Whole-genome sequencing for surveillance of antimicrobial resistance in Ecuador: present and future implications

William Calero-Cáceres[1], Nimer Ortuño-Gutiérrez[2], Temmy Sunyoto[3], Cícero-Armídio Gomes-Dias[4], Carlos Bastidas-Caldes[5], Ma. Soledad Ramírez[6], and Anthony D. Harries[7]

ABSTRACT

Whole-genome sequencing is becoming the gold standard for pathogen characterization and offers considerable advantages for understanding the evolution and dissemination of new determinants of antimicrobial resistance. Despite the benefits of whole-genome sequencing for pathogen characterization, implementation costs and lack of expertise may limit its use by public health laboratories. This article reviews the advantages of whole-genome sequencing for pathogen characterization and the current status of the use of whole-genome sequencing for antimicrobial resistance surveillance in Ecuador. A roadmap is suggested for including whole-genome sequencing for pathogen characterization based on the needs of the health reference institutions through alliances with Ecuadorian universities. Establishing a partnership between public health institutions and academia would be valuable for clinicians, policy-makers, and epidemiologists who could then take reasonable measures in those areas and establish a basis for adapting One Health strategies to tackle antimicrobial resistance in Ecuador.

Keywords Environmental health surveillance; drug resistance, microbial; whole genome sequencing; Ecuador.

In 2015, the World Health Organization (WHO) launched the Global Antimicrobial Resistance and Use Surveillance System (GLASS) to standardize antimicrobial resistance surveillance methods, including guidelines for the collection, analysis, interpretation, and sharing of antimicrobial resistance data on priority pathogens by countries at the clinical level (1). Between 2015 and 2021, 109 countries and territories joined this initiative. However, Ecuador has not yet participated (2).

The GLASS initiative provides advice and recommendations about how to use whole-genome sequencing approaches to complement the phenotypic characterization of priority pathogens. It is expected that this technique will lead to a better understanding of the transmission and inter-relatedness of microbial populations. Today, whole-genome sequencing is an integral part of the first- or second-line methods for surveillance of antimicrobial resistance in many European countries and the USA (3). However, implementing this technology into routine diagnosis and surveillance of pathogens in developing countries is challenging, constraints being implementation costs, infrastructure, capacity and expertise, and availability

1   UTA-RAM One Health, Department of Food and Biotechnology Science and Engineering, Universidad Técnica de Ambato, Ambato, Ecuador. ✉ William Calero-Cáceres, wr.calero@uta.edu.ec
2   Damien Foundation, Brussels, Belgium.
3   MSF OCB Luxembourg Operational Research (LuxOR) Unit, Luxembourg, Luxembourg.
4   Department of Basic Health Sciences, Federal University of Health Sciences of Porto Alegre, Porto Alegre, Brazil.
5   Faculty of Engineering and Applied Sciences, Universidad de las Américas, Quito, Ecuador.
6   Department of Biological Science, College of Natural Sciences and Mathematics, California State University Fullerton, Fullerton, USA.
7   International Union Against Tuberculosis and Lung Disease, Paris, France.

of reagents (4). Considering these points, the academic sector, including research institutions and universities, in low- and middle-income countries could play an important role in developing and adapting surveillance strategies to optimize the use of resources to fill knowledge gaps and, in this way, utilize the benefits of whole-genome sequencing for the surveillance of pathogens worldwide. This paper aims to review the advantages of whole-genome sequencing for pathogen characterization and the current situation on the use of whole-genome sequencing for antimicrobial resistance surveillance in Ecuador and suggests a roadmap for how to move forward with this technology.

WHOLE-GENOME SEQUENCING FOR PATHOGEN RESEARCH

The costs associated with whole-genome sequencing are decreasing rapidly, enabling broader and more affordable access to this new technology in most countries (5). Whole-genome sequencing is also becoming the gold standard for pathogen characterization at the research level. Based on a PubMed search with the term "Whole-genome sequencing of bacterial pathogens," published papers have increased substantially from 93 in 2012 to 767 in 2021. In addition, the number of biosample bacterial sequences deposited at the National Center for Biotechnology Information database was 2 042 430 up to May 2022 (6). Among the many benefits of whole-genome sequencing, this technology characterizes bacteria by identifying the species, serotype, genotype, prophage, resistance and virulence genes, genome annotation, and phylogeny, all within a single laboratory workflow (7). Despite these advantages for identifying antimicrobial resistance genotypes, some enhancements must be developed to improve their use as a tool for clinical decision-making (8). Even though whole-genome sequencing is not a substitute for phenotypic methods for detecting antimicrobial resistance for public health purposes, the many advantages of this technology are crucial for understanding the evolution and dissemination of new determinants of antimicrobial resistance (9).

