting that month. Submissions entered after the 5th of each month will be considered for review at the following month. Meeting dates are posted on the IBC website. Any questions contact the IBC office at ibc@utep.edu.

A. PROJECT INFORMATION

SUBMISSION TYPE

⊗	**Initial or Triennial Submission**	☐	**Modification/Amendment** Make any changes in ***red italicized font*** within the most updated protocol

ADMINISTRATIVE DATA

Principal Investigator	DELFINA DOMINGUEZ (MENTOR) MARIA FUENTES (MPH STUDENT)	Lab/Office Phone: Lab Room Number:	
Department:	DEP. OF PUBLIC HEALTH (UTEP)	Emergency Phone: Back-up Emergency #	
Email Address:	DELFINA@UTEP.EDU / MFUENTES5@MINERS.UTEP.EDU		
Project Title:	"A STUDY OF EPIGENETIC FACTORS AFFECTING ANTIBIOTIC RESISTANCE OF BACTERIA FROM THE RIO GRANDE RIVER IN THE EL PASO, TX-CD. JUAREZ, MEXICO BORDER."		
Funding Source:	☐ NSF ☐ NIH/PHS ⊗ Other (include account #):		
Grant Title:	THE EDWARD N. AND MARGARET G. MARSH FOUNDATION		
Grant Proposal Number:			
IACUC Protocol #: **If applicable**		IRB Protocol number: **If applicable**	

B. PROJECT INFORMATION:

1.1a Closing Summary (If the project is being renewed (*de novo*), please include a summary of the project for the last three years. Describe in non-technical lay terms the purpose of the project):

1.1b Summary (Describe in non-technical lay terms the purpose of the project for the next three years):

To assess the extent of antimicrobial resistant bacteria populations through the identification of genes for Extended sprecturm Beta-Lactamases (ESBL) bacteria in surface water of the Rio Grande River in the El Paso, Texas/Cd. Juarez, Mexico border region (Courchesne, Anapra and Riverbend).

1.2 What do you believe is the required Biosafety Containment Level (e.g., BSL1, BSL2, BSL3)?

BSL2

1.3 List and describe the organisms to be used in your project. Include bacteria, parasites, viruses, cell lines, animals and toxins. Include specific name, strain, sub-species or serotype as necessary:

Check all that apply:	MATERIALS USED:	List/Describe:
☐	Genetically modified animal, plant, or insect	
☐	Parasites or insects	
☐	Whole Plant	
☒	Bacteria	ESBL resistant E. coli, other bacteria unknown
☐	Fungi	
☐	Viruses	
☐	Recombinant or Synthetic Nucleic Acid Molecules	
☐	Human or NHP blood, bloodborne pathogens, bodily fluids, blood products, tissue, or cells (including cell cultures), list.	
☒	Environmental samples (e.g., soil, water, sewer, air), list type and source	Water collected from the Rio Grande River (Anapra, Courchesne and Riverbedn areas) along the border c TX and Juarez, Mexico.
☐	Select Agents:	
☐	Other:	

1.4 Do you intend to ship infectious substances or hazardous chemicals? If YES, please, contact Environmental Health and Safety (EH&S x7124) to make arrangements.	YES	☐
	NO	☒
1.5 Would this research project demonstrate how to render a vaccine ineffective or confer resistance to therapeutically useful antibiotics/antiviral agents?	YES	☐
	NO	☒
1.6 Would this project potentially increase transmissibility of a pathogen or potentially enable the evasion of diagnostic/detection modalities of an agent?	YES	☐
	NO	☒
1.7 Would this project alter the host range of a pathogen or potentially enable the weaponization of a biological agent or toxin?	YES	☐
	NO	☒

C. WILL YOUR PROJECT INVOLVE:

☐	Creation of transgenic animals, organisms, recombinant or synthetic DNA **(complete Section II below)**
☐	Use or creation of lentiviral vectors **(complete APPENDIX A)**
☒	Risk Group 2 Organisms **(complete APPENDIX B)**
☐	Risk Group 3 Organisms **(complete APPENDIX C)**
☐	CRISPR- Genome Editing Technologies **(complete APPENDIX D)**
☐	NIH Exemption **(complete APPENDIX E)**

D. LAB SAFETY, DECONTAMINATION AND DISPOSAL:

Check the disinfectants used for surface decontamination and spills:

☐	**Cavicide**	☒	**Bleach**	☐	**Other:**
☐	**Vesphene**	☒	**70% Alcohol**		

Check the protective clothing or equipment used when handling this agent(s):

☒	Gloves	☐	Double Gloves	☐	Lab Coat
☐	Booties	☐	Double booties	☒	Closed front gown
☐	Surgical mask	☒	Safety Glasses	☐	
☐	PAPR	☐	Hair cover	☐	Centrifuge ☐
☐	N95 respirator	☐	Grinder	☐	Splash shields
☐	Sharps containers	☐	Blunt ended forceps/scissors	☐	Face shied
☐	Ear plugs	☐	Capillary tubes	☐	Safety needles/scalpel
☐	Sonicator	☐	Cell sorter	☐	Other:
☐	Chemical Fume Hood, Rm. Location:	☒	Biological Safety Cabinet, Rm location: HSSN RM #471	☐	Comments:

Check the method of biohazardous waste disposal:

☐	Placed in **single** red biohazard bag and autoclaved
☐	Placed in **double** red biohazard bag and autoclaved
☒	Chemical disinfection then placed in red biohazard bag
☐	Chemical disinfection of bulk liquid then poured down sanitary sewer

☐	Autoclaved and placed in bag for incineration

E. Study Personnel

Personnel listed on the project:

Please list the names of the individuals who are covered under this protocol. Include their full name, check off their role in the study, and include their UTEP e-mail address.

Project Team Members – Identify each current person involved in the design, conduct, or reporting of the research

Name: *First and Last Name*	**E-Mail:** *Mostly used*	**Team members role on the Project:** 1. Principal Investigator 2. Co-investigator 3. Student 4. Faculty 5. Staff 6. Outside Collaborator **Check all that apply**						
		1	**2**	**3**	**4**	**5**	**6**	**Experience:**
Delfina Dominguez	delfina@utep.edu	☒	☐	☐	☒	☒	☐	Clinical/molecular microbiologist (UTEP)
Maria Fuentes	mfuentes5@miners.utep.edu	☒	☐ ☒	☒ ☒	☐	☐	☐	Physician/graduate student of MPH (UTEP)
Luis Cruz	lacruz4@miners.utep.edu	☐	☐	☐	☐	☐	☐	CLS (UTEP)
		☐	☐	☐	☐	☐	☐	
		☐	☐	☐	☐	☐	☐	
		☐	☐	☐	☐	☐	☐	
		☐	☐	☐	☐	☐	☐	
		☐	☐	☐	☐	☐	☐	
		☐	☐	☐	☐	☐	☐	
		☐	☐	☐	☐	☐	☐	

IMPORTANT: If project personnel is greater than 10 individuals, please add additional rows.

1.8 Has your staff read the entire protocol?	YES	☒ ☐
	NO	☐
1.9 Have you educated your staff regarding safe handling and decontamination procedures for all of the agents or materials listed in the protocol?	YES	☒ ☐
	NO	☐

Personnel removed from the project (if applicable):

Please list the names of the individuals who are no longer involved in this protocol and needs to be removed. Include their full name, email, and reason of removal.

Project Team Members – Identify each current person involved in the design, conduct, or reporting of the research

Name: *First and Last Name*	**E-Mail:** *Mostly used*	**Reason of removal:** *(ex. Left the lab, graduated, etc.)*

F. SECTION II: RECOMBINANT OR SYNTHETIC DNA

2.1 Will your project involve the creation of recombinant DNA, synthetic DNA or transgenic animals?	**YES**- Complete the reminder of the form	☐
	NO- proceed to **Section G**	⊗

2.2 Where will the recombinant or synthetic DNA experiments be performed? (List all locations applicable and specify building and room number):

