Escherichia coli as a Potential Reservoir of Antimicrobial Resistance Genes on the Island of O'ahu

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Abstract

The problem of antimicrobial-resistant bacteria has not been adequately explored in the tropical island environment. To date, there has not been a systematic investigation into the prevalence and distribution of antimicrobial resistance determinants in the Hawaiian Islands. Urinary isolates are the most common bacterial pathogens encountered in the clinical laboratory. Therefore, the antimicrobial resistance determinant profiles of these organisms can serve as a sentinel of the overall antimicrobial resistance situation in a localized patient population. In this study, 82 clinical isolates of Escherichia coli *derived from 82 distinct patients were collected at a large medical center on the island of O'ahu. Each isolate was evaluated for the presence of antimicrobial resistance genes using a microarray-based approach. A total of 36 antimicrobial resistance genes covering 10 classes of antimicrobial compounds were identified. Most isolates were found to harbor between 3 and 5 antimicrobial resistance genes. Only a few isolates were found to harbor more than 12 genes. Significantly, a high rate of phenotypic resistance to one of the first-line treatments for uncomplicated urinary tract infection (sulfamethoxazole) was identified. This phenotype was correlated to the presence of sulfonamides and trimethoprim resistance determinants. Since* E. coli *is one of the most encountered pathogens in the hospital environment, the presence of clinically relevant resistance determinants in isolates of this organism from a clinical setting on O'ahu is a significant finding that warrants further investigation.*

Keywords

antimicrobials, antimicrobial resistance determinants, E. coli*, microarray*

Abbreviations and Acronyms

AMR = antimicrobial resistance
ARDM = antimicrobial resistance determinant microarray
AST = antimicrobial sensitivity testing
ESBL = extended-spectrum β-lactamase
SXT = sulfamethoxazole
TAMC = Tripler Army Medical Center

Introduction

Antimicrobial resistance is a serious threat to the continuation of modern antibacterial chemotherapy. Bacterial populations can develop antibacterial resistance by various mutational events on the chromosome that result in increased survivability of the population in the presence of an antibacterial agent. They can also develop resistance by acquiring exogenous genes that travel from cell to cell on mobile genetic elements or plasmids or by becoming infected by bacterial phages or other viruses that ferry various antibacterial resistance genes from strain to strain.[1,2] Since the genes carried on the bacterial chromosome are typically non-mobile, they are not likely to spread to nearby populations of bacteria, while genes carried on mobile genetic elements, plasmids, or viruses can be readily spread between populations. It is, therefore, important to identify the number and types of antibacterial resistance genes that are present in a local bacterial population and to identify whether or not they reside on the chromosome to inform the rational development of clinical interventions and preventive measures. Although numerous bacterial species cause human disease, it is impractical to study them all simultaneously. Therefore, it is essential that sentinel or indicator organisms be used to monitor the development and spread of antibacterial resistance.

Escherichia coli is a ubiquitous organism that can often lead to fatal infections in immunocompromised humans.[3,4] The abundance of this organism in the clinical laboratory makes it an ideal subject for surveying the antibacterial resistance landscapes of small clinics, community hospitals, and surrounding populations. Although it has been historically investigated as an indicator of human fecal contamination, *E. coli* can propagate in the environment outside of a human host and colonize numerous animal species.[5] Further, it is a naturally competent organism that can incorporate extracellular DNA in the ambient conditions present in environmental waters.[6-8] Regional temperature variation has been associated with increases in the rates of *E. coli* infection in the Pacific region, and it has been suggested that global warming may lead to further increases in overall infection rates.[9]

The propensity for *E. coli* to serve as a reservoir for antimicrobial determinants genes has been previously demonstrated.[10] Indeed, macrolide resistance genes that have been found to hinder the treatment of *Shigella* infections have been identified in *E. coli,* and the horizontal of these genes have led to recent increases in the detection of macrolide resistance in *Shigella* species.[11] In addition, sulfonamide resistance genes have been detected in *E. coli* strains that harbor the transmissible genetic structures that facilitate the dissemination and integration of these genes into a wide range of bacterial pathogens. It is suspected that these strains may eventually lead to an increase in the overall levels of antimicrobial resistance in currently circulating bacterial communities.[12] These properties of *E. coli* indicate that it has the potential to serve as a sentinel or indicator of the overall antimicrobial resistance profile of a localized patient population in the community hospital and large medical center setting.