Nowadays, several bioinformatic tools that do not require strong programming skills are available. For antimicrobial resistance surveillance, the online services of the Center for Genomic Epidemiology allow the typing, phenotyping, and elucidation of phylogeny in an easy interface (10). Other bioinformatics

FIGURE 1. Minimum spanning tree of the core genome multilocus sequence type (cgMLST) allelic profiles of *mcr*-1 positive *Escherichia. coli* isolates from Ecuador with whole-genome sequencing
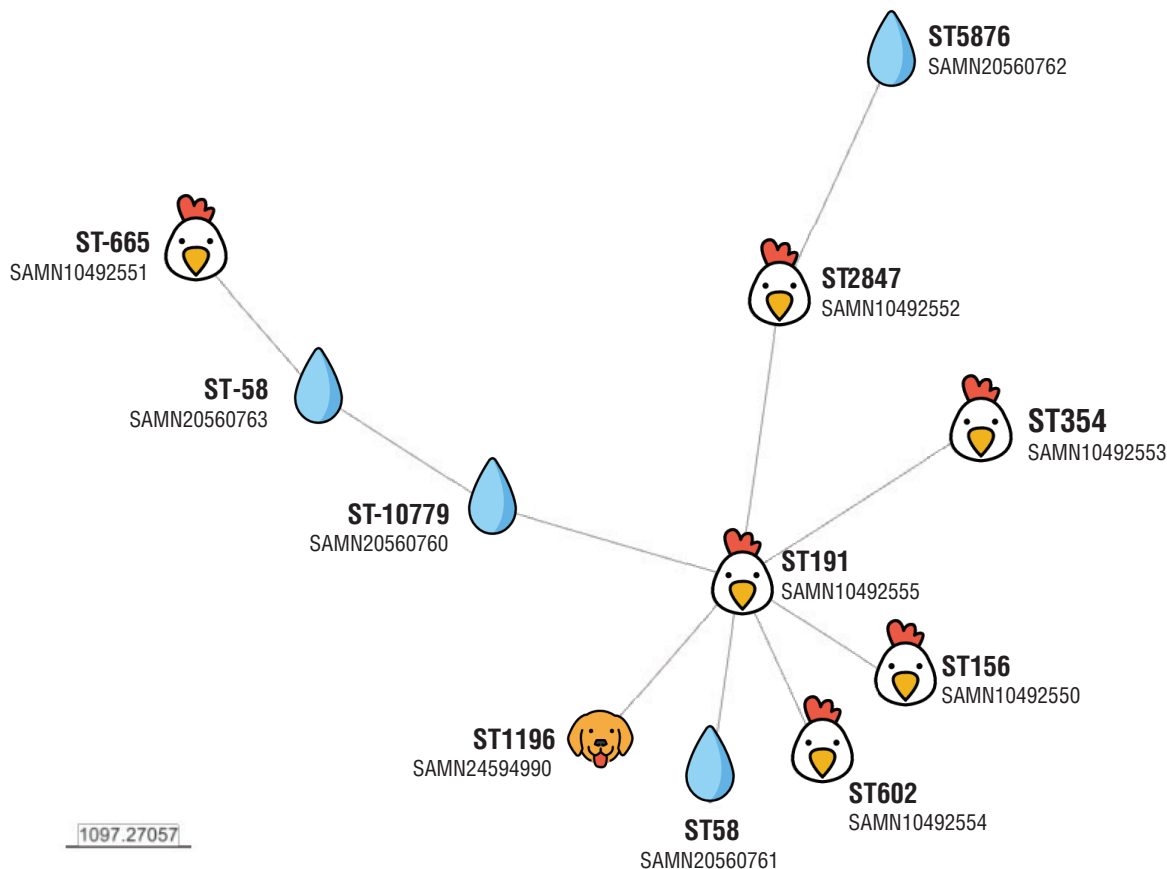
Notes: Data retrieved from at the National Center for Biotechnology Information database until June 2022.
The origin of the isolates (poultry, dog and environment) is represented using free icons (getemoji.com).
The multilocus sequence type (Warwick scheme) and the biosample number are shown.
Source: Prepared by authors based on information retrieved from the BacWGST 2.0 repository (22).

