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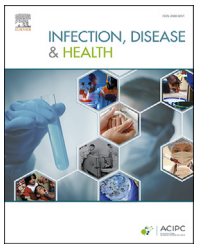

Research paper

Microbial laden mobile phones from international conference attendees pose potential risks to public health and biosecurity

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Abstract *Introduction:* Mobile phones, contaminated with pathogenic microorganisms, have the potential to act as "trojan horses". The microbial signatures present on their surfaces most probably vary across different geographical regions. As a result, mobile phones belonging to international conference attendees may serve as a model for global microbial dissemination, posing potential risks to public health and biosecurity.
Aim: This study aimed to profile the microbes present on mobile phones belonging to delegates attending an international scientific conference through use of metagenomic shotgun DNA sequencing.
Methods: Twenty mobile phones, representing ten different geographical zones from around the world, were swabbed and pooled together into ten geographical-specific samples for high definition next-generation DNA sequencing. WONCA council members were invited to participate and provided verbal consent. Following DNA extraction, next generation sequencing, to a depth of approximately 10Gbp per sample, was undertaken on a v1.5 Illumina NovaSeq6000 system. Bioinformatic analysis was performed via the CosmosID platform.

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Results: A total of 2204 microbial hits were accumulated across 20 mobile phones inclusive of 882 bacteria, 1229 viruses, 88 fungi and 5 protozoa. Of particular concern was the identification of 65 distinct antibiotic resistance genes and 86 virulence genes. Plant, animal and human pathogens, including ESKAPE and HACEK bacteria were found on mobile phones.
Discussion/conclusion: Mobile phones of international attendees are contaminated with many & varied microorganisms. Further research is required to characterize the risks these devices pose for biosecurity and public health. Development of new policies which appropriately address and prevent such risks maybe warranted.

Highlights

- Metagenomic analysis of 20 Mobile phones of conference attendees showed the presence of 2204 microbes.
- Of these 20 mobile phones were found 882 bacteria, 1229 viruses, 88 fungi and 5 protozoa.
- Public health associated human pathogens, including ESKAPE and HACEK microorganism were retrieved from these mobile phones.
- Plant and animal pathogens were retrieved from these mobile phones which pose a bio security concern.
- 65 distinct antibiotic resistance factors and 86 virulent factors that were present on the surface of these mobile phones.

Introduction

Biosecurity organizations, targeted with mitigating the significant biological threats facing global security, seek to prevent the introduction of pathogens (micro-organisms) and pests into particular environments or geographical zones [6]. Active containment and restricting the entrance of potentially harmful microbes, are imperatives in managing potential risks to national or continental biodiversity, the health of humans, state of flora and fauna and the preservation of the environments fragile bio-balance [1]. Biosecurity measures serve as the frontline defense to industries such as agriculture and tourism, protecting countries from economic harm and resource waste. Numerous biosecurity and quarantine strategies have been developed to prevent vectorization of identified microbial threats from arthropods, plants [2,3], animals (including zoonotic pathogens) [4] and, for person-to-person transmissions [5]. However, the number of identified biological threats continues to increase.

Increases in global trade and tourism, in conjunction with the continual evolution and adaptation of microorganisms to new hosts, presents a variety of avenues by which the biosecurity of a country may be compromised [6,7]. The transport of cargo and commodities provides avenues for the introduction of invasive alien species via land, sea and air [8]. Additionally, a growing international travel industry which sees over a billion travelers embarking and disembarking from cruise ships and airplanes annually, presents a vector for the global spread of non-indigenous species and infectious diseases [9]. The rise of emerging and re-emerging infectious diseases globally because of climate change is predicted to further complicate the biosecurity landscape [10,11]. Together these dynamic factors present complex challenges requiring international cooperation of governments and relevant stakeholders [12].

Australia, the world's largest island with a surface area of 7688287 m^2, has positioned itself as a leader in biosecurity to protect its people, unique fauna, flora and agricultural industry from pathogens that pose a significant risk to its economy. In order to address the threats posed by its unique geographical position, Australia has established a rigorous biosecurity system consisting of advanced surveillance systems at its ports of entry, educational messaging, advanced sensors and imaging, risk assessments and training to identify and respond to potential threats and enhance optimal recovery [13]. This robust and costly biosecurity system is necessitated to prevent negative impacts to the country. The entry of foot-and-mouth disease is estimated to cost AUD $5 billion, while the impact of *Xylella fastidiosa* to Australia's wine industry would represent an estimated loss of AUD $2.8 billion to the economy [14]. Ongoing vigilance, research, innovation and review of new control methods is critical to the preservation and maintenance of the fragile Australian landscape and viability of its primary production industries [9]. Whilst existing issues such as climate change, the spread of arthropod vectors, and the dissemination of existing and emerging plant and animal pathogens remain of significant importance, it is imperative regulatory agencies routinely consider new alternative pathways for the introduction of biosecurity threats.

