

Research Paper

SElfies and CELLfies: Whole Genome Sequencing and Annotation of Five Antibiotic Resistant Bacteria Isolated from the Surfaces of Smartphones, An Inquiry Based Laboratory Exercise in a Genomics Undergraduate Course at the Rochester Institute of Technology

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Abstract

Are touchscreen devices a public health risk for the transmission of pathogenic bacteria, especially those that are resistant to antibiotics? To investigate this, we embarked on a project aimed at isolating and identifying bacteria that are resistant to antibiotics from the screens of smartphones. Touchscreen devices have become ubiquitous in society, and it is important to evaluate the potential risks they pose towards public health, especially as it pertains to the harboring and transmission of pathogenic bacteria that are resistant to antibiotics. Sixteen bacteria were initially isolated of which five were unique (four *Staphylococcus* species and one *Micrococcus* species). The genomes of the five unique isolates were subsequently sequenced and annotated. The genomes were analyzed using *in silico* tools to predict the synthesis of antibiotics and secondary metabolites using the **ant**ibiotics and **S**econdary **M**etabolite **A**nalysis **S**Hell (antiSMASH) tool in addition to the presence of gene clusters that denote resistance to antibiotics using the **R**esistance **G**ene **I**dentifier (RGI) tool. *In vivo* analysis was also done to assess resistance/susceptibility to four antibiotics that are commonly used in a research laboratory setting. The data presented in this manuscript is the result of a semester-long inquiry based laboratory exercise in the genomics course (BIOL340) in the Thomas H. Gosnell School of Life Sciences/College of Science at the Rochester Institute of Technology.

Key words: smartphones, touchscreen, public health, antibiotic resistance, *Staphylococcus*, *Micrococcus*, secondary metabolites

Introduction

Antibiotic resistance is one of the major public health challenges of the 21st century. The recent death of a United States citizen who became ill with an infection caused by *Klebsiella pneumoniae* that was resistant to twenty-six antibiotics highlights this important issue (1). Pathogenic bacteria can colonize a

number of everyday objects such as dishwashers, personal computers and smartphones, which have become ubiquitous in daily life (2-4). They act as potential vectors for the transmission of bacteria, many of which could be pathogenic and/or resistant to clinically relevant antibiotics. Mobile phones have

already been shown to be frequently contaminated with potential pathogens in universities and hospitals (5, 6). Studies have even found that the harboring of bacteria that are resistant to antibiotics is more prevalent on smartphones with touchscreens compared to phones that do not have touchscreens (7). The surfaces of electronic devices in hospitals have already been identified as potential infection threats to patients (3). Alongside personal mobile phones, devices with touchscreens are increasingly being used in public settings. The prevalence of shared public touchscreen devices meant for usage as payment kiosks and entertainment presents a particular risk for transmission of potentially pathogenic bacteria amongst vulnerable segments of the population. For example, touchscreens in chain restaurants are becoming very popular and are often used by young children for entertainment. Many children prefer “finger foods” and may not practice sufficient hand hygiene before food consumption. Therefore, such devices have the potential to become vectors for bacteria and other organisms. Studies conducted so far regarding the presence of bacteria on touchscreen devices have yielded mixed results. There is not a significant difference between the rates of incidence of microbial contamination on touchscreen cell phones versus cell phones that are of the keypad variety (8). A separate study concluded that touchscreen phones have even a lower risk of contamination than keypad phones (9). This difference in the literature pertaining to which type of device is more prevalent regarding the harboring bacteria is not settled. However, because of how widespread touchscreens have become, including and extending beyond mobile phones, the specific types of bacterial contamination are a potential source for concern. Many bacteria isolated from personal smartphones and other touchscreen devices are deemed a normal constituent of the human skin microbiome. For instance, a study conducted at a German university found that Gram-positive cocci associated with then human skin were among the most common microbes isolated from touchscreen smartphones (10). In addition, a 2014 study using Illumina-based next generation sequencing showed an 82% overlap of the dominant operational taxonomic unit (OTU) being shared between mobile phone surfaces and the index finger skin microflora of the respective owners (11). Resistance to both commonly used and last-resort antibiotics has been found in various environmental settings. A study assessing the bacterial diversity from the phones of students and health care workers in Gaza found that over a quarter of the *Staphylococcus aureus* isolates

were resistant to methicillin, and almost 4% were resistant to vancomycin (12). Another study found hospital touchscreens that were contaminated with vancomycin resistant *Enterococcus* bacteria (13). The presence of antibiotic resistance in hospital settings is especially disconcerting since some patients are particularly susceptible to opportunistic infections.

