Assessment of Antibiotic Levels, Multi-Drug Resistant Bacteria and Genetic Biomarkers in the Waters of the Rio Grande River Between the United States-Mexico Border

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Introduction

Antimicrobial resistance (AR) represents one of the most important global challenges in public health. It has been estimated that by the year 2050, deaths by complications related to infectious diseases will increase to as many as 10 million per year.1 The microorganisms currently reported to represent major health threats due to increased AR, not only in the US but around the world, include carbapenem resistant enterobacteria, methicillin-resistant Staphylococcus aureus, and extended spectrum β-lactamase (ESBL) producing bacteria.2,3 These multidrug resistant (MDR) bacteria are disseminating rapidly through mobile genetic elements not only in health care, but in the environment worldwide.

Background. The worldwide emergence of multi-drug resistant bacteria has become a health crisis, as fewer or sometimes no antimicrobial agents are effective against these bacteria. The Rio Grande River is the natural boundary between the United States (US) and Mexico. It spans a border region between Texas, New Mexico and Mexico. Underserved populations on the Mexican side use the river for recreational purposes, while on the US side, the river is used for irrigation and as a source of drinking water.

Objectives. The purpose of the present study was to evaluate the concentration of antibiotic residues, to determine the presence of genetic elements conferring antibiotic resistance and to characterize multi-drug resistant bacteria in the waters of the Rio Grande River.

Methods. Water samples were obtained from the Rio Grande River. Deoxyribonucleic acid (DNA) was extracted from both isolated bacteria and directly from the water. Amplification of selected genetic elements was accomplished by polymerase chain reaction. Identification and isolation of bacteria was performed through MicroScan autoSCAN-4. Fecal contamination was assessed by IDEXX Colilert. Antibiotic residues were determined by liquid chromatography and mass spectrometry.

Results. Antibiotics were found in 92% of both water and sediment samples. Antibiotic concentrations ranged from 0.38 ng/L - 742.73 ng/L and 0.39 ng/l - 66.3 ng/g dry weight in water and sediment samples, respectively. Genetic elements conferring resistance were recovered from all collection sites. Of the isolated bacteria, 91 (64.08%) were resistant to at least two synergistic antibiotic combinations and 11 (14.79%) were found to be resistant to 20 or more individual antibiotics. Fecal contamination was higher during the months of April and July.

Conclusions. The 26 km segment of the Rio Grande River from Sunland Park NM to El Paso, TX and Juarez, Mexico is an area of concern due to poor water quality. The presence of multi-drug resistant bacteria, antibiotics and mobile genetic elements may be a health hazard for the surrounding populations of this binational border region. Policies need to be developed for the appropriate management of the environmental natural resources in this border region.

Competing Interests. The authors declare no competing financial interests.

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carbapenems) inactive. Furthermore, these enzymes exhibit co-resistance to many other classes of antibiotics, limiting therapeutic options. Infections caused by ESBLs range from urinary tract infections to life threatening sepsis. Consequences of AR infections in humans include longer hospitalization, intake of more toxic and expensive medications resulting in higher medical costs and higher mortality rates, and patient complications, such as spreading of infection to different body sites. Due to technical issues, ESBL detection represents a challenge. However, a combination of culture-based methods and molecular technology offers the best detection. Among the most commonly antimicrobial resistant genes (ARG) encoding ESBL enzymes include TEM, SHV, and CTX-M. The most frequently isolated ESBL-resistant bacteria are Escherichia coli (E. coli), Klebsiella pneumoniae and Pseudomonas aeruginosa.

While there are numerous reports on AR research in health care settings, limited work has been performed examining the environment as a major reservoir of antibiotic resistant bacteria and the impact on public health. With the goal of understanding the extent of AR in the ecosystem, the present study aimed to first identify multidrug resistant organisms, ARG and the most common antimicrobial residues found in waters of the Rio Grande River, which is the main source of potable water for a population of nearly 3 million people in the United States-Mexico border region.

### Driving factors of resistance in the environment

Although AR has been largely attributed to the overuse and misuse of antibiotics, there are many other factors involved in the antibiotic resistance crisis that contribute to the development and dissemination of resistant genes in the environment. Some of these include the use of antibiotics in agriculture, aquaculture and in cattle as prophylaxis and growth promoters for commercial purposes. Animals can excrete between 50% and 100% of the administered dose of some antimicrobial compounds within several days of treatment. The resulting agricultural pollution can easily reach humans via fish/meat consumption. Surface water runoff and other natural physical forces can disseminate the compounds in animal feces throughout the environment. Human antibiotic intake can also enter surface waters directly from the effluents of wastewater treatment plants (WWTPs) by excretion, flushing of old prescriptions and medical waste from clinics and hospitals. Hence, surface water is now identified as an important reservoir of AR bacteria contributing to the evolution and further spread of resistant organisms.

Other anthropogenic activities that contribute to the spread of AR bacteria include urbanization, worldwide travel, WWTPs and industrial effluents contaminated with pharmaceutical products and heavy metals. Genetic factors contributing to the rapid dissemination of AR involve the ability of ARG and mobile genetic elements such as plasmids, transposons and integrons to transfer easily into other bacteria by a variety of genetic mechanisms. The transfer of genetic material between bacterial cells is induced by stressors such as antibiotics, biocides and heavy metals. Furthermore, reports indicate that sub-lethal concentrations of antibiotics and heavy metals found in polluted environments can contribute to bacterial resistance by recruitment of resistant genes carried in mobile elements, and by maintaining MDR plasmids in host bacteria and allowing them to survive.