The prevalence and distribution of antimicrobial resistance genes have not been adequately characterized in the Pacific region, and there have been no recent antimicrobial resistance studies on the island of Oʻahu. This island is characterized by a warm and humid tropical environment, the presence of numerous recreational and coastal waters, and a large military population.[13-15] Since seawater can serve as a source of multi-drug resistant *E. coli*, it is expected that residents and visitors may be exposed to one or more of the environmental reservoirs of this pathogen at some point during their time on the island.[16] Exposure can occur during recreational activities, by contact with miscellaneous surfaces in public and non-public spaces, and by iatrogenic exposure in clinics and hospitals.[17-19] The goal of this study was to determine the potential for *E. coli* to serve as a reservoir for antimicrobial resistance genes on Oʻahu by evaluating 82 clinical isolates that were obtained from urinary cultures collected between 2014 and 2015, analyzed between 2016 and 2017, and evaluated between 2018 and 2019.

Materials and Methods

Patient Population

This study was conducted at the Tripler Army Medical Center (TAMC) on the island of Oʻahu, in the state of Hawai**ʻ**i (21.4389°N, 158.0001°W). TAMC is the only federal medical center in the Pacific Basin; it serves a patient population of 200 000 military beneficiaries annually, including active duty and retired military, their families, and members of a variety of indigenous Pacific Islander groups. Significantly, TAMC regularly receives samples and patients from throughout the Pacific region to include the Republic of the Marshall Islands, the Republic of Palau, and the Federated States of Micronesia.

Bacterial Isolates

Since urine samples are the most common samples submitted to the clinical laboratory and since *E. coli* is the most common isolate with a potential to serve as a sentinel species, a total of 82 urinary isolates of *E. coli* were obtained from the pathology laboratory at TAMC. They consisted of pre-existing, anonymous, clinical diagnostic isolates that were stripped of all identifiers. All isolates were obtained from urine collected as part of the routine care of patients with suspected urinary tract infections. The clinical laboratory procedure for the isolation of bacterial pathogens from urine is as follows: after collection, the urine was transported to the clinical laboratory where it was used to inoculate blood agar and MacConkey plates. The plates were then incubated aerobically at 35˚C for 24 hours, and the resulting isolates were collected and re-streaked onto blood agar plates. The isolates were then supplied to the research group as pure colonies on blood agar plates. To avoid skewing our results, isolates were selected only based on their identification as *E. coli* and not based on phenotypic antimicrobial susceptibility patterns or demonstrated resistance criteria.

Antimicrobial Sensitivity Testing

Antimicrobial sensitivity testing (AST) was performed on a total of 22 out of 82 isolates due to the availability of materials and instrument availability using the Vitek 2 (bioMérieux, Inc.: Hazelwood, MO). All testing was performed following the manufacturer's instructions.

DNA Isolation and Microarray Hybridization

DNA was extracted from all 82 bacterial isolates using the MasterPure DNA and RNA Complete Purification Kit (Epicenter Biotechnologies: Madison, WI) and quantified using the Qubit fluorimeter (Invitrogen/Life Technologies: Grand Island, NY). Extracted DNA was whole genome amplified, fragmented using DNase I, biotin-labeled, and hybridized on the Antimicrobial Resistance Determinant (ARDM) Microarray (version 2 [v.2]) as previously described.[18] Microarray pre-hybridization and hybridization were performed at 60˚C in a rotisserie incubator, followed by washing and labeling with polymeric streptavidin horseradish peroxidase (S104PHR, Fitzgerald Industries: North Acton, MA). ARDM interrogation was accomplished using the ElectraSense Reader (CustomArray: Woodinville, WA). The ARDM v.2 comprises 2 240 probes, corresponding to 236 antimicrobial resistance determinant genes. Content of the microarray is described in Taitt, et al.[20]

Data Analysis

Signal processing and evaluation of the signal to noise ratio was accomplished utilizing previously established algorithms. Briefly, 2 signal thresholds were established: a high stringency threshold resulting from the mean of the lowest 2 128 probes with 3 standard deviations and a low stringency threshold resulting from the mean of the lowest 2 016 probes with 3 standard deviations. A gene detection was considered positive if at least half the probes for that gene were above the high stringency threshold, or 70% of the probes were above the low stringency threshold. A chi-square test with a significance level set at $P < .05$ was used to evaluate possible correlations between bacterial genotype and antimicrobial resistance phenotype.