platforms that have a large number of different tools and workflows for characterization of antimicrobial resistance linked to epidemiological surveillance have been developed by the Centre for Genomic Pathogen Surveillance of the United Kingdom of Great Britain and Northern Ireland (Pathogenwatch, Microreact, Data-flo, and Epicollect5) (11). This software allows the prediction of antimicrobial resistance genotypes, geospatial analysis of dissemination of antimicrobial resistance, evaluation of potential hotspots of resistance, and visualization of antimicrobial resistance patterns in specific geographical locations. Despite the advantages of whole-genome sequencing for pathogen characterization, several challenges associated with costs and lack of expertise may limit its use by public health laboratories (12).

ANTIMICROBIAL RESISTANCE SURVEILLANCE AND ONE HEALTH IN ECUADOR

In Ecuador, the surveillance of bacterial pathogens and antimicrobial resistance in the clinical context is mainly carried out by the Reference Laboratory of Antimicrobial Resistance of the National Institute of Public Health (Instituto Nacional de Investigación en Salud Pública), using both conventional and molecular techniques (13). This laboratory serves as a support for the country's hospital network in the characterization of human pathogens. In addition, the laboratory collaborates with Ecuadorian universities by complementing specific analyses for characterization of antimicrobial resistance using both phenotypic and genotypic methods, which allows enhanced pathogen surveillance (14, 15). The laboratories of the Agency for Phytosanitary and Zoosanitary Regulation and Control (Agencia de Regulación y Control Fito y Zoosanitario AGROCALIDAD) are the reference laboratory in Ecuador for pathogen characterization in the animal sector. This institution analyzes and detects plant and veterinary pathogens using molecular and conventional techniques (16). The laboratories do not currently implement whole-genome sequencing as part of their surveillance procedures. In addition, no official analysis has been done using whole-genome sequencing by public authorities investigating antimicrobial resistance in the environment.

Implementing whole-genome sequencing techniques in Ecuador to characterize antimicrobial resistance in pathogens has been mainly the prerogative of the universities, often in collaboration with the National Institute of Public Health and foreign research institutions. For instance, the research group UNIETAR of the Central University of Ecuador maintains

TABLE 1. Genomic and general characteristics of the *Escherichia coli* harboring *mcr-1* gene whole-genome sequences from Ecuadorian samples