### United States-Mexico border region

The United States (US)-Mexico border encompasses 100 km north and south of the international boundary and is comprised of two sovereign nations, four states in the US and six states in Mexico. Along the border, 95% of the population lives in sister communities. It is estimated that about 29% of US border residents live below the poverty level. The Texas border region has high poverty rates, higher rates of uninsured and is medically underserved. The poverty rate in El Paso, Texas is estimated to be 23% and approximately 30% of

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### Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| AR           | Antibiotic resistance |
| ARG          | Antimicrobial resistant genes |
| MPN          | Most probable number |
| m/z          | Mass over charge |
| DNA          | Deoxyribonucleic acid |
| TCEQ         | Texas Commission of Environmental Quality |
| WWTP         | Wastewater treatment plants |
| WWTP         | Wastewater treatment plants |
| MDR          | Multidrug resistance |
| MPN          | Most probable number |
| m/z          | Mass over charge |
| DNA          | Deoxyribonucleic acid |
| TCEQ         | Texas Commission of Environmental Quality |
| WWTP         | Wastewater treatment plants |

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residents are uninsured. The Rio Grande River is the natural boundary between the United States and Mexico. It spans the border region between Texas, New Mexico and Mexico. The Rio Grande River provides a major source of potable and agricultural water in the New Mexico, Texas and Mexico border region. Previous reports from one of our laboratories showed that the Rio Grande exceeds standards for fecal contamination and chemical toxicity including heavy metals. Factors that may contribute to the microbial burden and the release of antimicrobial agents and pharmaceuticals into the Rio Grande include cattle farming and ranching, horse racetracks, WWTPs, septic disposal systems, animal feeding operations and other activities related to urbanization along the river. The presence of antibiotic resistant bacteria in the Rio Grande River may lead to an increased number and severity of infections, frequency of treatment failure, allergies, and alteration of intestinal flora.

The presence of antimicrobial residues in the environment is a growing public health concern. Antibiotic residues can persist in the environment after direct disposal of unused or expired medication from pharmaceutical plants, hospitals and the community. It is estimated that up to 90% of an antibiotic is not completely metabolized in humans after ingestion and can be excreted through urine or feces into the environment via domestic sewage and WWTPs. These compounds can remain in the soil and water (both surface and groundwater) for long periods of time and appear to have the ability to generate new by-products under the new aquatic environment. The presence of these antibiotic residues and their metabolites may be a novel source for antibiotic resistance development in re-emergent pathogens favoring mutations, genetic recombination and horizontal gene transfer.

To manage the antibiotic resistance crisis, it is important to identify and quantify antibiotic residues, AR bacteria and mobile genetic elements in the environment; to investigate the different mechanisms of bacterial spreading in the environment and their possible effects on human health.

**Methods**

Sampling sites were selected according to urbanization and other anthropogenic activities that could play an important role in the dissemination of AR in the environment. This project was approved by the Institutional Biosafety Committee at the University of Texas at El Paso.

**Water sampling**

Water samples were collected in one-quart cubitainers using the grab method during the months of February, April, July, September, and December 2017. Three river sites were sampled along a 26 km segment of the Rio Grande River between Sunland Park, NM and El Paso, TX. River areas selected were: Site 1) Sunland Park, NM area, upstream from the Sunland Park Drive Bridge and impacted by effluents from Sunland Park WWTP, which is responsible for the waste water system of Sunland Park and Santa Teresa, NM (coordinates: 31.798843, -106.557647); Site 2) Courchesne Bridge area, located 50 m downstream from Courchesne Bridge in El Paso, TX and 1 km downstream from the Montoya Drain, a canal in the county of El Paso where many dairy, cotton, cattle and farming products are processed (coordinates: 31.801885, -106.540540); and Site 3) River Bend area near Sunset Heights in El Paso, TX located on the US side of the river across from a public park located on the Mexican side, and selected for the high recreational activities of families and children on the river (Coordinates: 31.768277, -106.511933). Water samples were sent to laboratory facilities at the University of Texas at El Paso and El Paso Community College for molecular/chemical and microbiological analysis, respectively (Figure 1).

**Assessment of fecal contamination**

The most probable number (MPN) of E. coli/100 ml was determined using the IDEXX Colilert system (IDEXX Laboratories, Inc., Westbrook, Maine USA) as an indicator of fecal contamination according to manufacturer’s instructions.

**Identification of bacterial isolates and antibiotic profiles**

Collected water samples were filtered through 0.45 μm Millipore membrane filters. Filters were placed on selective and differential media including bile esculin azide agar, modified mTEC agar (Difco), mEndo agar and mannitol salt agar and incubated for 24 hours at 37°C. Isolated colonies were re-streaked for isolation and gram stained. Once isolated, colonies were analyzed using the MicroScan autoSCAN-4 automated bacterial identification system. MicroScan panels NBPC 34 were used to identify gram negative isolates and PBPC 20 panels for gram positive isolates as well as for determination of their corresponding antimicrobial susceptibility patterns.

**Deoxyribonucleic acid extraction from AR bacterial isolates**

Gram negative bacterial isolates showing resistance to two or
more β-Lactam antibiotics such as penicillins, broad spectrum cephalosporins and carbapenems were selected for further molecular characterization of ESBL genes (TEM, CTX-M and SHV). Resistant bacteria were cultured in Luria-Bertani broth and incubated at 35°C for 24 hours. Deoxyribonucleic acid (DNA) extraction was performed by suspending a colony from an overnight grown culture on trypticase soy agar, in 50 μl water and boiling at 100°C for 10 minutes. The integrity of the genomic DNA was assessed by electrophoresis in a 1.5% agarose gel in Tris-acetate-EDTA buffer.

Direct DNA extraction from water samples

A sample of 1500 ml of water was collected each month. Water samples were filtered through a 0.45 μm Millipore filter using a vacuum filtration unit. Deoxyribonucleic acid extraction was performed using the Rapid Water DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA, USA) following the manufacturer’s instructions.

Identification of resistance genes and mobile genetic elements by polymerase chain reaction

Multiplex polymerase chain reaction amplification was used to investigate the presence of the most common genes encoding ESBLs, TEM, SHV and CTX-M. Polymerase chain reaction amplification was performed following the protocol of Monstein et al. with slight modifications. Polymerase chain reaction amplification conditions were as follows: initial denaturation at 95°C for 15 minutes and 30 cycles at 95°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 2 minutes, followed by a final extension at 72°C for 5 minutes. Deoxyribonucleic acid from control ATCC organisms (CTX BAA-2326, SHV BAA199 and TEM BAA-196) were included in each run. Polymerase chain reaction samples were run in 1.5% agarose gel Tris-acetate-ethylenediaminetetraacetic acid (EDTA) buffer for 90 minutes at 75 volts. Polymerase chain reaction for Class 1 and 2 integrons were performed following Kotlarska’s protocol.