Results

Patient Population and Phenotypic Antimicrobial Resistance

This study was conducted on the island of Oʻahu at the only military tertiary medical facility in the Pacific Basin. The patient population consists of men and women who were active duty service members, retired service members, military family members, and indigenous Pacific Islanders from throughout the region. A total of 82 *E. coli* isolates were collected.

Phenotypic susceptibility testing was only performed on a subset of the isolates due to resource limitations (n=22). Over

half of those isolates produced extended-spectrum β-lactamases (ESBLs, 59%); a large number were found to be resistant to ampicillin (86%), and over half were resistant to first-, second-, third-, or fourth-generation cephalosporins. Resistance to fluoroquinolones and trimethoprim/sulfamethoxazole (SXT) was present in 40% of the isolates and was of concern as they have been used as a treatment for uncomplicated urinary tract infections in the past (Table 1).[21]

Antimicrobial Resistance Gene Detection

A total of 36 unique antimicrobial resistance (AMR) genes were detected by the microarray within the tested population (Figures 1 and 2). These genes covered 10 classes of antimicrobials: β-lactams, aminoglycosides, macrolides, tetracyclines, phenicols, fluoroquinolones, quaternary amines, streptothricin, fluoroquinolones, sulfonamides, and trimethoprim. The number of genes detected in the tested population in this study was skewed, with most isolates harboring between 3 and 5 AMR genes and a small number of isolates harboring 10 or more AMR genes (Figure 3). There was an average of 4.4 and a median of 3 AMR gene detections per isolate. Five isolates harbored an astonishing 14 genes. The AMR determinants most detected were the chromosomal genes, *cmr* (70%), *mac*(A) (57%), *mac*(B) (48%), and bla_{TEM} (29%).

Seven β-lactamase genes were detected throughout the isolate set. Twenty-four isolates harbored bla_{TEM}. AST data were available for half of the bla_{TEM}-positive strains, and all but 1 was resistant to both ampicillin and ampicillin/sulbactam (data not shown), suggesting that these isolates harbored inhibitor-resistant TEM-type β-lactamases. One isolate carrying bla_{TEM} was susceptible to ampicillin and all other β-lactam antibiotics, suggesting that the bla_{TEM} gene was not transcribed or that the gene product was non-functional. Significantly, 24 isolates were found to carry genes from the $bla_{CTX\text{-}M\text{-}1}$ (13%) or $bla_{CTX\text{-}M\text{-}9}$ families of ESBLs. Members of this family are typically transmitted on plasmids, and carriage of these genes positively correlated with

Table 1. Antimicrobial Resistance Profile of Bacterial Isolates

Antimicrobial	n (%) N=22
ESBL Producer[a]	13 (59%)
Ampicillin alone	19 (86%)
Ampicillin + Sulbactam	14 (64%)
Cefazolin (first generation)	14 (64%)
Ceftazidime (third generation)	13 (59%)
Ceftriaxone (third generation)	14 (64%)
Cefepime (fourth generation)	13 (59%)
Imipenem	0 (0%)
Ertapenem	0 (0%)
Amikacin	0 (0%)
Gentamicin	1 (4%)
Tobramycin	1 (4%)
Ciprofloxacin	9 (40%)
Levofloxacin	9 (40%)
Trimethoprim-sulfamethoxazole	9 (40%)

