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Antibiogram profile prediction of selected bacterial strains by in silico determination of acquired antimicrobial resistance genes from their whole-genome sequence

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Abstract

Background: The continuous increase in the resistance of pathogenic bacteria to antimicrobial agents elicits a source of concern for public health. Developing a method that allows for swift evaluation of the antibiotic sensitivity profile of bacteria is a major leap in antimicrobial research and could be one of the deciding factors in providing a lasting solution to antimicrobial resistance. The gradual and continuous reduction in the cost and turnaround time of whole-genome sequencing (WGS) has enabled scientists to develop WGS-based antimicrobial susceptibility testing using computational methods. The genes present on the ResFinder database were blasted against the WGS of the bacterial isolates obtained from NCBI database, and the best-matching genes were automatically generated by the system.

Results: Antimicrobial resistance genes were detected from the strains tested though not innate, thereby suggesting that they must have been acquired through horizontal gene transfer. Additionally, it was revealed that specific genes confer resistance to specific group of antibiotics.

Conclusion: The in silico method of antimicrobial resistance research provides for easy interpretation and reproducibility of results thereby reducing the cost and time utilized.

Keywords: Antimicrobial resistance, Whole-genome sequence, Resistance genes, ResFinder, Resistance database

Background

The emergence of multidrug-resistant bacterial strains (MDR) is limiting the effectiveness of antimicrobial therapy and making antimicrobial resistance an important area of research in biomedical science due to the threat posed by these bacteria to public health (Andersson et al. 2020). Projections have it those global annual deaths as a result of antimicrobial resistance (AMR) could rise to 10 million by the year 2050 (Mahfouz et al. 2020).

One of the measures targeted at reducing the global burden is by developing effective antimicrobial chemotherapy, and this is largely dependent on the successful testing of drug resistance of pathogenic microorganisms. Conventionally, disc diffusion, agar well diffusion, and broth microdilution (BMD) are the standard assays used for determining the antibiotic sensitivity profile of bacterial isolates, with turnaround times ranging between 24 and 72 h. However, these methods are prone to error which can occur during the preparation of the inoculum or at the culturing stage (Stoesser et al.

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2013; Su et al. 2019; Tamma et al. 2018). An error can also occur when reading the results, and this can ultimately hinder the reproducibility of results (Pedersen et al. 2018). These setbacks experienced while carrying out the standard antimicrobial susceptibility tests (ASTs) have necessitated the development of faster and more precise techniques for the detection of resistance.

Also, high-throughput automated bacterial characterization systems, such as the Vitek 2, that also perform antimicrobial sensitivity testing, are increasingly being used to characterize the resistance phenotype of bacteria to various antimicrobial compounds. These automated methods are relatively quick (when compared to the conventional AST methods), taking about 12 h after the initial isolation of bacteria (Anjum 2015). Similarly, BD Phoenix™ M50 is an automated system that has been used in the identification of bacterial species and has been validated for the determination of bacterial susceptibility (Hong et al. 2019; Sivaraman et al. 2021). Jayol et al. (2018) further stated that automated systems such as BD Phoenix, MicroScan, and Vitek2 cannot be used to detect colistin resistance due to high error rates when compared to BMD. Bayode et al. (2022) reviewed the use of isothermal microcalorimetry (IMC) calScreener and extensively discussed its use in the determination of susceptibility of Gram-positive and Gram-negative bacteria as well as biofilms of polymicrobial nature.

The gradual and continuous reduction in the cost and turnaround time of whole-genome sequencing (WGS) has enabled scientists to develop WGS-based antimicrobial susceptibility testing using computational methods (Pesesky et al. 2016; Stoesser et al. 2013; Mahfouz et al. 2020). With the ease of obtaining the WGS data of bacterial strains, it has become very easy to identify the determinants of antibiotic resistance from specific databases (Moradigaravand et al. 2018) such as ResFinder (McArthur et al. 2013), PointFinder (Zankari et al. 2012) and Comprehensive Antibiotic Resistance Database (CARD) (Zankari et al. 2017).