Controls for integrons were included in each run (Int-1 E. cloacae E705 and Int-2 A. baumannii A98). Carbapenem-producing isolates were screened using BBL™ CHROMagar™ KPC, which is a chromogenic medium designed to detect reduced sensitivity to carbapenem agents. Multidrug resistant isolates were sub-cultured onto this chromogenic medium, incubated overnight at 35°C and interpreted as directed by the manufacturer.

Chemical analysis for antimicrobial residues

Upon arrival, the aqueous samples were filtered using vacuum filtration. The samples were poured into a funnel and passed through Whatman 70 mm circular glass microfiber filters. After filtration, the filtrate was transferred...
into new, clean 1 L amber vials where the pH was adjusted to 2.0 ± 0.5 using 2 M hydrochloric acid and followed by solid phase extraction.

**Solid samples**

For each sediment sample, 20 g (wet weight) was placed inside a centrifuge tube and stored in a -20°C freezer prior to freeze drying. The sample was left in the lyophilizer for approximately 48 hours. Once the sediment was completely free of moisture, approximately 1 g (dry weight) was carefully weighed out and transferred to a clean centrifuge tube, to which 20 ml of LCMS grade acetonitrile was added. A blank with no sediment was used as the laboratory control. Next, the sediment and solvent mixtures were sonicated for 30 minutes and then centrifuged for 5 minutes at 3000 rpm. The supernatant was decanted into a clean 250 ml round bottom flask, while the sediment was further mixed with 15 ml of phosphate buffer, pH adjusted to 2.0 ± 0.5 using 2 M hydrochloric acid and additional phosphate buffer, and an additional 20 ml of acetonitrile was added. The mixtures were sonicated and centrifuged again. The supernatants were decanted and combined with the first supernatants collected in the round bottom flasks. An additional extraction using 15 ml of acetonitrile was performed to each sediment sample with sonication and centrifugation as aforementioned. The combined supernatants were concentrated using a rotary evaporator to reach a final volume of approximately 20 - 30 ml. Finally, 200 ml of reagent water and 500 mg of EDTA was added into the concentrated supernatants which were subjected to solid phase extraction.

**Solid phase extraction**

Chromabond HR-X cartridges (Macherey-Nagel Inc. Bethlehem, PA, USA) were conditioned using 5 ml methanol and 5 ml of acidified (pH 3) deionized water at 3 ml/min. The sample extracts were loaded into the solid phase extraction cartridges at 5 ml/min and then washed with 5 ml of acidified deionized water and dried for 30 minutes. The analytes were then eluted using 5 ml of methanol, 5 ml of the 50:50 vol/vol methanol and acetone at 1 ml/min. The extracts were collected in new clean centrifuge tubes and dried under nitrogen. Finally, the samples were resuspended in 200 ml of LCMS grade water with 0.1% formic acid and ammonium acetate with simetone (internal standard at 1 μM). They were sonicated to homogenize and transferred to 2 ml vials for chemical analysis.

**Calibration curves**

The internal standard and antibiotics (simetone (internal standard), azithromycin, ciprofloxacin, doxycycline, erythromycin, sulfamethoxazole, tetracycline, and trimethoprim) were re-suspended in Optima grade acetonitrile; diluted to concentrations of 0.001 μM, 0.01 μM, 0.1 μM and 1 μM, respectively, with the internal standard at a concentration of 1 μM. Sample peak areas were collected using the Thermo Xcalibur Qual Browser (Thermo Fisher Scientific, Waltham, MA, USA) with selection criteria based on the single reaction monitoring parameters as shown in Table 1. The linear regression line $R^2$ values for all standards were above 0.95 generated in Microsoft Excel.

**Ultra-high performance liquid chromatography-mass spectrometry**

Ultra-high performance liquid chromatography separation was performed on a Dionex Ultimate 3000 (Thermo Fisher Scientific). The injection volume of water samples and standards was 30 μl loaded onto a pre-equilibrated Luna Omega 1.6 μm Polar C18, 100 Å, 100 x 2.1 mm (Phenomenex, Torrance, CA) with the respective security guard column, at 10% solvent B (60% acetonitrile, 38% methanol, 2% water, 20 mm ammonium acetate, 0.2% formic acid) and 90% solvent A (100% water, 20 mm ammonium acetate, 0.2% formic acid) at a constant flow rate of 0.4 ml/min. The column temperature was set at 55°C throughout the entirety of the run. Injected samples were washed
for 1 minute in 10% solvent B before beginning the elution gradient. Solvent B was increased from 10% to 75% over 1 minute, maintained at 75% for 6 minutes, increased to 95% over 1 minute and maintained at 95% solvent B for the remainder of the 10-minute run. To re-equilibrate the column to starting conditions, a blank injection (95% solvent B, 5% solvent A) was inserted between each sample. The blank injection began at 95% solvent B for 1 minute. The gradient decreased to 10% solvent B over 1 minute and remained constant for 3 minutes. Tandem mass spectrometry data were acquired using TSQ Endura™ triple quadrupole mass spectrometer (Thermo Fisher Scientific) equipped with a heated electrospray ionization source with the following ionization parameters: positive ion (V): 4300; sheath gas (arb): 10; aux gas (arb): 5; sweep gas (arb): 1; ion transfer tube temperature (°C): 275; vaporizer temperature (°C): 20. Samples and standards were analyzed using single reaction monitoring mode with a retention time window of 3 minutes. Internal standard and antibiotics were optimized for product ion and collision energies. Table 1 lists the mass spectrometer parameters used to set up the single reaction monitoring method on the TSQ Endura™. In instances where the mass over charge (m/z) value was the same between two standards, an additional m/z value was applied to confirm detection.

Statistical analysis

All data were entered into Microsoft Excel. Statistical Package for the Social Sciences software (IBM SPSS Statistics 24) was used to determine descriptive analysis of ESBL genes and carbapenem resistant enterobacteria isolates, and the frequency distribution according to geographical sampling sites and susceptibility patterns among bacterial isolates. For inferential analysis, one sample T-test was selected to compare the mean of all water samples with the standard value of 126/100 ml. Probabilities less than 5% (p < 0.05) were considered statistically significant.