ID NCBI	Strain name	MLST (classic Warwick)	Clonal complex	Sampling point		Year	Sample	Antibiotic resistance genes detected (ResFinder database)
				Latitude	Longitude			
SAMN10492550	1CT 86A	156	156	–0,18	–78,46	2014	Poultry	*aac(3)-VIa, aac(6')-Ib, aadA1, aph(3'')-Ib, bla$_{CTX-M-2}$, bla$_{TEM-1B}$, catA1, catB3, dfrA1, dfrA14, mcr-1.1, mdf(A), sul1, sul2, tet(B)*
SAMN10492551	1CT 109B	665	None	–0,18	–78,46	2014	Poultry	*aac(3)-VIa, aph(3'')-Ib, aph(6)-Id ,bla$_{CTX-M-140}$, bla$_{TEM-1B}$, catA1, dfrA1, erm(B), mcr- 1.1, mdf(A), mph(B), sul1, sul2, tet(A)*
SAMN10492552	1CT 136A	2847	None	–0,18	–78,46	2014	Poultry	*aac(3)-IV, aadA1, aph(3'')-Ib, aph(4)-Ia, aph(6)-Id, bla$_{CTX-M-65}$, bla$_{KPC-3}$, dfrA17, floR, fosA3, mcr-1.1, mdf(A), sul2, tet(A)*
SAMN10492553	1CT 22A	354	354	–0,18	–78,46	2014	Poultry	*aac(3)-IId, aph(3'')-Ib, aph(3')-Ia, aph(6)-Id, bla$_{CMY-2}$, bla$_{TEM-1B}$, dfrA17, floR, fosA, mcr-1.1, mdf(A), sul2*
SAMN10492554	1CT 160A	602	446	–0,18	–78,46	2014	Poultry	*aac(3)-IV, aph(4)-Ia, aph(6)-Id, bla$_{CTX-M-65}$, bla$_{TEM-1B}$, catA1, dfrA17, floR, fosA3, fosA7, mcr-1.1, mdf(A), oqxA, oqxB, tet(B)*
SAMN10492555	1CT 188B	191	None	–0,18	–78,46	2014	Poultry	*aadA1, aadA2, bla$_{CMY-2}$, cmlA1, dfrA1, floR, mcr-1.1, mdf(A), qnrB19, sul3, tet(A)*
SAMN20560760	UTA20	10779	None	–1,19	–78,60	2018	Environment	*aadA1, aadA2, bla$_{CTX-M-55}$, cmlA1, dfrA1, fosA3, mcr-1.1, mdf(A), sul3, tet(B)*
SAMN20560761	UTA30	58	155	–0,94	–78,61	2018	Environment	*aac(3)-IV, aph(3'')-Ib, aph(3')-Ia, aph(4)-Ia, aph(6)-Id, bla$_{CTX-M-65}$, bla$_{TEM-1B}$, dfrA5, floR, fosA3, mcr-1.1, mdf(A), qnrB19, sul2, tet(A)*
SAMN20560762	UTA144	5876	None	–0,94	–78,61	2018	Environment	*aac(3)-IV, aph(3'')-Ib, aph(4)-Ia, aph(6)-Id, bla$_{CTX-M-65}$, bla$_{TEM-1B}$, floR, mcr- 1.1, mdf(A), tet(B)*
SAMN20560763	UTA175	10	10	–0,95	–78,61	2018	Environment	*aac(3)-IIa, aadA1, aadA2, aph(3'')-Ib, aph(6)-Id, bla$_{CTX-M-15}$, bla$_{TEM-1B}$, cmlA1, dfrA14, floR, mcr-1.1, mdf(A), qnrS1, sul2, sul3, tet(A), tet(M)*
SAMN24594990	UTA069	1196	1196	–1,27	–78,64	2018	Dog	*aadA1, aadA2, bla$_{CTX-M-55}$, cmlA1, dfrA12, fosA3, mcr-1.1, mdf(A), sul3, tet(A)*

MLST, multilocus sequence type.
Note: Available at the National Center for Biotechnology Information database until June 2022 (22).
Source: Prepared by authors based on information retrieved from the National Center for Biotechnology Information database (6) and BacWGST 2.0 repository (22).

a close partnership with the New York State Department of Agriculture and Markets (USA) and McGill University (Canada). Together they have worked on the characterization by whole-genome sequencing (Illumina®) of zoonotic pathogens (*Salmonella enterica* serovar Infantis and *Escherichia coli*) from the poultry sector in northern Ecuador (17). This collaboration has also been extended to other universities. For example, the research group UTA-RAM-One Health at the Technical University of Ambato (central Ecuador) used whole-genome sequencing with Illumina® sequencing to analyze strains of *S.* Infantis and *S. enterica* serovar Kentucky circulating in the layer poultry farms in this geographical area (18). This group is also investigating the dissemination of the mobile colistin resistance gene (*mcr-1*) in *E. coli* from environmental sources in collaboration with the US Food and Drug Administration, the National Institute of Public Health, the Catalan Institute for Water Research (Spain), and the Structured Operational Research and Training Initiative (SORT-IT), again using Illumina® sequencing (19). Another institution that uses whole-genome sequencing to characterize *E. coli* and *Salmonella* from animal, human, food, and environmental sources is the University San Francisco of Quito, which uses Illumina® and Oxford Nanopore Sequencing. In addition, this institution works in collaboration with the University of Minnesota (USA) (20). The study results from these university collaborations have been primarily published in journals classified as Q1 according to the ranking Scimago Scopus, which includes the most important scientific journals worldwide.