Among the many benefits of the WGS-based AST approach, they allow the enumeration of virtually all known AMR genes, and also allow the storage of sequenced data indefinitely for possible future analysis when new phenotypes are discovered (Stubberfield et al. 2019). As catchy as genotypic AST methods are, the main downside is characterized by the fact that only known AMR mechanisms can be detected, with a very high chance of skipping resistance caused by a variety of gene expression or new mechanisms (Bortolaia et al. 2020).

In this paper, we present an in silico antibiogram profile of selected pathogenic bacteria which have been reported in articles using the ResFinder 4.1 tool to predict their phenotypic properties to commercially-available antibiotics.

Methods

Collection of whole-genome sequence of selected pathogens

The sequences of some pathogenic bacterial strains were obtained from the National Centre for Biotechnological Information (NCBI) nucleotide database (http://www.ncbi.nlm.nih.gov), and they were appropriately referenced through a PubMed search (www.pubmed.ncbi.nlm.nih.gov). The sequences were then downloaded from the NCBI database in fasta format (.fna). The specifications of the bacterial strains are given in Table 1.

ResFinder 4.1 interface

ResFinder 4.1 is available for free at the Centre for Genomic Epidemiology online server (https://cge.cbs.dtu.dk/services) and has been embedded using the same interface as previous versions of ResFinder. The interface which is user-friendly prompts the user to make a selection from an array of bacterial species, to select the type of reads, the threshold for percentage (%) identity and the minimum length of the matching gene (s) to the whole genome. A perfect match in ResFinder is 100%, but it also covers the entire length of the resistance genes. Additionally, the interface allows the user to select the

Table 1 Specification of bacterial strains used for the study

S/N	Species	Strain	Gen Bank assembly accession	Reference literature
1	*Salmonella enterica*	PNUSAS252393	GCA_021313215.1	–
2	*S. enterica*	P-stx-12	GCA_000245535.1	Ong et al. (2012)
3	*Pseudomonas aeruginosa*	PAO1	GCA_000006765.1	Winsor et al. (2005)
4	*P. aeruginosa*	NCTC 10332	GCA_001457615.1	–
5	*Campylobacter jejuni*	NCTC 11168	GCA_000009085.1	Gundogdu et al. (2007)
6	*Klebsiella pneumoniae*	184468	GCA_022117155.1	Souvorov et al. (2018)
7	*Escherichia coli*	K-12 substr. MG1655	GCF_000005845.2	Hayashi et al. (2006)

basis for ARGs between "Acquired" and "Chromosomal Point-Mutation". In this study, our focus is on acquired ARGs. There is also a drop-down option to select from the groups of antimicrobial agents intended for the study. However, a deep knowledge of intrinsic and acquired resistance is important to aid the careful interpretation of results (Bortolaia et al. 2020).

The interface requires the user to upload the bacterial sequence in FASTA format, which has already been obtained from the NCBI database (https://www.ncbi.nlm.nih.gov).

Identification of resistance genes in whole-genome of bacteria

As described by Zankari et al. (2012), the genes present on the ResFinder 4.1 database were BLAST against the whole-genome sequences of the bacteria, and the system automatically generated the best-matching genes as output. For a gene to be considered as a best match, it has to cover not less than 2/5 of the length of the gene in the ResFinder database. The ResFinder interface allows for selection of a percentage (%) identity threshold which is the percentage of nucleotides that are identical between the best-matching resistance genes in the ResFinder interface and the corresponding sequence in the bacterial genome.

Results

The ResFinder results obtained for the 2 strains of *S. enterica*, 2 strains of *P. aeruginosa*, 1 strain each of *C. jejuni*, *K. pneumoniae* and *E. coli* are presented in Tables 2, 3, 4, 5 and 6, respectively, detailing the ARGs, percentage identity/similarity of the detected genes to the genes available on the ResFinder server and their predicted resistance phenotypes.