2.3 Does your project include the deliberate transfer of a drug resistance trait to pathogenic microorganisms that are not known to acquire the trait naturally? (Section III-A-1-a, experiments falling under this section of the NIH Guidelines require IBC approval, Recombinant DNA Advisory (RAC) Committee review and NIH Director Approval before initiation; For example the introduction of the gene encoding chloramphenicol resistance into the pathogen Rickettsia conorii)?	YES	☐
	NO	☐
2.4 Does your project include cloning toxin molecules with a lethal dose (LD50) of less than 100 nanograms per kilogram body weight? (Section III-B-1, experiments falling under this section of the NIH Guidelines require IBC approval, and submission to the NIH Office of Biotechnology Activities [OBA] before initiation; For example the cloning of the gene coding for the botulinum toxin.)?	YES	☐
	NO	☐
2.5 Does your project include experiments involving the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into one or more human research participants? (Section III-C-1, experiments falling under this section of the NIH Guidelines require IBC approval, Recombinant DNA Advisory (RAC) Committee review and IRB approval before initiation)?	YES	☐
	NO	☐
2.6 Does your project include experiments using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as host-vector systems (Section III-D-1) that would require BSL-2 or BSL-3 containment (experiments falling under this section of the NIH Guidelines require IBC approval before initiation)?	YES	☐
	NO	☐
2.7 Does your project include experiments in which DNA from Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems (Section III-D-2, experiments falling under this section of the NIH Guidelines require IBC approval)?	YES	☐
	NO	☐
2.8 Does your project include experiments involving the use of infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper virus in tissue culture systems (Section III-D-3, experiments falling under this section of the NIH Guidelines require IBC approval)?	YES	☐
	NO	☐
2.9 Does your project include experiments involving whole animals in which the animal's genome has been altered by the introduction of DNA into the germ line or experiments involving rDNA modified microorganisms tested on whole animals (Section III-D-4, III-E-3 – If only Section III-E-3 is applicable then these experiments may be initiated at the same time as IBC registration is in process)?	YES	☐
	NO	☐
2.10 Does your project include experiments involving whole plants (Section III-D-5, III-E-2, If only Section III-E-2 is applicable then these experiments may be initiated at the same time as	YES	☐

IBC registration is in process)?	NO	☐
2.11 Does your project include experiments involving more than 10 liters of culture (Section III-D-6)?	YES	☐
	NO	☐
2.12 Does your project include experiments involving the formation of recombinant DNA molecules containing two-thirds or more of the genome of any eukaryotic virus (Section III-E-1, If only Section III-E-1 is applicable then these experiments may be initiated at the same time as IBC registration is in process)?	YES	☐
	NO	☐
2.13 Does your project include cloning into an E. coli K-12 strain or K-12 derivative (If Yes, this work falls under Section III-F-6 and Appendix C-II)?	YES	☐
	NO	☐
2.14 What is the source of rDNA, DNA, RNA to be inserted or cloned? (Include species of organism from which it is derived):		
2.15 What is the nature of rDNA, DNA, RNA to be inserted or cloned? (For example is it a structural gene or oncogene?):		
2.16 What is the host system to be used? (For example, Rat cardiomyocytes and HEK-293 cells; or E. coli strain BL21; or yeast):		
2.17 What is the vector(s) to be used? Include information such as product literature, or vector map describing construction of vector. (For example, Lentiviral vector, Invitrogen ViraPower TM):		
2.18 List any helper virus or packaging cells used? (For example 293FT cell line):		

G. EXPERIMENTAL AIMS:

Experimental Aim #1 (describe the experimental goal and the specific experiment(s) that will be carried out to accomplish the goal):

1. To determine the presence of genes: SHV, TEM and CTX-M for Extended spectrum Beta-Lactamases (ESBL) bacteria from water collected at Rio Grande River.

Experimental Aim #2 (describe the experimental goal and the specific experiment(s) that will be carried out to accomplish the goal):

2. To determine the presence of genetic markers: integrons INTL-1 and INTL-2 in bacteria from water collected at Rio Grande River.

Experimental Aim #3 (describe the experimental goal and the specific experiment(s) that will be carried out to accomplish the goal):

3. To characterize the bacterial communities carrying resistant bacterial populations along the Rio Grande River and to determine the environmental and public health implications.

Principal Investigator Assurances-Conflict of Interest and Fiscal Responsibility

Do you or any person responsible for the design, conduct, or reporting of this study have an economic interest in, or act as an officer or director of any outside entity whose financial	YES	☐

interests may reasonably appear to be affected by this research? If yes, please explain any potential conflict of interest	NO	⊗
Do you or any person responsible for this study have existing financial holdings or relationships with the sponsor of this study? If yes, please explain any potential conflict of interest	YES NO N/A	☐ ⊗ ☐

Principal Investigator Certifications:

With this submission I certify that:

⊗ The information provided or attached is accurate and complete

⊗ I am familiar with and agree to abide by provisions of the current NIH guidelines for Research Involving Recombinant DNA Molecules and accept the responsibilities listed in Section IV-B-7

⊗ I accept responsibility for making sure all laboratory personnel involved in the project have been appropriately trained.

⊗ I will ensure that all research personnel are familiar with and understand the potential hazards and relevant biosafety practices, techniques, and emergency procedures associated with this research protocol as dictated by the CDC and NIH document, Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition (http://www.cdc.gov/biosafety/publications/bmbl5/index.htm

⊗ I further certify that I will immediately report any injuries or spills that occur while conducting research covered by this IBC protocol to the UTEP Biosafety Officer (747-7124) and the IBC Chair (747-6889) or IBC Coordinator (747-6056)

ADMINISTRATIVE USE ONLY

NIH GUIDELINES CLASSIFICATIONS:

Please select all categories that apply. See Risk Group (RG) definitions. Full text of the NIH Guidelines can be found at http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

Risk Group Definitions

Risk Group 1 (RG1): Agents that are not associated with disease in healthy adult humans.

Risk Group 2 (RG2): Agents are associated with human disease which is rarely serious and for which preventative or therapeutic interventions are often available.

Risk Group 3 (RG3): Agents that are associated with serious or lethal human disease for which preventative or therapeutic interventions may not be available.

Risk Group 4 (RG4): Agents are likely to cause serious or lethal human disease for which preventative or therapeutic interventions are not usually available.

☐	**Section III-E:** Experiments not included in Sections III-A, III-B, III-C, III-D, III-F; and experiments in which all components are derived from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes and may be conducted at BSL1.
☐	**Section III-E-1:** Recombinant or synthetic nucleic acid molecules containing no more than two-thirds of the genome of any eukaryotic virus may be propagated and maintained in cells in tissue culture (BSL1). For such experiments, it must be demonstrated that the cells lack helper virus for the specific families of defective viruses being used.

☐	**Section III-E-2:** Experiments involving nucleic acid molecule-modified whole plants, and/or experiments involving recombinant or synthetic nucleic acid molecule-modified organisms associated with whole plants.		
☐	**Section III-E-3:** Experiments involving the generation of rodents in which the animal's genome has been altered by stable introduction of recombinant or synthetic nucleic acid molecules, or nucleic acids derived therefrom, into the germ-line (transgenic rodents). BSL1 containment only; experiments BSL2 or higher are covered under Section III-D-4		
☐	**Section III-D-1:** Experiments Using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems.	**RG2:**	☐
		RG3:	☐
		RG4:	☐
☐	**Section III-D-2:** Experiments in Which DNA from Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems.	**RG2:**	☐
		RG3:	☐
☐	**Section III-D-3:** Experiments involving the use of infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper virus in tissue culture systems.	**RG2:**	☐
		RG3:	☐
☐	**Section III-D-4:** Recombinant or synthetic nucleic acid experiments involving whole animals (e.g., non-human vertebrate or invertebrate organism, including arthropods).	**RG1: (Section II-D-4-a)**	☐
		RG2 or RG3: (Section II-D-4-a)	☐
☐	**Section III-D-5:** Experiments involving whole plants or insects. Experiments to genetically engineer plants by recombinant or synthetic nucleic acid molecule methods, to propagate such plants, or to use plants together with microorganisms or insects containing recombinant or synthetic nucleic acid molecules (BSL2 or higher).		
☐	**Section III-D-6:** Experiments involving more than 10 liters of culture.		
☐	**Section III-D-7:** Experiments involving influenza viruses.		

IBC Risk Group 2 Organism Form

The University of Texas at El Paso
Institutional Biosafety Committee
Appendix B Form

Instructions: Appendix needs to be submitted with Protocol Form and submitted via IRBNet prior to the 5th of each month to be considered for review at the IBC meeting that month. Submissions entered after the 5th of each month will be considered for review at the following month. Meeting dates are posted on the IBC website. Any questions contact the IBC office at ibc@utep.edu.

B. APPENDIX B: RISK GROUP 2 ORGANISMS
See also, the NIH guidance document, Biosafety Considerations for Research with Risk Group 2 Organisms,
https://www.cdc.gov/biosafety/publications/bmbl5/bmbl5_sect_ii.pdf

Question:	**Agent:** ESBL E coli	**Agent:**	**Agent:**
B.1 Where will the experiments involving the use of Risk Group 2 agents be performed? (List all locations applicable and specify building and room number):	UTEP HSSN Bulding Rm#471		
B.2 Will needles, glassware or other sharps be used while working with risk group 2 organisms (to include work with animals)?	⊗ **NO** ☐ **YES** If **yes,** describe what precautions will be taken by the PI and lab personnel to minimize the exposure risk.	☐ **NO** ☐ **YES** If **yes,** describe what precautions will be taken by the PI and lab personnel to minimize the exposure risk.	☐ **NO** ☐ **YES** If **yes,** describe what precautions will be taken by the PI and lab personnel to minimize the exposure risk.