Here we report the isolation, identification, whole genome sequencing and annotation of five antibiotic resistant bacteria from the surfaces of student owned smartphones. This study was facilitated by an inquiry based laboratory exercise in a genomics course (BIOL 340) in the Thomas H. Gosnell School of Life Sciences on the campus of the Rochester Institute of Technology. The course is primarily designed to introduce undergraduate students to next generation sequencing employing *in vivo* and *in silico* aspects of bacterial genomics using questions related to antibiotic resistance as the overarching theme.

Methods

Isolation of bacteria from the screens of smartphones

Sixteen student owned smartphones were used in this study. Bacteria were initially isolated on agar plates of tryptic soy (TS), potato dextrose (PD), Reasoner’s 2A (R2A), and Luria broth (LB) using sterile cotton swab moistened with R2A broth. The plates were incubated for 30°C for 48-96 hours. Figure 1 shows the diversity of organisms cultured on a TS agar plate from the screen of one of the smartphone devices.

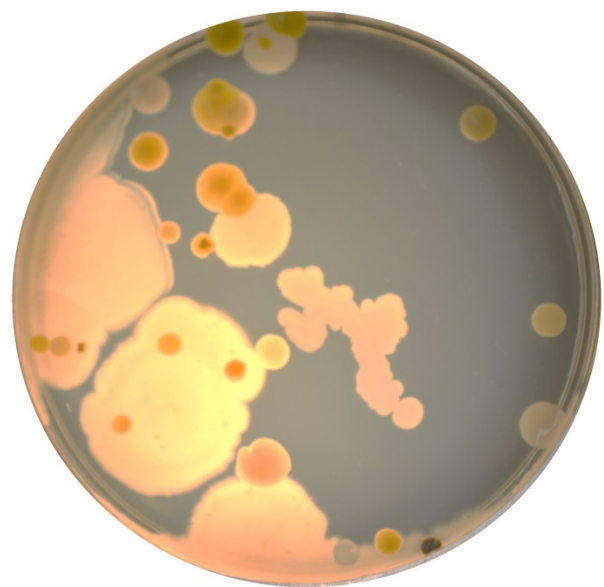


Figure 1. Example of bacteria cultured from one of the smartphones used in this study. The bacteria were cultured on a tryptic soy (TS) agar plate incubated at 30°C for 48 hours.

Table 1. Genome annotation information for the isolated strains.

Organism	Accession no.	Genome size (bp)	% GC Content	Genome Coverage (X)	No. of contigs	No. of ORFs	No. of tRNAs	No. of rRNAs
<i>Staphylococcus pasteurii</i> sp RIT 605	RJLY01000000	2,546,404	31.47	202	28	2,502	60	4
<i>Micrococcus</i> sp RIT 608	RJLX00000000	2,482,291	72.97	243	140	2,332	48	3
<i>Staphylococcus epidermis</i> sp RIT 611	RJMA01000000	2,483,518	31.98	251	49	2,470	56	4
<i>Staphylococcus hominis</i> sp RIT 612	RDSL00000000	2,146,799	31.44	239	46	2,179	53	4
<i>Staphylococcus cohnii</i> sp RIT 614	RJLZ01000000	2,728,720	32.43	209	44	2,653	53	4

The genome size and the GC content were obtained from the Quality Assessment Tool for Genome Assemblies (QUAST) and the other annotation features were obtained from the Prokaryotic Genome Annotation Pipeline (PGAP).