Results

Fecal contamination was found to be consistently higher than the standard set by the Texas Commission of Environmental Quality (TCEQ) (126 MPN/100 ml), exception for samples collected from Site 2 during the months of September and December. Fecal contamination appears to increase during irrigation season in the months of April and July, specifically in Site 3, where levels exceed the standard by 13.7 times. For the months of September, December and February the MPN values decreased closer to the TCEQ standard. The mean MPN value (751.5) was higher than the TCEQ set standard of 126 per colony-forming unit/ml for this segment of the Rio Grande River (p<0.01) (Figure 2).

Extended spectrum β-lactamase encoding genes and mobile genetic elements directly from water samples and bacterial isolates

Fifteen water samples were collected from three sites described in the Methods section. Extended spectrum β-lactamase genes were detected in 11 water samples (73.0%). From these, 7 (46.7%) tested positive for the identification of the CTX-M gene, and 9 (60.0%) for the TEM gene. The SHV gene was not identified in any of the water samples that were directly analyzed for the identification of ESBL genes (Figure 3). Class 1 and Class 2 integrons were identified in 11 samples.
(73.3%). Class 1 integrons were the most prevalent (Figure 4).

A total of 28 bacterial isolates were selected for molecular analysis of ESBL genes. Four (14.3%) carried the SHV gene and 13 (46.4%) the TEM gene. Four isolates (14.3%) carried both SHV and TEM genes and only one isolate (3.6%) carried both TEM and CTX-M genes, as shown in Table 2.

Identification and antimicrobial susceptibility of bacterial isolates

A total of 310 isolates were collected and processed by the MicroScan autoSCAN-4 system. Genus and species of 142 gram negative and gram positive isolates belonging to 18 bacterial genera were identified with probabilities of correct identification ranging from 92.72-99.99% as shown in Supplemental Material. From these, 91 isolates showed resistance to at least two synergistic antibiotic combinations (amoxicillin/potassium clavulanate, piperacillin/tazobactam, trimethoprim/sulfamethoxazole, ticarcillin/clavulanate and ampicillin/sulbactam). One hundred and one (101) isolates were resistant to at least four individual antibiotics. Eleven of the isolates were identified as ESBL-producing bacteria by MicroScan and 21 of the isolates were identified to be resistant to 20 or more individual antibiotics. Fifteen of these isolates were isolated in Sites 1 and 3. Multidrug resistant isolates were found in all three sites. Fourteen out of the 21 multi-resistant isolates were identified as E. coli or Klebsiella pneumonia.

A description of 142 bacterial isolates identified in this study and antibiograms can be found in the Supplemental Material. Extended spectrum β-lactamase-producing organisms and carbapenem-resistant enterobacteria are shown in Table 3.
Chemical analysis for antibiotics

The analytical method implemented was able to detect azithromycin, ciprofloxacin, doxycycline, erythromycin, sulfamethoxazole, tetracycline, and trimethoprim. The limit of detection was 0.001 µM for all antibiotics. Antibiotics were present in water and sediment in the range of 0.38 ng/L - 742.73 ng/L and 0.39 ng/L - 66.3 ng/g dry weight, respectively. From the seven antibiotics analyzed, ciprofloxacin was the most commonly detected in sediment and trimethoprim was most frequently detected in water. As shown in Table 4 and 5, at least one antibiotic was found in 80% and 100% of all sediment and water samples, respectively. Samples collected in February usually had the highest level of antibiotics and samples collected in September had the least amount of antibiotics. Site 3 had the highest levels of antibiotics found in water and sediment (Figures 5 and 6). Antibiotics were found in 92% of both water and sediment samples. Site 3, in general, had the highest levels of antibiotics found in both water and sediment and the most commonly found antibiotics in water and sediment combined were trimethoprim, ciprofloxacin, doxycycline, with 63%, 58%, and 21% occurrence, respectively.

Discussion

The present pilot study identified and characterized MDR bacteria, ESBL genes (TEM, CTX-M, SHV), Class 1 and 2 integrons, and measured antimicrobial residues present in waters of the Rio Grande River along El Paso, TX, Sunland Park, NM and Juarez, Mexico, which borders this area.

Water quality in the Rio Grande varies significantly along the border region throughout the year. Fecal coliforms and *E. coli* values ranged between 123 to 1732.87 colony-forming unit/ml. The TCEQ standard for *E. coli* is set at 126 colony-forming unit/ml. The *E. coli* counts observed during the 12-month water sample collection period changed greatly (Figure 2). All water samples collected were above the standard set by TCEQ, except during the month of December, possibly due to climate change conditions such as lower temperatures and/or water flow. The highest *E. coli* numbers occurred during the months of February, April, July and September. Site 1 and Site 2 exceeded the limit by 13.7 and 7.8 times, respectively, for the month of April. Site 3 exceeded the standard limit by 13.7 times during the month of July. The lowest *E. coli*/coliform bacteria
numbers were found in the month of December for all of the sites. These results are in agreement with recent reports from the National Park Service, which finds that the water quality in the Rio Grande River is highly variable and high numbers of bacteria occur during the months of November and December.²⁻⁶,³⁸ This significant difference shows the great variability of water flow in the Rio Grande River as influenced by climatological conditions, which dictates irrigation seasons.⁴⁰ An additional factor influencing fecal contamination could be that the adherence to regulations on water quality standards differs significantly in the sampled area, which includes three states (New Mexico, Texas, Chihuahua) and two countries (US and Mexico), impacting coliform levels. Water pollution awareness must be disseminated to people who use the river for recreational purposes as they may be exposed to MDR bacteria, leading to changes in their microbiota and they may become chronic carriers of these AR enterobacteria. Additional public health concerns may include a higher risk of gastrointestinal infections and possible lower response to antimicrobial therapy.