B.3 Will risk group 2 organisms be injected into animals?	⊗ **NO** ☐ **YES**	☐ **NO** ☐ **YES**	☐ **NO** ☐ **YES**
B.4: List and describe the experiments that will be conducted with the selected agent(s) and animals (e.g., how will animals be infected, infection monitored, risks to animals/humans)	Water samples will be collected from different areas along the Rio Grande River. Then DNA extracted from bacteria will be analyzed for genetic markers for resistant bacteria that could represent a health threat to the population along El Paso, TX and Juarez, Mexico.		
B.5 Can this agent infect or cause disease in immunocompromised individuals?	☐ **NO** ⊗ **YES** If **yes,** provide a description of potential mechanism of laboratory transmission Through inappropriate use of infectious agents and no following lab safety measures by researchers. If **yes,** is the infection associated with replication in humans or is it abortive (no progeny)? ⊗ **Replication** ☐ **Abortive**	☐ **NO** ☐ **YES** If **yes,** provide a description of potential mechanism of laboratory transmission If **yes,** is the infection associated with replication in humans or is it abortive (no progeny)? ☐ **Replication** ☐ **Abortive**	☐ **NO** ☐ **YES** If **yes,** provide a description of potential mechanism of laboratory transmission If **yes,** is the infection associated with replication in humans or is it abortive (no progeny)? ☐ **Replication** ☐ **Abortive**
B.6 Please list or describe any known vaccine or chemo prophylactic drugs available to treat infections from the proposed risk group 2 organisms?	Some antibiotics may be used in case of presenting symptoms related to infectious agent.		
B.7 Provide agents Safety Data Sheet **(SDS)** (Formerly known as Material Safety Data Sheets [MSDS]) if			

available, upload into IRBNet or with application.

DNA Water Extraction (MOBIO) Protocol

Steps

Experienced User Protocol Please wear gloves at all times Warm Solution PW1 prior to use at 55°C for 5-10 minutes. Use Solution PW1 while still warm. Check Solution PW3 and warm at 55°C for 5-10 minutes if necessary. Solution PW3 can be used while still warm.

1. Filter water samples using a reusable or disposable filter funnel attached to a vacuum source. Disposable filter funnels, containing 0.22 µm or 0.45 µm filter membranes, can be ordered from MO BIO Laboratories (see page 3). The volume of water filtered will depend on the microbial load and turbidity of the water sample. (Please see Types of Water Samples in the Hints and Troubleshooting Guide section of the Instruction Manual).

2. If using a reusable filter funnel, remove the upper portion of the apparatus. If using a MO BIO Laboratories filter funnel, remove the 100 ml upper portion of the filter cup from the catch reservoir by snapping it off.

3. Using two sets of sterile forceps, pick up the white filter membrane at opposite edges and roll the filter into a cylinder with the top side facing inward. Note: Do not tightly roll or fold the filter membrane. To see a video of this technique, please visit the PowerWater® DNA Isolation Kit product page on www.mobio.com.

4. Insert the filter into the 5 ml PowerWater® Bead Tube.

5. Add 1 ml of Solution PW1 to the PowerWater® Bead Tube. Note: Solution PW1 must be warmed to dissolve precipitates prior to use. Solution PW1 should be used while still warm. For samples containing organisms that are difficult to lyse (fungi, algae) an additional heating step can be included. See Alternate Lysis Method in the Hints and Troubleshooting Guide.

6. Secure the PowerWater® Bead Tube horizontally to a MO BIO Vortex Adapter, catalog number 13000-V1-15 or 13000-V1-5.

7. Vortex at maximum speed for 5 minutes.

8. Centrifuge the tubes ≤ 4000 x g for 1 minute at room temperature. The speed will depend on the capability of your centrifuge. (This step is optional if a centrifuge with a 15 ml tube rotor is not available, but will result in minor loss of supernatant) .

9.Transfer all the supernatant to a clean 2 ml Collection Tube (provided). Draw up the supernatant using a 1 ml pipette tip by placing it down into the beads. Note: Placing the pipette tip down into the beads is required. Pipette more than once to ensure removal of all supernatant. Any carryover of beads will not affect subsequent steps. Expect to recover between 600-650 µl of supernatant depending on the type of filter membrane used.

10. Centrifuge at 13,000 x g for 1 minute.

11. Avoiding the pellet, transfer the supernatant to a clean 2 ml Collection Tube (provided).

12. Add 200 µl of Solution PW2 and vortex briefly to mix. Incubate at 4°C for 5 minutes.

13. Centrifuge the tubes at 13,000 x g for 1 minute.

14. Avoiding the pellet, transfer the supernatant to a clean 2 ml Collection Tube (provided).

15. Add 650 µl of Solution PW3 and vortex briefly to mix. Note: Check Solution PW3 for precipitation prior to use. Warm if necessary. Solution PW3 can be used while still warm.

16. Load 650 µl of supernatant onto a Spin Filter and centrifuge at 13,000 x g for 1 minute. Discard the flow through and repeat until all the supernatant has been loaded onto the Spin Filter. Note: A total of two loads for each sample processed are required.

17. Place the Spin Filter basket into a clean 2 ml Collection Tube (provided).

18. Shake to mix Solution PW4 before use. Add 650 µl of Solution PW4 and centrifuge at 13,000 x g for 1 minute.

19. Discard the flow through and add 650 µl of Solution PW5 and centrifuge at 13,000 x g for 1 minute.

20. Discard the flow through and centrifuge again at 13,000 x g for 2 minutes to remove residual wash.

21. Place the Spin Filter basket into a clean 2 ml Collection Tube (provided).

22. Add 100 µl of Solution PW6 to the center of the white filter membrane.

23. Centrifuge at 13,000 x g for 1 minute.

24. Discard the Spin Filter basket. The DNA is now ready for any downstream application. No further steps are required. We recommend storing the DNA frozen (-20°C to -80°C). Solution PW6 contains no EDTA. To concentrate the DNA, see the Hints and Troubleshooting Guide.

Bacterial Isolates

February Isolates

In the month of February, 17 out of 39 isolates were identified with a probability of correct ID of 99.99% as determined by the Microscan autoSCAN 4 system . Two isolates displayed resistance or intermediate resistance to to 2 or more synergistic combinations including Trimeth/Sulfa, Amox/K Clav, and Pip/Tazo

All doses are in µg/ml and dual antibiotic doses are designated as a/b.

Isolate/ Probability of Correct ID	Site/source	Resistance (µg/ml)	Intermediate Resistance (µg/ml)
1. *Enterococcus durans/hirae*/99.99%	One/water	Tetracycline > 8	
2. *Enterococcus durans/hirae*/99.99%	One/water	Cefazolin >16 Cefepime>16 Cefotaxime >32 Ceftriaxone >32 Cephalothin>16 Tetracycline>8 Trimeth/Sulfa<=2/8	
3. *Enterococcus durans/hirae* 99.99%	One/water	Cefazolin >16 Cefepime>16 Cefotaxime >32 Ceftriaxone >32 Cephalothin>16 Tetracycline>8 Trimeth/Sulfa<=2/8	
4. *Enterococcus durans/hirae*/99.99%	Two/sediment	Tetracycline >8	
5. *Enterococcus durans/hirae* 99.99%	Two/water	Cefazolin >16 Cefepime>16 Cefotaxime >32 Ceftriaxone >32 Cephalothin>16 Linezoid >4 Tetracycline>8 Trimeth/Sulfa<=2/8	
6. *Enterococcus*	Two/water	Linezoid >4,	

durans/hirae/99.99%		Tetracycline>8	
7. *Aeromonas hydrophila* complex/99.99%	Three/water	Cefazolin >16	Amox/K Clav 16/8 Gentamicin 8
8. *Aeromonas hydrophila* complex/99.99%	Three/water		Amox/K Clav 16/8 Cefazolin 16
9. *Yersinia enterocolitica* group/99.99%	Three/sediment	Ampicillin >16 Gentamicin >8 Tobramycin >8 Trimeth/Sulfa >2/38	Amikacin 32 Cefazolin 16 Tetracycline 8
10. *Escherichia coli*/99.99%	Three/sediment		
11. *Aeromonas hydrophila* complex 99.99%	Three/sediment	Cefazolin >16	Amox/K Clav 16/8
12. *Vibrio fluvialis* 99.99%	Three/sediment	Amp/8 >16/8 Ampicillin >16 Ceftazidime >16 Trimeth/Sulfa	Amox/K Clav 16/8 Cefazolin >16 Gentamicin 8
13. *Aeromonas hydrophila* complex 99.99%	Three/water	Cefazolin >16	Amox/K Clav 16/8
14. *Aeromonas hydrophila* complex 99.99%	Three/water	Cefazolin >16	Amox/K Clav 16/8 Gentamicin 8
15. *Aeromonas hydrophila* complex 99.99%	Three/water	Cefazolin >16 Pip/Tazo >64	Amox/K Clav 16/8
16. *Aeromonas hydrophila* complex 99.99%	Three/water	Amox/K Clav 16/8 Cefazolin >16	
17. *Aeromonas hydrophila* complex 99.99%	Three/water	Cefazolin >16	Amikacin 32 Amox/K Clav 16/8 Gentamicin 8