[a] ESBL, extended-spectrum β-lactamase

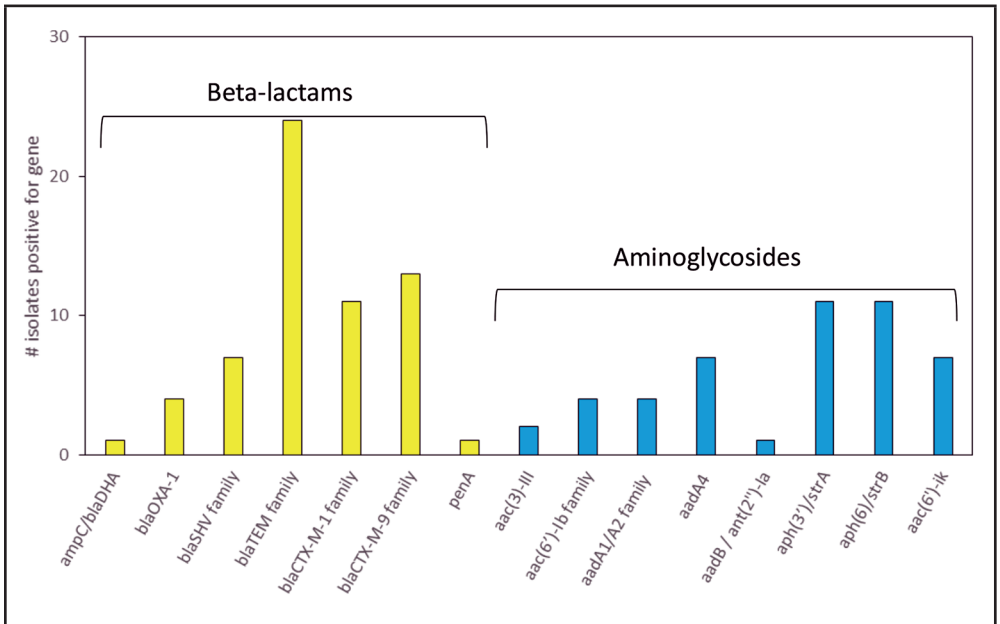

Figure 1. Distribution of Antimicrobial Resistance Genes in Bacterial Isolates[a,b]

[a] n=82 isolates; [b] Beta-lactams and aminoglycoside genes

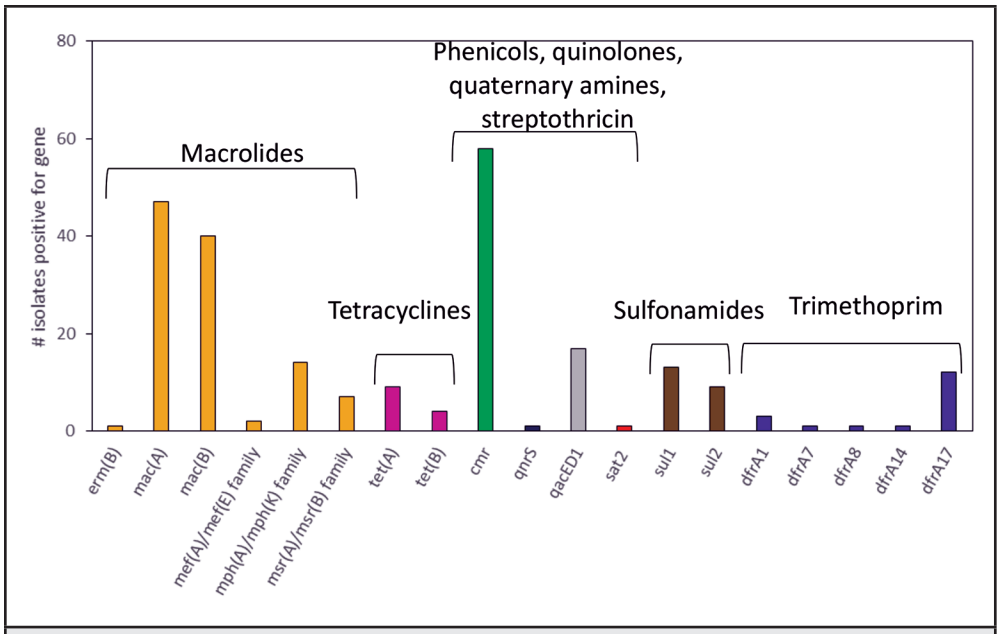

Figure 2. Distribution of Antimicrobial Resistance Genes in Bacterial Isolates[a,b]

[a] n=82 isolates; [b] Macrolides, tetracyclines, phenicols, quinolones, quaternary amines, streptothricin, sulfonamides, and trimethoprim genes