The identification of 142 isolates with 92–99.99% probability of correct identification, with the majority being highly resistant to multiple individual and synergistic antibiotic combinations used to treat serious infections, confirmed the potential health hazards of this segment of the river. The high number of enteric and other bacteria found is probably due to the effluents of wastewater treatment plants near the sampled area, as well as animal and human waste that impact the river. As previously reported, increased anthropogenic activities are contributors to AR bacteria, and water treatment plants are considered hotspots of AR.¹⁰,⁺⁻⁴⁻⁴³ This segment of the Rio Grande River is also impacted by migratory birds and Sites 1 and 2 are surrounded by agricultural fields, dairy farms and horse breeding facilities (Figure 1). Of greatest concern is Site 3, where children, mainly from the Mexican side, use this
The presence of plasmid-mediated β-lactamase and cephalosporin resistance was studied by the amplification of TEM, CTX and SHV genes by multiplex polymerase chain reaction. Results in the present study showed that the percentage of TEM (60%) and CTX-M (40%) correlate well with other reports from urban rivers around the world and areas where poor sanitation is a problem. In agreement with other studies, the SHV gene was only detected in Klebsiella species.

Class 1 and 2 integrons have been reported as a proxy of environmental pollution. In the present study, integrons were analyzed directly from water samples. Class 1 integron was the most prevalent (46.7%). Together, Class 1 and 2 integrons accounted for 73% of water samples collected. Previous chemical analyses in the Rio Grande River within the New Mexico-Texas region showed a high salinity and boron content. Both boron and salinity are described as markers of wastewater treatment plant effluents and correlate well with the presence of Class 1 integrons.

Antibiotics measured along the 26 km selected area of the Rio Grande were present in water and sediment in the range of 0.38 ng/L - 742.73 ng/L and 0.39 ng/g dry weight, respectively. From the seven antibiotics analyzed, ciprofloxacin was the most commonly detected in sediment and trimethoprim was most frequently detected in water. The levels of antibiotics detected for each site match the number of MDR bacteria-isolated antibiotic profiles during the months of April through September. Furthermore, the highest levels of antibiotics were found in Site 3, which is a hotspot for potential gastrointestinal and opportunistic infections and will likely affect surrounding populations that use the river for recreational activities. Consistent with our study, reports from antibiotic-contaminated water worldwide indicate that levels of trimethoprim, sulfamethoxazole followed by ciprofloxacin are high, and concentrations of ciprofloxacin have been reported to be as high as 6.5 mg/L. Since antibiotics are not completely degraded after wastewater treatment, effluents that reach the river along with agrochemicals and animal waste contribute to antibiotic contamination. Moreover, the low levels of antibiotics plus the presence of heavy metals will impact bacterial communities, exerting selection pressure to maintain plasmids and drug resistance.

Our findings suggest that the Rio Grande River is a hotspot for the presence of ARG, mobile genetic elements and antimicrobial residues. As water from the Rio Grande River is the main source of potable water and a site of recreation for some of the population, this area may represent a public health concern. Another important aspect of the border area is heavy traffic. The US-Mexico border, specifically between El Paso-Ciudad Juarez is one of the busiest international crossings in the world. In 2017 alone, the US Department of Transportation reported a total of 41 million crossings, excluding those related to commercial purposes. Metagenomic studies have shown that travel is an important factor that contributes to the rapid dissemination of AR among continents, as the human gut microbiome functions as a carrier and transport of MDR bacteria.

Currently, the health risks associated with continuous exposure to antimicrobial residues and ARG from water sources and through environmental contact is largely unknown and a very important topic of research. The challenge to health risk assessment and management is the lack of standardization and the need to establish baselines for minimal health risk exposure. More data is needed to investigate the fate and effects of antimicrobials in the environment.

Conclusions

The 26 km segment of the Rio Grande River from Sunland Park, NM to El Paso, TX and Juarez, Mexico is an area of concern due to poor water quality. The presence of multi-drug resistant bacteria, antibiotics and mobile genetic elements may be a health hazard for the surrounding populations of this binational border region. Further research is needed to critically evaluate antimicrobial gene transfer and evaluate the health risk in these border populations. Policies need to be developed for appropriate management of the environmental natural resources in this border region.

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References

1. O’Neill J. Review on antimicrobial resistance. Tackling drug-resistant infections globally: final report and recommendations [Internet]. London, UK: The Review; 2016 May [cited 2019 Jul 31]. 84 p. Available from: https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf

2. Antibiotic resistant threats in the United States, 2013 [Internet]. Atlanta, GA: Centers for Disease Control and Prevention; 2013 [cited Feb 18]. 114 p. Available from: https://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf

3. Roca I, Akova M, Baquero F, Carlet J, Canton R, Cavalieri M, Coenen S, Cohen J, Findlay D, Gysenens I, Heuer O, Kuhlmet G, Kruse H, Laxminarayan R, Liebana E, Lopez-Cerero L, MacGowan A, Martins M, Rodriguez-Bano J, Rolain JM, Segovia C, Sigauque B, Tacconelli E, Wellington E, Vila J. The global threat of antimicrobial resistance: science for intervention. New Microbes New Infect. 2015 Apr 16;6:22-9.

4. Canton R, Gonzalez-Alba JM, Galan JC. Extended-spectrum beta-lactamases: epidemiology, antibiotic resistance in gram-negative bacteria. Curr Opin Biotechnol. 2010 Sep;21(8):920-8. Available from: https://doi.org/10.1016/j.cobio.2009.11.003 Subscription required to view.

5. Rawat D, Nair D. Extended-spectrum β-lactamases in gram negative bacteria. J Glob Infect Dis [Internet]. 2010 Sep [cited 2019 Jul 31];2(3):263-74. Available from: http://www.jgid.org/text.asp?2010/2/3/263/68531

6. Nathiswesan S, Burgess DS, Lewis JS 2nd. Extended-spectrum beta-lactamas: epidemiology, detection, and treatment. Pharmacotherapy [Internet]. 2001 Aug [cited 2019 Jul 31];21(8):920-8. Available from: https://doi.org/10.1592/phco.21.11.920.34529 Subscription required to view.