April isolates

In the month of April, 56 isolates were processed and 27 isolates were identified with a probability of correct ID of 97% or above. Of these, 1 *E. coli* isolate was flagged as ESBL by the Microscan Autoscan system, 13 isolates showed resistance and two intermediate resistance to two or more synergistic combinations including Amox/K Clav , Amp/Sulbactam, Pip/Tazo, Trimeth/Sulfa and Ticar/K Clav

Isolate/ Probability of Correct ID	Site/source	Resistance (µg/ml)	Intermediate Resistance (µg/ml)
1.*Vibrio fluvialis*/99.99%	CW/water	Amox/K Clav>16/8 Amp/Sulbactam>16/8 Ampicillin>16 Imipenem>8	
2. *Staphylococcus cohnii* subsp. Cohnii/99.99%	KW/water	Amox/K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin<=0.25 Cefazolin<=8 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin<=8 Imipenem<=4 Oxacillin 1 Penicillin <=0.03	Erythromycin 4 Synercid 2
3. *Aeromonas hydrophila* complex/99.50%	CW/water	Cefazolin 16 Cefoxitin 16	
4. *Staphylococcus epidermidis*/99.99%	AW/water	Azithromycin>4 Erythromycin>4	
5. *Aeromonas hydrophila complex*/99.77%	CW/water	Amp/Sulbactam >16/8 Cefazolin<=8 Cefoxitin<=8	Amox/K Clav 16/8
6. *Aeromonas hydrophila* complex/99.12%	RB/water	Cefazolin 16 Cefoxitin<=8	
7. *Aeromonas hydrophila* complex/99.12%	RB/water	Cefazolin>16 Cefoxitin 16	
8. *Aeromonas hydrophila* complex/98.44%	RB/water	Amox/K Clav>16/8 Amp/Sulbactam>16/8 Cefazolin<=8 Cefoxitin<=8	
9. *Aeromonas hydrophila* complex/99.99%	RB/water	Amp/Sulbactam>16/8 Cefazolin<=8 Cefoxitin<=8	Amox/K Clav 16/8
10. *Vibrio fluvialis*/97.58%	AW/water	Ampicillin>16 Trimeth/Sulfa>2/38	Amox/K Clav 16/8 Cefazolin 16
11. *Aeromonas hydrophila* complex/99.99%	AS/water	Amox/K Clav>16/8 Cefazolin>16 Cefoxitin<=8 Ertapenem>4 Imipenem>8	Ciprofloxacin 2
12. *Aeromonas hydrophila* complex/99.99%	AS/water	Amox/K Clav>16/8 Cefazolin>16 Cefoxitin<=8 Imipenem>8	
13. *Aeromonas hydrophila* complex/99.99%	CS/water	Amox/K Clav>16/8 Cefazolin>16	

		Cefoxitin<=8 Imipenem>8	
14. *Vibrio fluvialis*/99.52%	AW/water	Amikacin>32 Amox/K Clav>16/8 Amp/Sulbactam>16/8 Ampicillin>16 Cefazolin>16 Cefepime>16 Cefotaxime>32 Cefoxitin>16 Ceftazidime>16 Cefuroxime>16 Ciprofloxacin>2 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Pip/Tazo>64 Tetracycline>8 Trimeth/Sulfa>2/38	
15. *Escherichia coli*/99.99%	CW/water	Amikacin>32 Amox/K Clav>16/8 Amp/Sulbactam>16/8 Ampicillin>16 Aztreonam>16 Cefazolin>16 Cefepime>16 Cefotaxime>32 Cefotetan>32 Cefoxitin>16 Ceftazidime>16 Ceftriaxone>32 Cefuroxime>16 Ciprofloxacin>2 Ertapenem>4 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Moxifloxacin>4 Pip/Tazo>64 Tetracycline>8 Ticar/K Clav>64 Tobramycin>8	
16. *Cedecea davisae*/99.99%	AW/water	Amikacin>32 Amox/K Clav>16/8 Amp/Sulbactam>16/8 Ampicillin>16 Aztreonam>16 Cefazolin>16 Cefepime>16	

		Cefotaxime>32 Cefotetan>32 Cefoxitin>16 Ceftazidime>16 Ceftriaxone>32 Cefuroxime>16 Ciprofloxacin>2 Ertapenem>4 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Moxifloxacin>4 Pip/Tazo>64 Tetracycline>8 Ticar/K Clav>64 Tobramycin>8 Trimeth/Sulfa>2/38	
17. *Escherichia coli*/99.99%	CW/water	Amikacin>32 Amox/K Clav>16/8 Amp/Sulbactam>16/8 Ampicillin>16 Aztreonam>16 Cefazolin>16 Cefepime>16 Cefotaxime>32 Cefotetan>32 Cefoxitin>16 Ceftazidime>16 Ceftriaxone>32 Cefuroxime>16 Ciprofloxacin>2 Ertapenem>4 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Moxifloxacin>4 Pip/Tazo>64 Tetracycline>8 Ticar/K Clav>64 Tobramycin>8	
18. *Leminorella species*/99.99%	RB/water	Amikacin>32 Amox/K Clav>16/8 Amp/Sulbactam>16/8 Ampicillin>16 Aztreonam>16 Cefazolin>16 Cefepime>16 Cefotaxime>32 Cefotetan>32	

		Cefoxitin>16 Ceftazidime>16 Ceftriaxone>32 Cefuroxime>16 Ciprofloxacin>2 Ertapenem>4 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Moxifloxacin>4 Pip/Tazo>64 Tetracycline>8 Ticar/K Clav>64 Tobramycin>8	
19. *Achromobacter xylosoxidans*/99.99%	RB/Water	Amikacin>32 Aztreonam>16 Cefepime>16 Cefotaxime>32 Ceftazidime>16 Ceftriaxone>32 Ciprofloxacin>2 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Pip/Tazo>64 Tetracycline>8 Ticar/K Clav>64 Tobramycin>8 Trimeth/Sulfa>2/38	
20.*Enterococcus faecium*/99.50%	RB/water	Cefazolin>16 Cefepime>16 Cefotaxime>32 Ceftriaxone>32 Cephalothin>16 Clindamycin>2 Trimeth/Sulfa<=2/38	Ciprofloxacin 2 Erythromycin 4 Linezolid 4
21. *Pseudomonas fluorescens*/putida/99.99%	KW/water	Aztreonam>16 Trimeth/Sulfa>2/38	Cefotaxime 32 Ceftriaxone 32 Chloramphenicol 16
22. *Escherichia coli*/99.99%	AW/water	Amp/Sulbactam>16/8 Ampicillin>16 Piperacillin>64 Tetracycline>8	
23. *Escherichia coli*/99.99%	AW/water	Amp/Sulbactam>16/8 Ampicillin>16 Piperacillin>64 Tetracycline>8	

24. *Pseudomonas aeruginosa*/99.99%	AW/water	Amox/K Clav>16/8 Amp/Sulbactam>16/8 Ampicillin>16 Cefazolin>16 Cefotaxime 32 Cefotetan>32 Cefoxitin>16 Ceftriaxone>32 Cefuroxime>16 Cephalothin>16 Chloramphenicol>16 Trimeth/Sulfa>2/38	
25. *Vibrio parahaemolyticus*/99.87%	AW/water	Amikacin>32 Amox/K Clav>16/8 Amp/Sulbactam>16/8 Ampicillin>16 Cefazolin>16 Cefepime>16 Cefotaxime>32 Cefoxitin>16 Ceftazidime>16 Cefuroxime>16 Chloramphenicol>16 Ciprofloxacin>2 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Pip/Tazo>64 Piperacillin>64 Tetracycline>8	
26. *Leminorella species*/99.99%	AW/water	Amikacin>32 Amox/K Clav>16/8 Amp/Sulbactam>16/8 Ampicillin>16 Aztreonam>16 Cefazolin>16 Cefepime>16 Cefotaxime>32 Cefotetan>32 Cefoxitin>16 Ceftazidime>16 Ceftriaxone>32 Cefuroxime>16 Chloramphenicol>16 Ciprofloxacin>2 Gatifloxacin>4 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8	