The ResFinder results obtained for the 2 strains of *P. aeruginosa* are presented in Table 3, detailing the ARGs, percentage identity and their predicted resistance phenotypes.

Discussion

For several decades after the discovery of antibiotics, the traditional methods for antimicrobial sensitivity testing have been disc diffusion, broth microdilution and agar well diffusion (Balouiri et al. 2016). More frequently used method for the phenotypic determination of the sensitivity of bacteria to antibiotics is the disc diffusion method, clearly showing the pattern of susceptibility or resistance to each antibiotic tested, measured through the zone of inhibition. Automated methods of testing antimicrobial resistance have been developed over the years, including the Vitek 2 system, BD Phoenix M50, Microscan, IMC calScreener among

Table 2 ResFinder results for resistance genes of 2 strains of *S. enterica*

S/N	Species	Strain	Resistance genes	Accession number	Percentage identity (%)	ResFinder predicted phenotype
1	*S. enterica*	PNUSAS252393	*aac(6')-laa*	NC_003197	97.95	AMK, TOB
2	*S. enterica*	P-stx-12	*aac(6')-laa*	NC_003197	97.47	AMK, TOB
			tet(B)	AF32677	97.00	DOX, TET, MIN

AMK Amikacin, *TOB* Tobramycin, *DOX* Doxycycline, *TET* Tetracycline, *MIN* Minocycline

Table 3 ResFinder results for resistance genes of 2 strains of *Pseudomonas aeruginosa*

S/N	Species	Strain	Resistance genes	Accession number	Percentage identity (%)	ResFinder predicted phenotype
1	*P. aeruginosa*	PAO1	*fosA*	ACWU01000146	99.51	FOS
			catB7	AFO36933	100.00	CHL
			bla_{PAO}	FJ666065	100.00	AMX, AMP, CEF, CFZ
2	*P. aeruginosa*	NCTC 10332	*fosA*	ACWU01000146	99.26	FOS
			catB7	AFO36933	98.75	CHL
			bla_{PAO}	AY08595	99.58	AMX, AMP, CEF, CFZ
			$bla_{OXA-396}$	AY306134	99.75	AMX, AMP, MER
			$bla_{OXA-494}$	AY597430	99.75	UBL
			bla_{OXA-50}	AY306130	99.75	AMX, AMP

FOS Fosfomycin, *CHL* Chloramphenicol, *AMX* Amoxicillin, *AMP* Ampicillin, *CEF* Cefepime, *CFZ* Ceftzidime, *MER* Meropenem, *UBL* Unknown beta-lactam

Table 4 ResFinder results for resistance genes of *Campylobacter jejuni*

Species	Strain	Resistance genes	Accession number	Percentage identity (%)	ResFinder predicted phenotype
C. jejuni	NCTC 11168	$bla_{OXA-193}$	CP013032	99.87	UBL
		bla_{OXA-61}	AY587956	99.87	AMX, AMP, AMX_C, AMP_C
		$bla_{OXA-489}$	CP013733	99.87	UBL
		$bla_{OXA-450}$	KR061502	99.87	UBL
		$bla_{OXA-452}$	KR061505	99.87	UBL
		$bla_{OXA-453}$	KR061507	99.87	UBL
		$bla_{OXA-451}$	KR061504	99.87	UBL

UBL Unknown beta-lactam, *AMX* Amoxicillin, *AMP* Ampicillin, AMX_C Amoxicillin + Clavulanic acid, AMP_C Ampicillin + Clavulanic acid