7. Perez F, Endimiani A, Hujer KM, Bonomo RA. The continuing challenge of ESBLs. Carr Opin Pharmacol [Internet]. 2007 Oct [cited 2019 Jul 31];7(5):459-69. Available from: https://doi.org/10.1016/j.coph.2007.08.003 Subscription required to view.

8. Penchovsky R, Traykovska M. Designing drugs that overcome antibacterial resistance: where do we stand and what should we do? Expert Opin Drug Discov [Internet]. 2015 Jun [cited 2019 Jul 31];10(6):631-50. Available from: https://doi.org/10.1517/17464415.2015.1048219 Subscription required to view.

9. Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev. 2010 Sep;74(3):37-33.

10. Bengtsson-Palme J, Kristiannsson E, Larsson DG. Environmental factors influencing the development and spread of antibiotic resistance. FEMS Microbiol Rev. 2018 Jan 1 [cited 2019 Jul 31];42(1). Available from: https://doi.org/10.1093/femsre/fux053

11. Done HY, Venkatesan AK, Halden RU. Does the recent growth of aquaculture create antibiotic resistance threats different from those associated with land animal production in agriculture? AAPS J [Internet]. 2015 May [cited 2019 Jul 31];17(3):513-24. Available from: https://doi.org/10.1208/s12248-015-9722-x Subscription required to view.

12. Mojica ER, Aga DS. Antibiotics pollution in soil and water: potential ecological and human health issues. In: Nriagu JO, editor. Encyclopedia of environmental health [Internet]. Amsterdam, Netherlands: Elsevier Science; 2011 [cited 2019 Jul 31]. p. 97-110. Available from: https://doi.org/10.1016/B978-0-444-52272-6.00038-X Subscription required to view.

13. Wellington EM, Boxall AB, Cross P, Feil EJ, Gaze Wellington EM. Selection of a multidrug resistance plasmid by sublethal levels of antibiotics and heavy metals. mBio [Internet]. 2014 Sep/Oct [cited 2019 Jul 31];5(5):Article e01918-14 [9 p.]. Available from: https://mbio.asm.org/content/5/5/e01918-14

14. Guan Y, Jia J, Wu L, Xue X, Zhang G, Wang Z. Analysis of bacterial community characteristics, abundance of antibiotics and antibiotic resistance genes along a pollution gradient of Ba River in Xi’an, China. Front Microbiol [Internet]. 2018 Jan 1 [cited 2019 Jul 31];9:Article 3191 [12 p.]. Available from: https://doi.org/10.3389/fmicb.2018.03191

15. Seiler C, Berendonk TU. Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. Front Microbiol [Internet]. 2012 Dec 14 [cited 2019 Jul 31];3:Article 339 [10 p.]. Available from: https://doi.org/10.3389/fmicb.2012.00339

16. Burket SR, White M, Ramirez AJ, Stanley JK, Banks KE, Weller WT, Chambliss CK, Brooks BW. Corbicula fluminea rapidly accumulate pharmaceuticals from an effluent dependent urban stream. Chemosphere [Internet]. 2019 Jun [cited 2019 Jul 31];224:873-83. Available from: https://doi.org/10.1016/j.chemosphere.2019.03.014 Subscription required to view.

17. Amos GC, Gozzard E, Carter CE, Mcad A, Bowes MJ, Hawkey PM, Zhang L, Singer AC, Gaze WH, Wellington EM. Validated predictive modelling of the environmental resistome. ISME J [Internet]. 2015 Jun [cited 2019 Jul 31];9(6):1467-76. Available from: https://doi.org/10.1038/ismej.2014.237

18. Domingues S, da Silva GJ, Nielsen KM. Integrons: vehicles and pathways for horizontal dissemination in bacteria. Mob Genet Elements [Internet]. 2012 Sep 1 [cited 2019 Jul 31];2(5):21-23. Available from: https://doi.org/10.4161/mge.22967

19. Gullberg E, Albrecht ML, Karlsson C, Sandegren L, Andersson DI. Selection of a multidrug resistance plasmid by sublethal levels of antibiotics and heavy metals. mBio [Internet]. 2014 Sep/Oct [cited 2019 Jul 31];5(5):Article e01918-14 [9 p.]. Available from: https://mbio.asm.org/content/5/5/e01918-14

20. Guan Y, Jia J, Wu L, Xue X, Zhang G, Wang Z. Analysis of bacterial community characteristics, abundance of antibiotics and antibiotic resistance genes along a pollution gradient of Ba River in Xi’an, China. Front Microbiol [Internet]. 2018 Jan 1 [cited 2019 Jul 31];9:Article 3191 [12 p.]. Available from: https://doi.org/10.3389/fmicb.2018.03191

21. Office of Border Public Health: Texas – Mexico border area. In: Health in the Americas. Vol. 2, countries [Internet]. Washington, D.C.;: Pan American Health Organization; 2007 [cited 2019 May 22]. p. 732-44. Available from: https://www.paho.org/hia2007/archivosvol2/paisesing/United%20States%20 Mexico%20Border%20Area%20English.pdf

22. Office of Border Public Health: Texas – Mexico border area. In: Health in the Americas. Vol. 2, countries [Internet]. Washington, D.C.;: Pan American Health Organization; 2007 [cited 2019 May 22]. p. 732-44. Available from: https://www.paho.org/hia2007/archivosvol2/paisesing/United%20States%20Mexico%20Border%20Area%20English.pdf

24. Office of Border Public Health: Texas – Mexico border area. In: Health in the Americas. Vol. 2, countries [Internet]. Washington, D.C.;: Pan American Health Organization; 2007 [cited 2019 May 22]. p. 732-44. Available from: https://www.paho.org/hia2007/archivosvol2/paisesing/United%20States%20Mexico%20Border%20Area%20English.pdf
border [Internet]. Austin, Texas: Texas Department of State Health Services; [updated 2019 Jun 11; cited 2019 May 18]. [about 2 screens]. Available from: https://www.dshs.texas.gov/borderhealth/

25. The uninsured in Texas [Internet]. Austin, TX: Texas Medical Association; c2019 [cited 2019 May 22]. [about 3 screens]. Available from: https://www.texmed.org/uninsured_in_texas/

26. Mendoza J, Botsford J, Hernandez J, Montoya A, Saenz R, Valles A, Vazquez A, Alvarez M. Microbial contamination and chemical toxicity of the Rio Grande. BMC Microbiol [Internet]. 2004 [cited 2019 Jul 31];4:Article 17 [16 p.]. Available from: https://doi.org/10.1186/1471-2180-4-17 Subscription required to view.