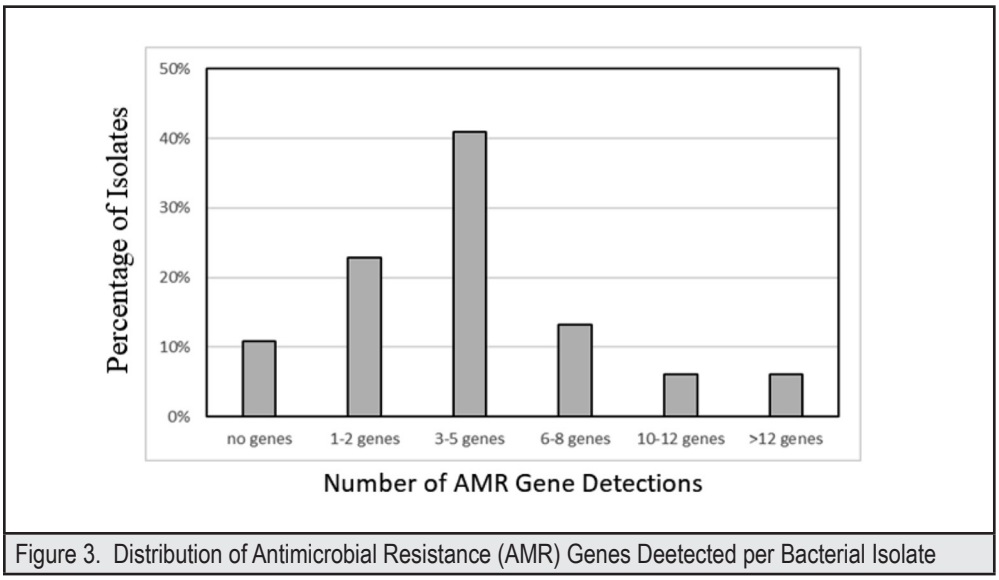

Figure 3. Distribution of Antimicrobial Resistance (AMR) Genes Deetected per Bacterial Isolate

ESBL phenotype (chi-square P=.008). Notably, none of the 83 tested strains harbored any of the 8 additional ESBL or 15 carbapenemase genes included in the ARDM v.2 chip content: bla_{BEL}, gla_{GES}, bla_{GIM}, bla_{IMI}, bla_{IMP}, bla_{KPC}, bla_{NDM}, $bla_{OXA-10/PSE}$, bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-48}, bla_{OXA-51}, bla_{OXA58}, bla_{PER}, bla_{SME}, bla_{SPM}, bla_{VEB}, $bla_{VIM.}$

A total of 11 aminoglycoside resistance genes were detected, but these were not found in a large proportion of the population under study; two-thirds of the isolates did not harbor any aminoglycoside resistance genes. The most detected aminoglycoside AMR genes were *strB* and *strA* that were often found to occur together. One isolate positive for *aac(6)-ib* was also resistant to ciprofloxacin and levofloxacin, suggesting that the detected *aac(6)-ib* gene may indeed be the fluoroquinolone resistant variant, *aac(6)-ib-cr*. However, we did not attempt to sequence this gene or the quinolone resistance-determining regions of *gyrA* and *parC*, most typically associated with high-level fluoroquinolone resistance. *QnrS*, observed in one isolate, typically confers only low-level fluoroquinolone resistance.

Although macrolides have reduced activity against gram-negative bacteria due to poor penetration of the outer membrane, it was noted that the *mac*(A)*/mac*(B) efflux pump genes were found in over 40% of the population.[22] However, the *mph*(A)/*mph*(K) genes that confer resistance to azithromycin, which is clinically useful in certain situations, were found in 16% of the study population.[23] Four tetracycline resistance genes were detected with a relatively low prevalence of 16%. Among these, the *tet*(A) gene was the most common (10%), followed by *tet*(B) (5%). The sulfonamide resistance genes, *sul1* and s*ul2*, were found in 12 and 10 isolates, respectively. Eighteen isolates harbored a trimethoprim resistance determinant, but none of them carried more than 1. Co-carriage of *sul1* or *sul2* with 1 of the trimethoprim resistance genes was observed in 13 strains and was correlated with phenotypic SXT resistance ($P = .003$).[24]