The combined strategy for analyzing circulating clones focuses on epidemiological connections between the origin of multidrug-resistant strains and the occurrence of these isolates in different sources in Ecuador. For instance, research on bacteria that harbor *mcr-1* has mainly focused on animal and environmental sources (21). Based on previous studies and the deposited sequences available at the National Center for Biotechnological Information database, a core genome multilocus sequence typing (cgMLST) analysis has been carried out, as shown by the minimum spanning tree method using the BacWGSTdb 2.0 repository (22). cgMLST phylogeny offers an accurate discriminatory capacity compared with other conventional phylogenetic methods. However, because of the limited number of isolates, it is currently not possible to find an epidemiological connection between the isolates given the complex diversity of the lineages, the scarcity of clinical isolates, and the bias about the origin of the samples, which are limited to Pichincha, Cotopaxi, and Tungurahua provinces (Figure 1). Comprehensive information about the characteristics of the samples is included in Table 1, including the antibiotic resistance genes detected *in silico* using BacWGSTdb 2.0.

WHOLE-GENOME SEQUENCING FOR PATHOGEN CHARACTERIZATION

Pathogen surveillance using whole-genome sequencing could enhance our understanding of the dissemination and evolution of antimicrobial resistance worldwide. This information would offer unprecedented opportunities to detect outbreaks, track microbial sources, detect reservoirs of antimicrobial resistance, and undertake clinical analysis. As noted above, some Ecuadorian universities are capable of performing whole-genome sequencing analysis, including both sequencing and bioinformatic analysis. Additionally, most universities and the antimicrobial resistance reference laboratories maintain solid collaborative networks between each other, as illustrated by their publication records. Although access to bioinformatics expertise and competence for routine whole-genome sequencing data analysis is still limited in Ecuador, the public entities (National Institute of Public Health, AGROCALIDAD, and

FIGURE 2. Tasks to be undertaken for implementation of WGS surveillance of antimicrobial resistance in Ecuador
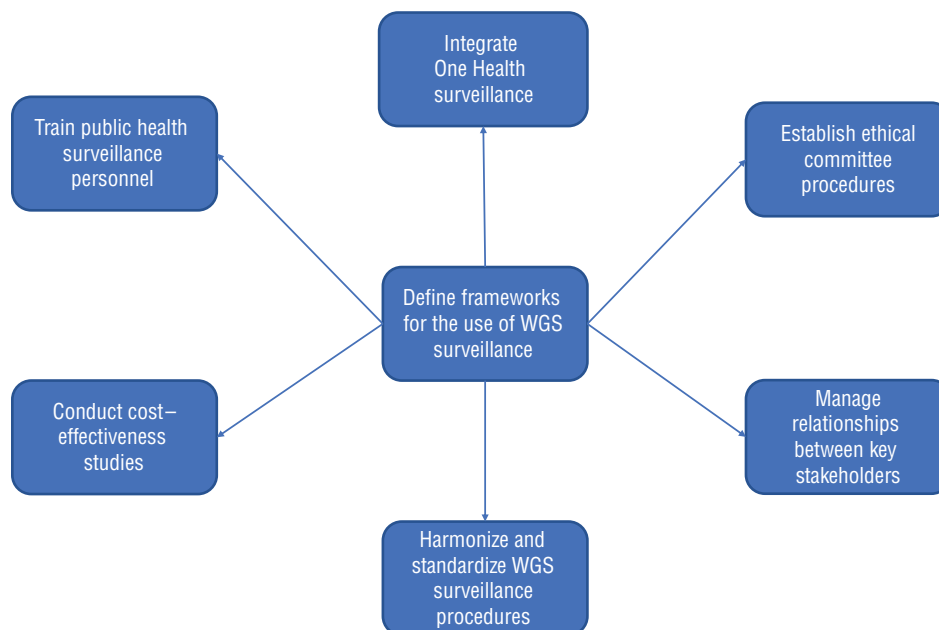
WGS, whole-genome sequencing.
Source: Prepared by the authors during the Structured Operational Research and Training Initiative (SORT-IT) course.

Ministry of Environment) could enhance collaboration with the universities that possess the skills and expertise to complement the genomic characterization.

The intrinsic characteristics of the health reference institutions and the Ecuadorian universities make it necessary to define suitable frameworks for using whole-genome sequencing surveillance (Figure 2) and establish specific responsibilities for each institution. The National Planification for the Prevention and Control of Antimicrobial Resistance (2019–2023), headed by the Ministry of Health, needs to be updated and include collaboration with academia for the surveillance of the critical pathogens. Guidelines are needed to define the participation of the ministries of health, environment, and agriculture and representation from the universities. These guidelines can be further developed to map the current situation, outline the strengths and weaknesses of both public health institutions and universities, and agree on the strategies that would combine and complement their roles in surveillance of antimicrobial resistance.