27. Rios-Arana JV, Walsh EJ, Gardea-Torresdey JL. Assessment of arsenic and heavy metal concentrations in water and sediments of the Rio Grande at El Paso-Juarez metropolitan region. Environ Int [Internet]. 2004 Jan [cited 2019 Jul 31];29(7):957-71. Available from: https://doi.org/10.1016/S0160-4120(03)00080-1 Subscription required to view.

28. Kotlarska E, Luczkiewicz A, Pisowacka M, Burzynski A. Antibiotic resistance and prevalence of class 1 and 2 integrons in Escherichia coli isolated from two wastewater treatment plants, and their receiving waters (Gulf of Gdansk, Baltic Sea, Poland). Environ Sci Pollut Res Int [Internet]. 2015 Feb [cited 2019 Jul 31];22(3):2018-30. Available from: https://doi.org/10.1007/s11356-014-3474-7

29. Zhang YL, Lin SS, Dai CM, Shi L, Zhou XF. Sorption-desorption and transport of trimethoprim and sulfonamide antibiotics in agricultural soil: effect of soil type, dissolved organic matter, and pH. Environ Sci Pollut Res Int [Internet]. 2014 May [cited 2019 Jul 31];21(9):5827-35. Available from: https://doi.org/10.1007/s11356-014-2493-8 Subscription required to view.

30. Hughes SR, Kay P, Brown LE. Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems. Environ Sci Technol [Internet]. 2013 Jan 15 [cited 2019 Jul 31];47(2):661-77. Available from: https://doi.org/10.1021/es3030148

31. Fatta-Kassinos D, Mercier C, Nikolau A. Pharmaceutical residues in environmental waters and wastewater: current state of knowledge and future research. Anal Bioanal Chem [Internet]. 2011 Jan [cited 2019 Jul 31];399(1):251-75. Available from: https://doi.org/10.1007/s00216-010-4300-9

32. Aguera A, Martinez Bueno MJ, Fernandez-Alba AR. New trends in the analytical determination of emerging contaminants and their transformation products in environmental waters. Environ Sci Pollut Res Int [Internet]. 2013 Jun [cited 2019 Jul 31];20(6):3496-515. Available from: https://doi.org/10.1007/s11356-013-1586-0 Subscription required to view.

33. Gao L, Yan Y, Zhang X, Hu J, Miao Z, Wei L, Chai T. Emissions of Escherichia coli carrying extended-spectrum β-lactamase resistance from pig farms to the surrounding environment. Int J Environ Res Public Health [Internet]. 2015 Apr 16 [cited 2019 Jul 31];12(4):4203-13. Available from: https://doi.org/10.3390/ijerph1204203

34. Scott GI, Porter DE, Norman RS, Scott CH, Uygur-Diaz MI, Maruya KA, Weisberg SB, Fulton MH, Wirth EF, Moore J, Pennington PL, Schlenk D, Cobb GP, Denslow ND. Antibiotics as CECs: an overview of the hazards posed by antibiotics and antibiotic resistance. Front Microsci [Internet]. 2016 Apr 20 [cited 2019 Jul 31];3:Article 24 [15 p.]. Available from: https://doi.org/10.3389/fmars.2016.00024

35. Franck SM, Bosworth BT, Moon HW. Multiplex PCR for enterotoxigenic, attaching and effacing, and Shiga toxin-producing Escherichia coli strains from calves. J Clin Microbiol [Internet]. 1998 Jun [cited 2019 Jul 31];36(6):1795-7. Available from: https://jcm.asm.org/content/36/6/1795.long

36. Monstein HJ, Ostholm-Balkhed A, Nilsson MV, Nilsson N, Dornbusch K, Nilsson LE. Multiplex PCR amplification assay for the detection of blaSHV, blaTEM and blaCTX-M genes in Enterobacteriaceae. APMIS [Internet]. 2007 Dec [cited 2019 Jul 31];115(12):1400-8. Available from: https://doi.org/10.1111/j.1600-0463.2007.00722.x Subscription required to view.

37. Texas Commission on Environmental Quality, Chapter 307, Texas Surface Water Quality Standards. Sect. 307.7 (2018)

38. Rio Grande: water quality and river users [Internet]. Big Bend National Park, TX: National Park Service; [updated 2017 Nov 7; cited 2019 Feb 27]. [about 1 screen]. Available from: https://www.nps.gov/rgir/planyourvisit/riverwaterquality.htm

39. Ryu H, Alum A, Alvarez M, Mendoza J, Abbaszadegan M. An assessment of water quality and microbial risk in Rio Grande basin in the United States-Mexican border region. J Water Health. 2005 Jun;3(2):209-18.

40. Kortw W. Climate change impacts on agriculture in the Rio Grande river basin [Internet]. Milwaukee, WI: Center for Water Policy; 2013 [cited 2019 Jul 31]. 5 p. Available from: https://uwcm.edu/centerforwaterpolicy/wp-content/uploads/sites/170/2013/10/Rio-Grande_Agriculture_Final.pdf

41. Stalder T, Barraud O, Casellas M, Dogat C, Ploy MC. Integron involvement in environmental spread of antibiotic resistance. Front Microbiol [Internet]. 2012 Apr 9 [cited 2019 Jul 31];3:Article 119 [14 p.]. Available from: https://doi.org/10.3389/fmicb.2012.00119

42. Finley RL, Collignon P, Larsson DG, McEwen SA, Li XZ, Gaze WH, Reid-Smith R, Timinouni M, Graham DW, Topp E. The scourge of antibiotic resistance: the important role of the environment. Clin Infect Dis [Internet]. 2013 Sep [cited 2019 Jul 31];57(5):704-10. Available from: https://doi.org/10.1093/cid/cit355

43. Rodrigues-Mozar S, Chamorro S, Marti E, Huerta B, Gros M, Sanchez-Melias A, Borrego CM, Barcelo D, Balcazar JL. Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. Water Res [Internet]. 2015 Feb 1 [cited 2019 Jul 31];69:234-42. Available from: https://doi.org/10.1016/j.watres.2014.11.021 Subscription required to view.