Table 5 ResFinder results for resistance genes of *Klebsiella pneumoniae*

Species	Strain	Resistance genes	Accession number	Percentage identity (%)	ResFinder predicted phenotype
K. pneumoniae	184468	*OqXA*	EUB70913	99.23	CHL, NAL, CPR, TRI
		dfrA1	X00926	100.00	TRI
		dfrA12	AM040708	100.00	TRI
		sul1	U12338	100.00	SMO
		sul2	AY034138	100.00	SMO
		OqXB	EU370913	98.79	CHL, NAL, CPR, TRI
		aac(3)-lld	EU022314	99.88	APR, GEN
		aadA1	JX185132	99.75	TOB, SPE, STP
		armA	AY220558	100.00	AMI, GEN, TOB, ISE
		aac(6')-lb-cr	DQ303918	100.00	CPR
		aadA2	JQ364967	100.00	SPE, STP
		aph(6)-ld	CP000971	100.00	STP
		aph(3')-VI	KC170992	100.00	AMK
		fosA	ACWO01000079	99.29	FOS
		mph(E)	DQ839391	99.89	ERY
		ere(A)	FN396877	100.00	ERY
		msr(E)	FR151518	100.00	ERY, AZT
		AAR-2	HQ141279	100.00	RFP
		bla_{SHV-56}	EU586041	99.54	AMX, AMP, AMX_C, AMP_C
		bla_{SHV-89}	DQ193536	99.54	AMX, AMP, CEP
		bla_{SHV-76}	AM176551	99.54	AMX, AMP, CEP
		$bla_{CTX-M-15}$	AY044436	100.00	AMX, AMP, CEF, CFT, CFZ

CHL Chloramphenicol, *NAL* Nalidixic acid, *CPR* Ciprofloxacin, *TMP* Trimethoprim, *SMO* Sulfamethoxazole, *APR* Apramycin, *GEN* Gentamycin, TOB Tobramycin, *SPE* Spectinomycin, *STP* Streptomycin, *AMK* Amikacin, *FOS* Fosfomycin, *ISE* Isepamicin, *ERY* Erythromycin, *AZT* Azithromycin, *RFP* Rifampicin, *AMX* Amoxicillin, AMX_C Amoxicillin + Clavulanic acid, *AMP* Ampicillin, AMP_C Ampicillin + clavulanic acid, *CEP* Cephalotin, *CEF* Cefepime, *CFT* Cefotaxim, *CFZ* Ceftazidime

Table 6 ResFinder results for resistance genes of *Escherichia coli*

Species	Strain	Resistance genes	Accession number	Percentage identity (%)	ResFinder predicted phenotype
E. coli	K-12 substr. MG1655	*formA*	X73835	81.09	FOR

FOR Formaldehyde

others (Anjum 2015; Hong et al. 2019; Jayol et al. 2018; Sivaraman et al. 2021; Bayode et al. 2022).

The traditional method of bacterial resistant genes testing often involves the use of polymerase chain reaction (PCR), in which only one or a few genes are tested using specific primers (Ochman et al. 1998). This method does not fully account for all the resistance

genes that are present in the MDR bacteria (Zankari et al. 2012).

Very recently, WGS-based sensitivity testing methods have been developed (Mahfouz et al. 2020), reducing the amount of time used in testing by predicting the phenotypes of resistance through the enumeration of the ARGs present in the genome of the bacteria and also reducing the cost of testing drastically. WGS-based methods give the advantage of providing complete information on all the genes present, and as a result cancels out the need of carrying out new experiments to search for novel genes (Zankari et al. 2012).

In this study, we have been able to identify all the ARGs in 2 strains of *Salmonella enterica*, 2 strains of *Pseudomonas aeruginosa* and 1 strain each of *Campylobacter jejuni*, *Klebsiella pneumoniae* and *Escherichia coli* using their whole-genome data collected from the NCBI database (https://www.ncbi.nlm.nih.gov). The 2 strains of *Salmonella enterica* studied contain the *aac(6')-laa* gene, which is predicted to confer resistance to amikacin and tobramycin. A study by de Toro et al. (2010) showed that *S. enterica* serovar Typhimurium DT104B strain, having acquired the *aac(6')-Ib-cr* gene located on a non-typeable plasmid was resistant to amikacin, tobramycin among other antibiotics.