Several potential assemblages of antimicrobial resistance genes were detected. S*ul1* was found to occur in combination with the quaternary amine resistance gene *qacE*D*1* in 12 isolates. These genes are often associated with the presence of class 1 integrons.[25] One isolate was found to harbor *dfrA1, aadA1/A2*, and *sat2*, a combination often associated with class 2 integrons.[26] Six isolates were found to harbor the same unique combination of 11 genes: *aadA4, aph3/str(A), aph6/str(B), mac*(A)*, mph*(A)*/mph*(K)*, tet*(A)*, cmr, qacE*D*1, sul1, sul2,* and *dfrA17*. While some members of these genes are typically chromosomal (*cmr*, *mac*(A)), others are often found on plasmids.[27,28] It is also interesting to note that 5 of the 6 isolates also harbored $bla_{\text{CTX-M-1}}$ (3 isolates) or $bla_{\text{CTX-M-9}}$ (2 isolates), which are also typically plasmid-borne.[29] These data suggest that there may be 1 or more multi-drug resistant plasmids circulating in Hawai‘i.

Discussion

To our knowledge, this is the first surveillance study of the prevalence and diversity of AMR determinants in the Hawaiian Islands. It is important to note that the only selection criteria used for the inclusion of isolates into this study was the identification of each isolate as *E. coli*. This limitation suggests that the data obtained in this study may be somewhat representative of the overall AMR status of urinary *E. coli* isolates in the overall patient population at the time of collection. It should also be noted that although several antimicrobial resistance determinants known to be transmitted by plasmids were identified, there was no effort to separate chromosomal DNA from plasmid DNA. Therefore, the localization of a particular determinant to an individual plasmid could not be demonstrated. Some of the AMR genes detected here are not clinically relevant, including *mac*(A), *mac*(B), and *cmr*.[30] However, a significant number of isolates harbored genes considered of clinical importance. Significantly, there was a high level of phenotypic resistance (>40%) to SXT, which was correlated with co-carriage of sulfonamide and trimethoprim resistance determinants. Importantly, SXT is a first-line therapeutic agent for uncomplicated urinary tract infections.[31]

Two observations may be of particular concern to clinicians and epidemiologists. There was a high rate of carriage of CTX-M type ESBLs (29% overall), and there is a strong possibility that at least some of their genes are being carried on plasmids.[32] The even higher rate of ESBL phenotypes (59% of the 22 isolates with AST results) may indeed portend the eventual failure of third- and fourth-generation cephalosporins within the local communities. The high rate of resistance to ciprofloxacin is also of great concern; ciprofloxacin is an alternative therapeutic for uncomplicated urinary tract infection in cases of known allergy to SXT or when resistance to SXT is suspected; SXT resistance was observed in 40% of our tested isolates. Increasing prevalence of fluoroquinolone resistance in a population with already high rates of SXT resistance would significantly limit the therapeutic options available to clinicians treating urinary tract infections.

Our overall sample size was small (n=82 for genotypic analysis, n=22 for phenotypic analysis), and therefore extrapolating this result to the general bacterial population in Hawai‘i is not appropriate. However, our results point to the potential for some alarming trends. Indeed, the level of horizontal transfer of resistance determinants within the geographic confines of a relatively small Pacific island should be monitored. The emerging concept of the “coalescence” of microbial communities suggests that horizontal gene transfer tends to occur because of environmental forces that bring communities of microorganisms into contact with one another.[33] It is possible that the effects of human activity are amplified in small island environments due to the close association between the microbial, animal, fungal, and plant communities.[34] The results presented here should be followed up with more in-depth studies involving larger numbers of isolates and plasmid analysis. These studies should aim at identifying the source of the antimicrobial resistance genes that were detected in this study, and an effort should be made to determine the spatial distribution and movement patterns of those genes on the island of O‘ahu and throughout the Pacific region so that the risks associated with antimicrobial resistance can be anticipated and mitigated.

Disclaimer: The views expressed herein are those of the authors and do not reflect the position of the United States Military Academy, the Department of the Army, the Department of the Navy, or the Department of Defense.

Conflict of Interest

None of the authors identify any conflicts of interest.

Acknowledgements

This work was funded by the Army Advanced Medical Technology Initiative of the US Army Medical Research and Development Command Telemedicine and Advanced Research Technology Center.

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