		Moxifloxacin>4 Pip/Tazo>64 Piperacillin>64 Tetracycline>8 Ticar/K Clav>64 Tobramycin>8	
27. *Escherichia coli*(ESBL)/99.99%	AW/water	Amikacin>32 Amox/K Clav>16/8 Amp/Sulbactam>16/8 Ampicillin>16 Aztreonam>16 Cefazolin>16 Cefepime>16 Cefotaxime>32 Cefotetan>32 Cefoxitin>16 Ceftazidime>16 Ceftriaxone>32 Cefuroxime>16 Chloramphenicol>16 Ciprofloxacin>2 ESBL-a Scrn>4 ESBL-b Scrn>1 Gatifloxacin>4 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Moxifloxacin>4 Pip/Tazo>64 Piperacillin>64 Tetracycline>8 Ticar/K Clav>64 Torbramycin>8	

June Isolates

In the month of June, Out of 60 isolates, 18 were processed with a probability of correct identification of 94% or above. Of these, 9 (Five *E. coli* and 4 *Klebsiella*) isolates were flagged as ESBL by the Microscan Autoscan system, while a total of 15 had resistance to two or more

synergistic combinations including Amox/K Clav , Amp/Sulbactam, Pip/Tazo, Trimeth/Sulfa and Ticar/K Clav.

Isolate/ Probability of Correct ID	**Site/source**	**Resistance (µg/ml)**	**Intermediate Resistance (µg/ml)**
1.*Escherichia coli*/99.99%	AW/water		
2. *S. Xylous*/99.99%	CW/water	Amox/K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin<=0.25 Cefazolin<=8 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin<=8 Imipenem<=4 Oxacillin 1 Penicillin0.12	Clindamycin 2
3. *Escherichia coli*/99.99%	AW/water		
4. *Escherichia coli*/99.99%	AW/water		
5. *Cedecea davisae* Carbonomase/99.99%	AW/water	Amikacin>32 Amox/K Clav>16/8 Amp/Sulbactam>16/8 Ampicillin>16 Aztreonam>16 Cefazolin>16 Cefepime>16 Cefotaxime>32 Cefotetan>32 Cefoxitin>16 Ceftazidime>16 Ceftriaxone>32 Cefuroxime>16 Chloramphenicol>16 Ciprofloxacin>2 Gatifloxacin>4 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Moxifloxacin>4 Pip/Tazo>64 Piperacillin>64 Tetracycline>8 Ticar/K Clav>64 Tobramycin>8	
6.*Escherichia coli*(ESBL)/99.99%	AW/water	Amikacin>32 Amox/K Clav>16/8	

		Amp/Sulbactam>16/8 Ampicillin>16 Aztreonam>16 Cefazolin>16 Cefepime>16 Cefotaxime>32 Cefotetan>32 Cefoxitin>16 Ceftazidime>16 Ceftriaxone>32 Cefuroxime>16 Chloramphenicol>16 Ciprofloxacin>2 ESBL-a Scrn>4 ESBL-b SCRN>1 Gatifloxacin>4 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Moxifloxacin>4 Pip/Tazo>64 Piperacillin>64 Tetracycline>8 Ticar/K Clav>64 Tobramycin>8 Trimeth/Sulfa>2/32	
7. *Aeromonas hydrophila* complex/99.99%	CW/water	Amikacin>32 Amox/K Clav>16/8 Amp/Sulbactam>16/8 Aztreonam>16 Cefazolin>16 Cefepime>16 Cefotaxime>32 Cefoxitin>16 Ceftazidime>16 Ceftriaxone>32 Cefuroxime>16 Chloramphenicol>16 Ciprofloxacin>2 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Pip/Tazo>64 Tetracycline>8	
8. *S. xylous*/99.99%	RB/water	Amox/K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin<=0.25 Cefazolin<=8 Cefepime<=8	Ciprofloxacin 2

		Cefotaxime<=8 Ceftriaxone<=8 Cephalothin<=8 Imipenem<=4 Oxacillin1 Penicillin0.12	
9. *Escherichia coli*(ESBL)/99.99%	RB/water	Amikacin>32 Amox/K Clav>16/8 Amp/Sulbactam>16/8 Ampicillin>16 Aztreonam>16 Cefazolin>16 Cefepime>16 Cefotaxime>32 Cefotetan>32 Cefoxitin>16 Ceftazidime>16 Ceftriaxone>32 Cefuroxime>16 Chloramphenicol>16 Ciprofloxacin>2 ESBL-a Scrn>4 ESBL-b Scrn>1 Gatifloxacin>4 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Moxifloxacin>4 Pip/Tazo>64 Piperacillin>64 Tetracycline>8 Ticar/K Clav>64 Tobramycin>8	
10. *S. xylous*/99.99%	RB/water	Amox/K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin<=0.25 Cefazolin<=8 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin<=8 Imipenem<=4 Oxacillin 1 Penicillin 0.12 Trimeth/Sulfa(TFG) <=2/38	
11. *S. xylous*/99.99%	RB/water	Amox/K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin<=0.25 Cefazolin<=8	

		Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin<=8 Imipenem<=4 Oxacillin 1 Penicillin 0.12 Trimeth/Sulfa(TFG) <=2/38	
12. *Escherichia coli**ESBL/94.40%	RB/water	Amikacin>32 Amox/K Clav>16/8 Amp/Sulbactam>16/8 Ampicillin>16 Aztreonam>16 Cefazolin>16 Cefepime>16 Cefotaxime>32 Cefotetan>32 Cefoxitin>16 Ceftazidime>16 Ceftriaxone>32 Cefuroxime>16 Chloramphenicol>16 Ciprofloxacin>2 ESBL-a Scrn>4 ESBL-b Scrn>1 Gatifloxacin>4 Gentamicin >8 Imipenem>8 Levofloxacin>4 Meropenem>8 Moxifloxacin>4 Pip/Tazo>64 Piperacillin>64 Tetracycline>8 Ticar/K Clav>64 Tobramycin>8	
13. *Escherichia coli**ESBL/99.90%	RB/water	Amikacin>32 Amox/K Clav>16/8 Amp/Sulbactam>16/8 Ampicillin>16 Aztreonam>16 Cefazolin>16 Cefepime>16 Cefotaxime>32 Cefotetan>32 Cefoxitin>16 Ceftazidime>16 Ceftriaxone>32 Cefuroxime>16	

		Chloramphenicol>16 Ciprofloxacin>2 ESBL-a Scrn>4 ESBL-b Scrn>1 Gatifloxacin>4 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Moxifloxacin>4 Pip/Tazo>64 Piperacillin>64 Tetracycline>8 Ticar/K Clav>64 Tobramycin>8	
14. *Klebsiella pneumoniae**ESBL/99.99%	AW/water	Amikacin>32 Amox/K Clav>16/8 Amp/Sulbactam>16/8 Ampicillin>16 Aztreonam>16 Cefazolin>16 Cefepime>16 Cefotaxime>32 Cefotetan>32 Cefoxitin>16 Ceftazidime>16 Ceftriaxone>32 Cefuroxime>16 Chloramphenicol>16 Ciprofloxacin>2 ESBL-a Scrn>4 ESBL-b Scrn >1 Gatifloxacin>4 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Moxifloxacin>4 Pip/Tazo>64 Piperacillin>64 Tetracycline>8 Ticar/K Clav>64 Tobramycin>8	
15. *Escherichia coli**ESBL/99.99%	RB/water	Amikacin>32 Amox/K Clav>16/8 Amp/Sulbactam>16/8 Ampicillin>16 Aztreonam>16 Cefazolin>16 Cefepime>16 Cefotaxime>32	

		Cefotetan>32 Cefoxitin>16 Ceftazidime>16 Ceftriaxone>32 Cefuroxime>16 Chloramphenicol>16 Ciprofloxacin>2 ESBL-a Scrn>4 ESBL-b Scrn>1 Gatifloxacin>4 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Moxifloxacin>4 Pip/Tazo>64 Piperacillin>64 Tetracycline>8 Ticar/K Clav>64 Tobramycin>8	
16. *Klebsiella pneumoniae**ESBL/99.99%	CW/water	Amikacin>32 Amox/K Clav>16/8 Amp/Sulbactam>16/8 Ampicillin>16 Aztreonam>16 Cefazolin>16 Cefepime>16 Cefotaxime>32 Cefotetan>32 Cefoxitin>16 Ceftazidime>16 Ceftriaxone>32 Cefuroxime>16 Chloramphenicol>16 Ciprofloxacin >2 ESBL-a Scrn>4 ESBL-b Scrn>1 Gatifloxacin>4 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Moxifloxacin>4 Pip/Tazo>64 Piperacillin>64 Tetracycline>8 Ticar/K Clav>64 Tobramycin>8	
17. *Klebsiella pneumoniae**ESBL/99.99%	AW/water	Amikacin >32 Amox/K Clav>16/8 Amp/Sulbactam>16/8	