The 2 strains of *P. aeruginosa* studied were predicted to exhibit resistance to amoxicillin, ampicillin, cefepime, ceftazidime and meropenem through the presence of bla_{PAO} *and* $bla_{\text{OXA-396}}$. They were also predicted to be resistant to chloramphenicol and Fosfomycin by the presence of *catB* and *fosA* genes, respectively. Hancock (1998) reported that *P. aeruginosa* is a highly difficult bacterium to treat with disinfectants or antibiotics, particularly the antimicrobial resistant types acquired by cystic fibrosis patients (Henwood et al. 2001). Berra et al. (2010) reviewed that fosfomycin was effective against 90% of MDR *P. aeruginosa*, a claim that is closely similar to the report of CDC (2009), where they reported that 375 of 385 (97.4%) of *Pseudomonas* sp. were susceptible to fosfomycin. However, the acquisition of the *fosA* gene by *P. aeruginosa* strains PAO1 and NCTC 10332 through horizontal gene transfer makes it very likely that they will be resistant to fosfomycin as predicted by ResFinder.

The *C. jejuni* strain NCTC 11168 is predicted to be resistant to beta-lactam drugs (e.g. amoxicillin, ampicillin and some unknown beta-lactam drugs) as a result of the expression of the bla_{OXA} genes. A study by Proietti et al. (2020) evaluated the β-lactamase-mediated resistance of *Campylobacter* sp. to β-lactam drugs. About 90% of the strains studied were resistant to amoxicillin/clavulanic acid. Their study concluded that the inhibitory action of ticarcillin combined with clavulanic acid is lowered in strains that had the $bla_{\text{OXA-61}}$ gene highly expressed.

The *K. pneumoniae* strain 184468 is predicted to be resistant to different classes of antibiotics, owing to the vast array of ARGs detected in its whole-genome sequence. Majority of the antimicrobial resistance encountered in *K. pneumoniae* is as a result of acquired ARGs through horizontal gene transfer (Rozwandowicz et al. 2018).

Conclusions

In conclusion, we have presented an in silico antibiogram profile of some selected pathogenic strains whose whole-genome sequences were obtained from the NCBI database, having detected the presence of some acquired ARGs using the ResFinder 4.1 tool. The results obtained can be easily interpreted and reproduced thereby solidifying the transition of antimicrobial resistance research from the traditional methods to computational methods. Also, culture-independent genotyping and virulence genes identification can be performed with the ResFinder.

Abbreviations

AMR: Antimicrobial resistance; ARG: Antimicrobial resistance gene; AST: Antimicrobial sensitivity test/testing; BMD: Broth microdilution; BLAST: Basic Local Alignment Search Tool; CDC: Centre for Disease Control and Prevention; IMC: Isothermal microcalorimetry; MDR: Multidrug-resistant; NCBI: National Centre for Biotechnology Information; PCR: Polymerase chain reaction; WGS: Whole-genome sequence; AMK: Amikacin; AMP: Ampicillin; AMP_C: Ampicillin + clavulanic acid; AMX: Amoxicillin; AMX_C: Amoxicillin + clavulanic acid; APR: Apramycin; AZT: Azithromycin; CEF: Cefepime; CEP: Cephalotin; CFT: Cefotaxim; CFZ: Ceftzidime; CHL: Chloramphenicol; CPR: Ciprofloxacin; DOX: Doxycycline; ERY: Erythromycin; FOR: Formaldehyde; FOS: Fosfomycin; GEN: Gentamycin; ISE: Isepamicin; MER: Meropenem; MIN: Minocycline; NAL: Nalidixic acid; RFP: Rifampicin; SMO: Sulfamethoxazole; SPE: Spectinomycin; STP: Streptomycin; TET: Tetracycline; TMP: Trimethoprim; TOB: Tobramycin; UBL: Unknown beta-lactam.

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Author contributions

OJB and AFO designed the study, developed the methodology and carried out the analysis. MTB, SOB and AMO verified the methodology and proof read the manuscript. OJB performed a similarity check on the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The authors are willing to share all data used in this study upon reasonable request to the corresponding author.

Declarations

Ethics approval and consent to participate

No ethics approval was required for this study.

Consent for publication

Not applicable.

Competing interests
The authors have declared no competing interest.

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