Genomic DNA isolation and PCR amplification of the 16S V3/V4 rDNA regions

Genomic DNA was extracted from 1 mL of individual bacteria grown in TSA broth using MolBio DNA extraction kit according to the manufacturer's instructions. The genera of the bacteria were initially identified through nucleotide sequence analysis of the variable 3 and 4 (V3/V4) regions of the 16S rRNA gene, using the 314f/805r primer set (14). The V3/V4 regions were amplified using 15 picomoles of forward and reverse primer (5'-CCTACGGGNGGCWGCAG-3' and 5'-GACTACHVGGGTATCTAATCC-3') using the GoTaq™ Green PCR master mix (Promega). The following PCR conditions were used: 1 cycle at 95°C for 5 minutes, followed by 30 cycles at 95°C for 30 seconds, 55°C for 30 seconds and 72°C for 1 minute. The ~500 bp V3/V4 amplicons were resolved by electrophoresis on a 0.8% (w/v) agarose gel followed by gel extraction using the QIAquick Gel Extraction Kit (Qiagen) followed by Sanger nucleotide sequencing in both directions using the primers that were used for amplification. The individual genera were initially identified using the Basic Local Alignment Search Tool (BLAST) querying the 16S ribosomal RNA sequence database (15).

Bacterial genome sequencing, assembly and annotation

For whole genome sequencing, the genomic DNA was processed using the Nextera XT (Illumina), quantified using a Qubit 2.0 fluorometer and sequenced using a V3 600 cycle cartridge on the MiSeq Illumina platform at the Rochester Institute of Technology Genomics Facility. Sequence quality was assessed using FASTQC version 0.11.6. Adapter trimming was performed on the raw paired-end reads using Trimmomatic version 0.36.5 (16). The trimmed reads were subsequently assembled *de novo* with Unicycler version 0.3.0b with a minimum contig length of 200 bp (17). Genome assembly metrics were evaluated using the Quality Assessment Tool for Genome Assemblies (QUAST) version 4.1 (18).

Completed FASTA assemblies were uploaded to

the NCBI Whole Genome Shotgun project for annotation via the Prokaryotic Genome Annotation Pipeline (PGAP). Secondary metabolite biosynthesis pathways were predicted by uploading completed genome assemblies to antiSMASH using the most recent version 4.6 as of January 7, 2019, using default parameters (20). Each assembled genome was also uploaded to the Comprehensive Antibiotic Resistance Database (CARD), and antibiotic resistance gene prediction was performed using the Resistance Gene Identifier (RGI) version 4.2.2 (21).

Antibiotic resistance/susceptibility assay

Bacteria were streaked on individual LB agar plates supplemented with the antibiotics ampicillin, kanamycin, chloramphenicol, or gentamicin to a final concentration of 50 µg/mL. To ensure consistency with respect to the number of bacterial colonies on each plate, one colony was chosen, suspended and mixed by vortexing in 100 µL of LB broth. Five microliters of the bacteria suspension was pipetted onto each plate, and was then streaked. The inoculated plates were incubated at room temperature for four days to assess resistance and/or susceptibility.

Results and Discussion

Growth was observed on tryptic soy, potato dextrose, and Luria broth after 48 hours when compared to Reasoner's 2A medium that showed growth after 96 hours. Based on the initial amplification and sequencing of the 16S rRNA gene, 5 unique bacteria from the screen were chosen for whole-genome sequencing and annotation. The annotation features for each of the bacterial genomes are summarized in Table 1. The antibiotic resistance/susceptibility assay revealed that the five isolated strains were resistant to ampicillin, but sensitive to gentamicin and chloramphenicol, while two of the *Staphylococcus* strains were also able to resist kanamycin (Table 2). Analysis employing antiSMASH showed the production of various secondary metabolites, including antibiotics (Table 3). The results show that all five isolates possess gene clusters for the synthesis of siderophores. Since the host

imposes iron limitation, the ability to produce siderophores offers a selective advantage for pathogens. The production of siderophores has been correlated with resistance to carbanepam antibiotics, particularly in *P. aeruginosa* clinical isolates (22). However, the occurrence of resistance to individual antibiotics did not follow any particular pattern (23). Moreover, siderophores have a wide variety of functions including the regulation of gene expression and quorum sensing (24). Interestingly, *Micrococcus* sp RIT 608 was the only isolate that possessed a gene cluster for ectoine, a compound that is involved in protecting bacteria from osmotic stress. Given the fact that the bacteria were resistant to antibiotics as per the results in Table 2, we wanted to assess if the isolates possess gene clusters that confer resistance to other antibiotics. The results in Table 4 show a number of predicted gene clusters with homology to antimicrobial resistance genes. Predicted gene clusters were not identified in RIT 608 even though the bacterium is resistant to both ampicillin and kanamycin. Please note that the bacteria in this study could be resistant to additional antibiotics that were not included in this study. Even if the isolates reported in this study are not virulent pathogens, the occurrence of terpene and NRPS biosynthesis gene clusters could indicate antibiotic production by skin commensal *Staphylococcus* strains. Lugdunin is a recently discovered antibiotic produced by *Staphylococcus lugdunensis*, which commonly colonizes the human nasal cavity and offers protection against *Staphylococcus aureus* (25).