		Ampicillin>16 Aztreonam>16 Cefazolin>16 Cefepime>16 Cefotaxime>32 Cefotetan>32 Cefoxitin>16 Ceftazidime>16 Ceftriaxone>32 Cefuroxime>16 Chloramphenicol>16 Ciprofloxacin>2 ESBL-a Scrn>4 ESBL-b Scrn>1 Gatifloxacin>4 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Moxifloxacin>4 Pip/Tazo>64 Piperacillin >64 Tetracycline>8 Ticar/K Clav>64 Tobramycin>8	
18. *Klebsiella pneumoniae**ESBL/99.99%	AW/water	Amikacin>32 Amox/K Clav>16/8 Amp/Sulbactam>16/8 Ampicillin>16 Aztreonam>16 Cefazolin>16 Cefepime>16 Cefotaxime>32 Cefotetan>32 Cefoxitin>16 Ceftazidime>16 Ceftriaxone>32 Cefuroxime>16 Chloramphenicol>16 Ciprofloxacin>2 ESBL-a Scrn>4 ESBL-b Scrn>1 Gatifloxacin>4 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Moxifloxacin>4 Pip/Tazo>64 Piperacillin>64 Tetracycline>8	

		Ticar/K Clav>64 Tobramycin>8	

September Isolates

In the month of September, out of 79 isolates, 37 isolates were identified with a probability of correct identification of 94% or better. There were no isolates flagged as ESBL, and 26 isolates were resistant to 2 or more synergistic combinations including Amox/K Clav , Amp/Sulbactam, and Trimeth/Sulfa.

Isolate/ Probability of Correct ID	**Site/source**	**Resistance (µg/ml)**	**Intermediate Resistance (µg/ml)**
1.*Enterobacter cloacae*/99.99%	Site two/water	Amox/K Clav>16/8 Amp/Sulbactam>16/8 Ampicillin>16 Cefazolin>16 Cefotetan<=16 Cefoxitin>16 Cefuroxime<=4	
2. *Klebsiella pneumoniae*/99.99%	Site two/water	Ampicillin<=8	
3.*Enterobacter cloacae*/99.99%	Site two/water	Amox/K Clav>16/8 Amp/Sulbactam16/8 Ampicillin<=8 Cefazolin>16 Cefotetan>32 Cefoxitin>16 Ceftriaxone>32 Cefuroxime 8	Levofloxacin 4
4. *Klebsiella pneumoniae*/99.99%	Site two/water	Ampicillin 16	
5. *Enterobacter cloacae*/99.99%	Site two/water	Amox/K Clav>16/8 Amp/Sulbactam 16/8 Ampicillin>16 Cefazolin>16 Cefotetan<=16 Cefoxitin>16	Chloramphenicol 16

		Cefuroxime>16	
6. *Staphylococcus sciuri*/99.99%	Site two/water	Amox/K Clav>4/2 Amp/Sulbactam>16/8 Ampicillin>8 Azithromycin>4 Cefazolin 16 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin>16 Clindamycin>2 Erythromycin >4 Imipenem<=4 Oxacillin>2 Penicillin>8 Rifampin>2 Synercid>2 Vancomycin>16	
7. *Staphylococcus sciuri*/99.98%	Site two/ water	Amox/K Clav>4/2 Amp/Sulbactam<=8/4 Ampicillin 4 Azithromycin>4 Cefazolin>16 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin>16 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin>8 Rifampin>2 Synercid>2 Vancomycin>16	Ofloxacin 4
8. *Staphylococcus sciuri*/99.99%	Site two/Water	Amox/K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin 2 Azithromycin>4 Cefazolin<=8 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin<=8 Clindamycin>2 Erythromycin >4 Imipenem<=4 Oxacillin>2 Penicillin>8	

		Rifampin>2 Synercid>2 Vancomycin>16	
9. *Staphylococcus sciuri*/99.99%	Site three/water	Amox/K Clav>4/2 Amp/Sulbactam>16/8 Ampicillin>8 Cefazolin>16 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin>16 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin >8 Synercid >2 Vancomycin>16	
10. *Staphylococcus sciuri*/99.60%	Site three/water	Amox/K Clav>4/2 Amp/Sulbactam<=8/4 Ampicillin>8 Azithromycin>4 Cefazolin>16 Cefepime<=8 Cefotaxime 32 Ceftriaxone<=8 Cephalothin>16 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin>8 Synercid>2 Vancomycin>16	Levofloxacin 4 Rifampin 2
11. *Staphylococcus sciuri*/99.99%	Site three/water	Amox/K Clav>4/2 Amp/Sulbactam>16/8 Ampicillin>8 Azithromycin>4 Cefazolin>16 Cefepime<=8 Cefotaxime 32 Ceftriaxone <=8 Cephalothin>16 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin>8 Synercid>2 Vancomycin>16	Levofloxacin 4 Rifampin 2

12. *Staphylococcus sciuri*/99.99%	Site two/water	Amox/K Clav>4/2 Amp/Sulbactam>16/8 Ampicillin>8 Cefazolin 16 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin>16 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin>8 Synercid>2 Vancomycin>16	Azithromycin 4 Rifampin 2
13. *Staphylococcus sciuri*/99.99%	Site three/water	Amox/K Clav>4/2 Amp/Sulbactam>16/8 Ampicillin>8 Cefazolin>16 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin>16 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin 1 Penicillin>8 Synercid>2 Vancomycin>16	Rifampin 2
14. *Staphylococcus sciuri*/99.60%	Site three/water	Amox/K Clav>4/2 Amp/Sulbactam>16/8 Ampicillin>8 Azithromycin>4 Cefazolin>16 Cefepime<=8 Cefotaxime>32 Ceftriaxone<=8 Cephalothin>16 Ciprofloxacin>2 Clindamycin>2 Gatifloxacin>4 Gentamicin>8 Imipenem<=4 Levofloxacin>4 Ofloxacin>4 Oxacillin>2 Penicillin>8 Synercid>2 Vancomycin>16	Erythromycin 4
15. *Staphylococcus*	Site two/water	Amox/K Clav<=4/2	Azithromycin 4

sciuri/99.99%		Amp/Sulbactam<=8 Ampicillin 4 Cefazolin<=8 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin 16 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin>8 Rifampin>2 Synercid>2 Vancomycin>16	
16. *Staphylococcus auricularis* /95.57%	Site three/water	Erythromycin>4 Synercid>2	Clindamycin 2
17. *Klebsiella ozaenae*/99.99%	Site two/water		
18. *Klebsiella pneumoniae*/99.99%	Site two/water	Ampicillin<=8	
19. *Klebsiella ozaenae*/99.99%	Site two/water		
20. *Staphylococcus sciuri*/99.35%	Site three/water	Amox/K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin 2 Cefazolin<=8 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin<=8 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin>8 Rifampin>2 Synercid>2 Trimeth/Sulfa>2/38 Vancomycin>16	Azithromycin 4
21. *Staphylococcus sciuri*/98.11%	Site three/water	Amox/K Clav>4/2 Amp/Sulbactam<=8/4 Ampicillin>8 Cefazolin<=8 Cefepime<=8 Cefotaxime>32 Ceftriaxone>32 Cephalothin 16 Clindamycin>2 Erythromycin>4	Rifampin 2

		Imipenem<=4 Oxacillin>2 Penicillin>8 Synercid>2 Vancomycin>16	
22. *Staphylococcus sciuri*/94.42%	Site two/water	Clindamycin>2 Erythromycin>4 Synercid>2 Vancomycin>16	
23. *Staphylococcus sciuri*/99.60%	Site two/water	Amox/K Clav>4/2 Amp/Sulbactam>16/8 Ampicillin>8 Cefazolin<=8 Cefepime<=8 Cefotaxime<=8 Ceftriaxone <=8 Cephalothin<=8 Clindamycin>2 Erythromycin>4 Imipenem 8 Oxacillin 2 Penicillin>8 Synercid>2 Vancomycin>16	
24. *Staphylococcus xylosus*/99.99%	Site two/water	Amox/K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin>8 Azithromycin>4 Cefazolin<=8 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin<=8 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin>8 Rifampin>2 Synercid>2 Vancomycin>16	
25. *Staphylococcus aureus*/95.24%	Site three/water	Erythromycin>4 Synercid>2	Clindamycin 2
26. *Staphylococcus sciuri*/99.99%	Site two/water	Amox/K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin 8 Azithromycin>4 Cefazolin<=8 Cefepime<=8	