44. Dhawale R, Macaden R, Saranath D, Nilgiriwala K, Ghadge A, Birdi T. Antibiotic resistance characterization of environmental E. coli isolated from River Mula-Mutha, Pune District, India. Int J Environ Res Public Health [Internet]. 2018 Jun 12 [cited 2019 Jul 31];15(6):Article 1247 [15 p.]. Available from: https://doi.org/10.3390/ijerph15061247

45. Bevan ER, Jones AM, Hawkey PM. Global epidemiology of CTX-M β-lactamases: temporal and geographical shifts in genotype. J Antimicrob Chemother [Internet]. 2017 Aug 1 [cited 2019 Jul 31];72(8):2145-55. Available from: https://doi.org/10.1093/jac/dkx146

46. Akiba M, Sanha B, Otagiri H, Prabhasankar VP, Taniyasu S, Yamashita N, Lee K, Yamamoto T, Tsutsui T, Joshua DI, Balakrishna K, Bairy I, Iwata T, Kusumoto M, Kannan K, Guruge KS. Impact of wastewater from different sources on the prevalence of antimicrobial-resistant Escherichia coli in sewage treatment plants in South India. Ecotoxicol Environ Saf [Internet]. 2015 May [cited 2019 Jul 31];115:203-8. Available from: https://doi.org/10.1016/j.ecoenv.2015.02.018

47. Zhao WH, Hu ZQ. Epidemiology and genetics of CTX-M extended-spectrum β-lactamases in Gram-negative bacteria. Crit Rev Microbiol [Internet]. 2013 Feb [cited 2019 Jul 31];39(1):79-101. Available from: https://doi.org/10.3109/1040841X.2012.691460

48. Ma L, Li AD, Yin XL, Zhang T. The prevalence of...
integrons as the carrier of antibiotic resistance genes in natural and man-made environments. Environ Sci Technol [Internet]. 2017 May 16 [cited 2019 Jul 31];51(10):5721-28. Available from: https://doi.org/10.1021/acs.est.6b05887 Subscription required to view.

49. Gillings MR, Gaze WH, Pruden A, Smalla K, Tiedje JM, Zhu YG. Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. ISME J [Internet]. 2015 Jun [cited 2019 Jul 31];9(6):1269-79. Available from: https://doi.org/10.1038/ismej.2014.226

50. Miyamoto S, Fenn LB, Swietlik D. Flow, salts, and trace elements in the Rio Grande: a review [Internet]. College Station, TX: Texas Water Resources Institute; 1995 [cited 2019 Jul 31]. Available from: https://oaktrust.library.tamu.edu/handle/1969.1/6160

51. Stephens DB. Evaluation of Rio Grande salinity, San Marcial, New Mexico to El Paso, Texas. Santa Fe, NM: New Mexico Environment Department; 2010 Jun 30 [cited 2019 Feb 18]. 202 p.

52. Sabri NA, Schmitt H, Van der Zaan B, Gerritsen HW, Zuidema T, Rijnaarts HH, Langenhoff AA. Prevalence of antibiotics and antibiotic resistance genes in a wastewater effluent-receiving river in the Netherlands. J Environ Chem Eng. Forthcoming.

53. Goel S. Antibiotics in the environment: a review. In: Kurwadkar S, Zhang X, Ramirez D, Mitchell FL. Emerging micro-pollutants in the environment: occurrence, fate, and distribution [Internet]. Washington, D.C.: American Chemical Society; 2015 [cited 2019 Jul 31]. p. 19-42. (ACS symposium series 1198). Available from: https://pubs.acs.org/doi/pdf/10.1021/bk-2015-1198.ch002?rand=sm3cuhih Subscription required to view.

54. Gullberg E, Albrecht LM, Karlsson C, Sandgren L, Andersson DI. Selection of a multidrug resistance plasmid by sublethal levels of antibiotics and heavy metals. mBio [Internet]. 2014 Sep/Oct [cited 2019 Jul 31];5(5):Article e01918-14 [9 p.]. Available from: https://mbio.asm.org/content/5/5/e01918-14

55. Marti E, Jofre J, Balcazar JL. Prevalence of antibiotic resistance genes and bacterial community composition in a river influenced by a wastewater treatment plant. PLoS One [Internet]. 2013 Oct 25 [cited 2019 Jul 31];8(10):Article e78906 [8 p.]. Available from: https://doi.org/10.1371/journal.pone.0078906

56. Brooks BW, Riley TM, Taylor RD. Water quality of effluent-dominated ecosystems: ecotoxicological, hydrological, and management considerations. Hydrobiol [Internet]. 2006 Feb [cited 2019 Jul 31];556(1):365-79. Available from: https://doi.org/10.1007/s10750-004-0189-7 Subscription required to view.

57. Larsson DJ. Antibiotics in the environment. Upsala J Med Sci. 2014 May;119(2):108-12.

58. 2017 Border crossing/entry data [Internet]. Washington, D.C.: Bureau of Transportation Statistics; [updated 2018 Feb 16; cited 2019 May 27]. Available from: https://www.bts.gov/newsroom/2017-border-crossingentry-data

59. Bengtsson-Palme J, Angelin M, Huss M, Kjellqvist S, Kristiansson E, Palmgren H, Larsson DG, Johansson A. The human gut microbiome as a transporter of antibiotic resistance genes between continents. Antimicrob Agents Chemother [Internet]. 2015 Oct [cited 2019 Jul 31];59(10):6551-60. Available from: https://aac.asm.org/content/59/10/6551.long

60. van der Bij AK, Pitout JD. The role of international travel in the worldwide spread of multiresistant Enterobacteriaceae. J Antimicrob Chemother [Internet]. 2012 Sep [cited 2019 Jul 31];67(9):2090-100. Available from: https://doi.org/10.1093/jac/dks214