Table 2. Summary of antibiotic resistance/susceptibility.

Strain	Ampicillin (50 µg/mL)	Kanamycin (50 µg/mL)	Gentamicin (50 µg/mL)	Chloramphenicol (50 µg/mL)
RIT 605	+	-	-	-
RIT 608	+	+	-	-
RIT 611	+	+	-	-
RIT 612	+	-	-	-
RIT 614	+	-	-	-

+ indicates resistance and - indicates susceptibility.

Table 3. Summary of the antibiotics and secondary metabolite analysis shell (antiSMASH) analysis showing the presence/absence of gene clusters involved in the synthesis of antibiotics and secondary metabolites.

Strain	Terpene	Ectoine	Siderophore	Non-ribosomal peptide synthetase
RIT 605	-	-	+	-
RIT 608	+	+	+	-
RIT 611	-	-	+	+
RIT 612	+	-	+	-
RIT 614	+	-	+	-

+ indicates the presence of a gene cluster and - indicates that a cluster was not identified.

Table 4. Summary of antimicrobial resistance gene prediction using the Comprehensive Antibiotic Resistance Database (CARD) database v3.0.0 and the Resistance Gene Identifier (RGI) tool v4.2.2.

Strain	Drug Class	% ID of matching region	% Length of Reference Sequence
RIT605	Fusidic acid	100	100
RIT608	-	-	-
RIT611	Streptogramin antibiotic, Macrolide antibiotic	100	100
	Tetracycline antibiotic, Fluoroquinolone antibiotic, Penam, Peptide antibiotic, Cephalosporin, Acridine dye	95.24	100
	Penam	94.66	100
	Diaminopyrimidine antibiotic	98.14	100
	Fluoroquinolone antibiotic, Acridine dye	99.48	100
RIT612	Streptogramin antibiotic, Macrolide antibiotic	99.98	100
	Fluoroquinolone antibiotic	99.03	100
	Macrolide antibiotic	90.3	100
RIT614	Macrolide antibiotic	90.3	100
	Streptogramin antibiotic, Macrolide antibiotic	98.98	100

The rise in the number of multidrug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin intermediate and resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, among others, has led to a significant increase in the morbidity and mortality of humans infected with pathogenic bacteria (26). In the UK, at least 10% of phones used by patients in a hospital were contaminated by hospital-associated pathogens (27). Several studies documented the risk of pathogenic contamination of mobile phones in hospitals with or without antibiotic resistance, and some suggested these as the source of hospital-acquired infections (28-31). One study reported a higher prevalence of MRSA on mobile phones vs the hands of hospital workers in the intensive care and surgical units, suggesting the enrichment of resistant strains rather than merely indicating the presence of skin commensals (32). Another study reported the prevalence of antibiotic resistant bacteria on the surface of mobile phones used by patients in a hospital (33). From a public health perspective, our study further supports the need for awareness of the threat that touchscreen devices may pose with respect to the harboring and/or transmission of pathogenic bacteria, especially those that are resistant to clinically relevant antibiotics. The regular disinfection of patient and hospital worker cell phones and clear usage guidelines for both patients and staff could minimize the risk of nosocomial infections. The current study lends further support to the observation that antibiotic resistant bacteria colonize mobile phone touchscreens that are potential vectors for the

transmission of bacteria in a number of settings, other than just the well-studied case of hospitals. It should be noted that in addition to smartphones, other kiosk-like devices at airports, automatic teller machines, restaurants, hotels among others are potential vectors for the transmission of antibiotic resistant pathogens especially since these devices are used by more than one individual and the potential for vectoring pathogens may depend on how often these devices are sanitized and or sterilized.

Nucleotide sequence accession numbers

The genome sequences of the strains described in this study have been deposited in the GenBank database with the accession numbers and annotation features described in Table 1. The version described in this paper is the first version.

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Competing Interests

The authors have declared that no competing interest exists.

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