		Cefotaxime<=8 Ceftriaxone<=8 Cephalothin<=8 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin>8 Rifampin>2 Synercid>2 Vancomycin>16	
27. *Staphylococcus sciuri*/99.99%	Site two/water	Amox/K Clav>4/2 Amp/Sulbactam>16/8 Ampicillin>8 Cefazolin<=8 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin<=8 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin>8 Synercid>2 Vancomycin>16	
28. *Staphylococcus xylosus*/99.99%	Site two/water	Amox/K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin 8 Azithromycin>4 Cefazolin<=8 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin<=8 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin>8 Rifampin>2 Synercid>2 Vancomycin>16	
29. *Staphylococcus sciuri*/99.99%	Site two/water	Amox/K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin>8 Azithromycin>4 Cefazolin<=8 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8	

		Cephalothin<=8 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin>8 Rifampin>2 Synercid>2 Vancomycin>16	
30. *Staphylococcus hominis* subsp. Hominis/99.99%	Site two/water	Amox/K Clav>4/2 Amp/Sulbactam 16/8 Ampicillin>8 Cefazolin>16 Cefepime>16 Cefotaxime>32 Ceftriaxone 32 Cephalothin>16 Imipenem<=4 Oxacillin>2 Penicillin>8	Clindamycin 2
31.*Staphylococcus schleiferi* subspecies schleiferi/97.76%	Site two/water	Amox/ K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin>8 Cefazolin<=8 Cefepime>16 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin<=8 Clindamycin>2 Erythromycin>4 Gentamicin>8 Imipenem<=4 Oxacillin>2 Penicillin>8 Synercid>2 Trimeth/Sulfa>2/38 Vancomycin>16	Chloramphenicol 16 Rifampin 2
32. *Staphylococcus sciuri*/99.99%	Site two/water	Amox/K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin 2 Azithromycin>4 Cefazolin>16 Cefepime<=8 Cefotaxime>32 Ceftriaxone>32 Cephalothin>16 Ciprofloxacin>2 Clindamycin>2 Erythromycin>4 Gatifloxacin>4 Imipenem<=4	

		Levofloxacin>4 Moxifloxacin>4 Ofloxacin>4 Oxacillin>2 Penicillin>8 Rifampin>2 Synercid>2 Vancomycin>16	
33. *Staphylococcus sciuri*/99.99%	Site two/water	Amox/K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin 2 Cefazolin<=8 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin<=8 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin>8 Rifampin>2 Synercid>2 Vancomycin>16	Azithromycin 4
34. *Staphylococcus aureus*/98.99%	Site two/water	Clindamycin>2 Erythromycin>4 Synercid>2 Vancomycin>16	Rifampin 2
35. *Staphylococcus sciuri*/99.99%	Site two/water	Amox/K Clav>4/2 Amp/Sulbactam>16/8 Ampicillin>8 Azithromycin>4 Cefazolin>16 Cefepime>16 Cefotaxime>32 Ceftriaxone>32 Cephalothin>16 Clindamycin>2 Erythromycin>4 Imipenem<=4 Moxifloxacin>4 Ofloxacin>4 Oxacillin>2 Penicillin>8 Rifampin>2 Synercid>2 Trimeth/Sulfa>2/38 Vancomycin>16	Gentamicin 8 Tetracycline 8

36.*Escherichia coli*/99.99%	Site 2/water		
37. *Escherichia coli*/99.99%	Site 2/water		

December Isolates

In the month of December, out of 76 isolates, 43 were identified with a probability of correct identification of 92% or better. One *Klebsiella pneumoniae* isolate was flagged as ESBL. There were 33 isolates resistant to two or more synergistic combinations including Amox/K Clav , Amp/Sulbactam, Pip/Tazo, Trimeth/Sulfa and Ticar/K Clav.

Isolate/ Probability of Correct ID	**Site/source**	**Resistance (µg/ml)**	**Intermediate Resistance (µg/ml)**
1. *Staphylococcus xylosus*/99.99%	Site one/water	Amox/K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin 2 Azithromycin>4 Cefazolin<=8 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin<=8 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin 8 Rifampin>2 Synercid>2 Vancomycin>16	
2. *Staphylococcus sciuri*/99.99%	Site one/water	Amox/K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin>8 Azithromycin>4 Cefazolin<=8 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin<=8	

		Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin>8 Rifampin>2 Synercid>2 Vancomycin>16	
3. *Staphylococcus sciuri*/99.27%	Site two/water	Amox/K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin 2 Azithromycin>4 Cefazolin<=8 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin<=8 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin>8 Rifampin>2 Synercid>2 Vancomycin>16	
4. *Staphylococcus sciuri*/99.35%	Site two/water	Amox/K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin 2 Cefazolin<=8 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin 16 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin>8 Rifampin>2 Synercid>2 Vancomycin>16	Azithromycin 4
5. *Klebsiella pneumoniae*/99.99% (ESBL)	Site three/water	Amikacin>32 Amox/K Clav>16/8 Amp/Sulbactam>16/8 Ampicillin>16 Aztreonam>16 Cefazolin>16 Cefepime>16 Cefotaxime>32 Cefotetan>32 Cefoxitin>16	

		Ceftazidime>16 Ceftriaxone>32 Cefuroxime>16 Ciprofloxacin>2 Ertapenem>4 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Moxifloxacin>4 Pip/Tazo>64 Tetracycline>8 Ticar/K Clav>64 Tobramycin>8	
6. *Staphylococcus sciuri*/99.35%	Site three/water	Amox/K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin 2 Cefazolin<=8 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin 16 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin>8 Rifampin>2 Synercid>2 Vancomycin>16	Azithromycin 4
7. *Staphylococcus aureus*/98.99%	Site one/water	Clindamycin>2 Erythromycin>4 Synercid>2 Vancomycin>16	
8. *Staphylococcus epidermidis*/99.99%	Site two/water	Clindamycin>2 Erythromycin>4 Synercid>2 Vancomycin>16	
9. *Pseudomonas luteola*/99.99%	Site two/water	Amikacin>32 Aztreonam>16 Cefepime>16 Cefotaxime>32 Ceftazidime>16 Ceftriaxone>32 Ciprofloxacin>2 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Pip/Tazo>64	

		Tetracycline>8 Ticar/K Clav>64	
10. *Staphylococcus hyicus*/99.99%	Site 2/water	Amox/K Clav<=4/2 Amp/Sulbactam>16/8 Ampicillin 8 Cefazolin<=8 Cefepime<=8 Ceftriaxone<=8 Cephalothin<=8 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin>8 Synercid>2 Vancomycin>16	
11. *Vibrio metschnikovii*/99.99%	Site two/water	Amikacin>32 Amox/K Clav>16/8 Amp/Sulbactam>16/8 Ampicillin>16 Cefazolin>16 Cefepime>16 Cefotaxime>32 Cefoxitin>16 Ceftazidime>16 Cefuroxime>16 Ciprofloxacin>2 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Pip/Tazo>64 Tetracycline>8	
12. *Burkholderia cepacia* Complex/99.99%	Site two/water	Ceftazidime>16 Levofloxacin>4 Meropenem>8 Ticar/K Clav>64	
13. *S. hominis*-novo/99.00%	Site two/water	Amox/K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin<=0.25 Cefazolin<=8 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin<=8 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin 2	

		Synercid>2 Vancomycin>16	
14.*Staphylococcus xylosus*/99.99%	Site 3/water	Amox/K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin>8 Azithromycin>4 Cefazolin<=8 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin<=8 Clindamycin>2 Erythromycin>4 Gentamicin>8 Imipenem<=4 Oxacillin>2 Penicillin>8 Rifampin>2 Synercid>2 Tetracycline>8 Trimeth/Sulfa>2/38 Vancomycin>16	
15. *Staphylococcus schleiferi subspecies schleiferi*/99.99%	Site three/water	Amox/K Clav>4/2 Amp/Sulbactam>16/8 Ampicillin>8 Azithromycin>4 Cefazolin>16 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin>16 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin>8 Rifampin>2 Synercid>2 Tetracycline>8 Vancomycin>16	
16. *Staphylococcus xylosus*/99.99%	Site three/water	Amox/K Clav>4/2 Amp/Sulbactam>16/8 Ampicillin>8 Azithromycin>4 Cefazolin>16 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin>16 Clindamycin>2 Erythromycin>4	