An excellent starting point could be the analysis of pathogens considered priority 1 by WHO (23) (carbapenem-resistant *Acinetobacter baumanii*, carbapenem-resistant *Pseudomonas aeruginosa,* and carbapenem-resistant, extended spectrum beta-lactamase-producing Enterobacterales) and isolates that harbor emerging antibiotic resistance genes to last-resort antibiotics (mobile colistin resistance and mobile tigecycline resistance genes) detected in the hospital network of laboratories of the National Institute of Public Health. This information would be valuable for clinicians, policy-makers, and epidemiologists, who could then take reasonable measures in those areas and establish a basis for adapting One Health strategies to tackle antimicrobial resistance in Ecuador.

CONCLUSION

We hope that this opinion paper will encourage the public health authorities in Ecuador to work more closely with academia in order for this body to play an active role in the future National Planification for the Prevention and Control of Antimicrobial Resistance, based on their experience with genomic analysis and bioinformatics, and their involvement in the One Health approach. In addition, we outline a roadmap for including whole-genome sequencing in antimicrobial resistance surveillance in Ecuador that would allow the characterization of outbreaks, assessment of reservoirs, and dissemination routes of pathogens of critical interest.

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Conflicts of interest. None declared.

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Secuenciación del genoma completo para la vigilancia de la resistencia a los antimicrobianos en Ecuador: implicaciones actuales y futuras

RESUMEN

La secuenciación del genoma completo, que está pasando a ser el estándar de referencia para la caracterización de agentes patógenos, ofrece ventajas considerables para comprender la evolución y la diseminación de los nuevos determinantes de la resistencia a los antimicrobianos. Sin embargo, a pesar de los beneficios que genera, los costos de ejecución y la falta de experiencia pueden limitar su uso por parte de los laboratorios de salud pública. En este artículo se evalúan las ventajas de la secuenciación del genoma completo para la caracterización de agentes patógenos y el estado actual del uso de la secuenciación del genoma completo en la vigilancia de la resistencia a los antimicrobianos en Ecuador. Se propone una hoja de ruta para incluir la secuenciación del genoma completo para la caracterización de agentes patógenos según las necesidades de las instituciones de salud de referencia, lo que se haría por medio de alianzas con universidades ecuatorianas. Establecer una asociación entre las instituciones de salud pública y los círculos académicos sería sumamente valioso para los médicos, los responsables de las políticas y los epidemiólogos, que podrían adoptar medidas razonables en sus ámbitos y sentar una base para adaptar las estrategias de "Una salud" a fin de abordar la resistencia a los antimicrobianos en Ecuador.

Palabras clave

Vigilancia sanitaria ambiental; farmacorresistencia microbiana; secuenciación completa del genoma; Ecuador.

Sequenciamento do genoma completo para vigilância da resistência aos antimicrobianos no Equador: implicações presentes e futuras

RESUMO

O sequenciamento do genoma completo está se tornando o padrão ouro para a caracterização de patógenos e oferece vantagens consideráveis para a compreensão da evolução e disseminação de novos determinantes de resistência aos antimicrobianos. Apesar dos benefícios do sequenciamento do genoma completo para a caracterização de patógenos, os custos de implementação e a falta de especialização podem limitar seu uso pelos laboratórios de saúde pública. Este artigo analisa as vantagens do sequenciamento do genoma completo para a caracterização de patógenos e a situação atual do uso desta técnica para a vigilância da resistência aos antimicrobianos no Equador. Sugere-se um roteiro para incluir o sequenciamento de genomas completos para caracterização de patógenos com base nas necessidades das instituições de saúde de referência, por meio de alianças com universidades equatorianas. A criação de uma parceria entre instituições de saúde pública e entidades acadêmicas seria valiosa para clínicos, formuladores de políticas e epidemiologistas, que poderiam, assim, tomar medidas razoáveis nessas áreas e estabelecer uma base para adaptar estratégias de Saúde Única para combater a resistência aos antimicrobianos no Equador.

Palavras-chave

Vigilância sanitária ambiental; resistência microbiana a medicamentos; sequenciamento completo do genoma; Ecuador.