		Imipenem<=4 Oxacillin>2 Penicillin>8 Rifampin>2 Synercid>2 Vancomycin>16	
17. *Klebsiella pneumoniae*/99.99%	Site 3/water	Amikacin>32 Amox/K Clav>16/8 Amp/Sulbactam>16/8 Ampicillin>16 Aztreonam>16 Cefazolin>16 Cefepime>16 Cefotaxime>32 Cefotetan>32 Cefoxitin>16 Ceftazidime>16 Ceftriaxone>32 Cefuroxime>16 Ciprofloxacin>2 Ertapenem>4 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Moxifloxacin>4 Pip/Tazo>64 Tetracycline>8 Ticar/K Clav>64 Tobramycin>8	
18. *Staphylococcus xylosus*/99.99%	Site three/water	Amox/K Clav>4/2 Amp/Sulbactam<=8/4 Ampicillin 2 Azithromycin>4 Cefazolin 16 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin 16 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin>8 Rifampin>2 Synercid>2 Vancomycin>16	
19. *Staphylococcus sciuri*/99.99%	Site three/water	Amox/K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin>8 Azithromycin>4	

		Cefazolin<=8 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin<=8 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin>8 Rifampin>2 Synercid>2 Vancomycin>16	
20. *S. epidermidis*/99.99%	Site three/water	Amox/K Clav>4/2 Amp/Sulbactam<=8/4 Ampicillin<=0.25 Cefazolin<=8 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin<=8 Imipenem<=4 Oxacillin 1 Penicillin<=0.03	Clindamycin 2
21. *Staphylococcus haemolyticus*/99.99%	Site three/water	Amox/K Clav>4/2 Amp/Sulbactam<=8/4 Ampicillin>8 Azithromycin>4 Cefazolin<=8 Cefepime>16 Cefotaxime 32 Ceftriaxone<=8 Cephalothin 16 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin>8 Rifampin>2 Synercid>2 Vancomycin>16	
22. *S. Schleif-schlf*/99.99%	Site three/water	Amox/K Clav>4/2 Amp/Sulbactam>16/8 Ampicillin>8 Cefazolin<=8 Cefepime>16 Cefotaxime>32 Ceftriaxone<=8 Cephalothin<=8 Clindamycin>2 Erythromycin>4	Rifampin 2

		Imipenem<=4 Oxacillin>2 Penicillin>8 Synercid>2 Vancomycin>16	
23. *Sphingobacterium multivorum*/99.99%	Site three/water	Amikacin>32 Aztreonam>16 Cefepime>16 Cefotaxime>32 Ceftazidime>16 Ceftriaxone>32 Ciprofloxacin>2 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Pip/Tazo>64 Tetracycline>8 Ticar/K Clav>64 Tobramycin>8	
24. *Enterobacter cloacae*/99.99%	Site one/water	Amikacin>32 Amox/K Clav>16/8 Amp/Sulbactam>16/8 Ampicillin>16 Aztreonam>16 Cefazolin>16 Cefepime>16 Cefotaxime>32 Cefotetan>32 Cefoxitin>16 Ceftazidime>16 Ceftriaxone>32 Cefuroxime>16 Ertapenem>4 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Moxifloxacin>4 Pip/Tazo>64 Tetracycline>8 Ticar/K Clav>64 Tobramycin>8	Ciprofloxacin 2
25. Strenotrophomonas maltophilia/99.99%	Site one/water	Ceftazidime>16 Levofloxacin>4 Ticar/K Clav>64	
26. *S. epidermidis*/99.99%	Site two/water	Amox/K Clav<=4/2 Amp/Sulbactam 16/8 Ampicillin 4 Azithromycin>4	Rifampin 2

		Cefazolin>16 Cefepime>16 Cefotaxime 32 Ceftriaxone<=8 Cephalothin>16 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin>8 Synercid>2 Vancomycin>16	
27. *Chryseobacterium indologenes*/99.99%	Site two/water	Amikacin>32 Aztreonam>16 Cefepime>16 Cefotaxime>32 Ceftazidime>16 Ceftriaxone>32 Ciprofloxacin>2 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Pip/Tazo>64 Tetracycline>8 Ticar/K Clav>64 Tobramycin>8	
28. *Cedecea neteri*/99.77%	Site two/water	Aztreonam>16 Ceftazidime>16	Cefepime 16 Cefotaxime 32
29. *S. schleif*-coag/99.99%	Site two/water	Erythromycin>4	Clindamycin 2
30. *S. haemolyticus*/99.99%	Site two/water	Amox/K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin 2 Azithromycin>4 Cefazolin<=8 Cefepime 16 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin<=8 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin 2 Penicillin 8 Synercid >2 Tetracycline>8 Vancomycin>16	Chloramphenicol 16 Rifampin 2
31. *Staphylococcus aureus*/99.99%	Site two/water	Azithromycin>4 Clindamycin>2 Erythromycin>4	Synercid 2

32. *Vibrio cholerae*/99.99%	Site one/water	Ampicillin>16 Tetracycline>8	
33. *S. xylosus*/99.99%	Site one/water	Amox/K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin>8 Azithromycin>4 Cefazolin<=8 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin<=8 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin>8 Rifampin>2 Synercid>2 Vancomycin>16	
34. *Acinetobacter baumanni* complex/haemolyticus/99.78%	Site one/water	Amp/Sulbactam>16/8 Cefepime>16 Ceftazidime>16 Ciprofloxacin>2 Meropenem>8 Tetracycline>8 Ticar/K Clav>64 Tobramycin>8	Cefotaxime 32 Ceftriaxone 32
35. *S. xylosus*/99.99%	Site three/water	Amox/K Clav>4/2 Amp/Sulbactam<=8/4 Ampicillin>8 Azithromycin>4 Cefazolin<=8 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin<=8 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin>8 Rifampin>2 Synercid>2 Vancomycin>16	
36. *S. haemolyticus*/99.99%	Site one/water	Clindamycin>2 Erythromycin>4 Synercid>2 Vancomycin>16	
37. *S. auricularis*/92.76%	Site three/water	Amox/K Clav>4/2 Amp/Sulbactam<=8/4	

		Ampicillin 2 Cefazolin>16 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin>16 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin 1 Penicillin 8 Synercid>2 Vancomycin>16	
38. *S. multivorum*/99.99%	Site three/water	Amikacin>32 Ampicillin>16 Cefepime>16 Cefotaxime>32 Ceftazidime>16 Ceftriaxone>32 Ciprofloxacin>2 Gentamicin>8 Imipenem>8 Levofloxacin>4 Meropenem>8 Pip/Tazo>64 Tetracycline>8 Ticar/K Clav>64 Tobramycin>8	
39. *Sphingomonas paucimobilis*/99.99%%	Site 3/water	Aztreonam>16 Cefepime>16 Cefotaxime>32 Ceftazidime>16 Ciprofloxacin>2 Imipenem>8 Meropenem>8 Pip/Tazo>64 Tetracycline>8 Ticar/K Clav>64	Ceftriaxone 32
40. *Staphylococcus auricularis*/99.16%	Site 1/water	Amox/K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin>8 Cefazolin>16 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin>16 Clindamycin>2 Erythromycin>4 Imipenem=4 Oxacillin>2 Penicillin>8	Tetracycline 8

		Synercid>2 Trimeth/Sulfa>2/38 Vancomycin>16	
41. *Staphylococcus epidermidis*/92.72%	Site 2/water	Azithromycin>4 Clindamycin>2 Synercid>2 Vancomycin>16	Rifampin 2
42. *Staphylococcus xylosus*/99.99%	Site 3/water	Amox/K Clav<=4/2 Amp/Sulbactam<=8/4 Ampicillin 2 Cefazolin<=8 Cefepime<=8 Cefotaxime<=8 Ceftriaxone<=8 Cephalothin 16 Clindamycin>2 Erythromycin>4 Imipenem<=4 Oxacillin>2 Penicillin 8 Rifampin>2 Synercid>2 Vancomycin>16	Azithromycin 4
43. *Staphylococcus schleiferi* subspecies schleiferi/99.99%	Site 3/water	Clindamycin>2 Erythromycin>4 Synercid>2 Vancomycin>16	

Vita

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Dr. Maria Fuentes obtained her Medicine degree at Universidad Autónoma de Ciudad Juárez in May 2009. Her internship was completed at Instituto Mexicano del Seguro Social Hospital#35 and her social service at Servicios Estatales de Salud Department of Epidemiology in Cd. Juarez, Mexico. In 2012 she obtained her certificate from United States Educational Commission for Foreign Medical Graduates. After gaining experience in the medical field and epidemiology, she decided to study at the University of Texas at El Paso (UTEP) and obtain her Master in Public Health (MPH) to focus on research. Her main interests are environmental sciences, infectious diseases, and public health aiming to create prevention programs for the Hispanic community of El Paso, Texas. Maria Fuentes will now continue with her PhD in Interdisciplinary Sciences at UTEP.