One such example are mobile phones which, in the hands of billions of people, are important contaminated fomites (literal mobile "petri dishes") hosting a large variety of viable microorganisms including bacteria, fungi, viruses, protozoa and bacteriophages [15,16]. In 2023, there

were approximately 6.84 billion mobile phones worldwide with current estimates anticipating the smartphone mobile network to reach 7.7 billion users by 2028 [17]. Mobile phones are high touch devices [16] which once contaminated from the user's hands facilitates the persistence of microbial growth and reproduction due to their warm operating temperature, regular exposure to humidity and droplets from the user's mouth and airways [17,18]. As a result, mobile phones can act as reservoirs facilitating the continued vectorization of pathogens as the user navigates and perform his daily life whereabout [19]. This includes the transportation of mobile phones across borders via modern transport pathways potentially negatively impacting global public health and biosecurity efforts surrounding plant, animal and human pathogens. As an example, two recent reviews reported that the SARS-CoV-2 virus, responsible for over seven million COVID-19 deaths, was found on the surface of 45% of mobile phones [18] and that mobile phones may have contributed to the pandemic [19].

This present research aims to utilize an international medical conference as a case study to evaluate the potential biosecurity risk posed by mobile phones as a fomite and potential vector for microbial dissemination across international borders. Mobile phones belonging to attendees at an international conference held in Sydney (Australia), the WONCA 2023 conference were swabbed.

Methods

Overview

Mobile phones of the Council members attendees of the World Organization of National Colleges, Academies and Academic Associations of General Practitioners/Family Physicians (WONCA) 2023 international conference in Sydney were swabbed to profile microorganism present on the surface of these devices. This biennial conference was chosen as it attracts thousands of local and international attendees. Microorganisms were detected and analyzed for phylogenetic taxonomic identification by means of metagenomic derived shotgun DNA sequencing.

Participants

A participant information sheet was made available detailing the nature of the research study to all participants and ensuring that no personal identifying information would be collected. Informed consent was provided verbally by participants on the day.

Additionally, with the help of a world map (Fig. 1), participants were asked where they travelled from to attend the WONCA conference. The world map was divided into 10 geographical zones consisting of A: North America, B: Central (including Caribbean) and South America, C: West and East Europe (including Belarus and Ukraine), D: Northern Africa (Cameroon, Central African Republic, Ethiopia and South Sudan were included in D by author LT), E: Southern Africa (including Gabon, Congo, Uganda and Kenya were included in E by author LT), F: Middle East (including Iran and Turkey), G: Upper Asia (including Kazakhstan and Mongolia), H: Southern Asia (including China, Myanmar, India and Pakistan), I: South East Asia (including Vietnam and Thailand), J: Oceania (including Australia, New-Zealand and New Caledonia).

Sampling

Participants in the study provided their mobile phones for sampling. Swabs were performed on the front surface of the phones. Gloves were worn at all times and changed after each sampling to prevent potential cross-contamination. Each swab was placed in a pre-labelled tube corresponding to the participant's port of origin (A to J as per Fig. 1). A total of 20 swabs were collected, but no sample was collected from geographical zone G and the largest pool corresponded to the European geographical zone C (Table 1).

DNA extraction of swabs

DNA was prepared and sequenced by the Australian Centre for Ecogenomics (ACE) sequencing facility (University of Queensland, Brisbane, Australia). The cotton ends of the swabs were aseptically cut and transferred into a 2 mL screw cap tube with an O-ring (Sarstedt, cat #72.694.006) filled with 800 μl of CD1 (DNeasy Powersoil Pro Kit, Qiagen, cat #47016) and 300 mg 0.1 diameter glass beads (BioSpec Products, cat #11079101). DNA was lysed by bead beating using the Powerlyser 24 homogenizer (Mo-Bio #1315) for 5 min at 2000 RPM. The tubes were heated at 65 °C for 10 min with shaking at 1000 RPM on the Eppendorf ThermoMixer (Eppendorf, cat #5382000031), followed by centrifugation for 1 min at 10,000×*g*. The resulting lysate was transferred to a 1.5 mL Eppendorf tube (supplied), and samples were frozen and thawed to further lyse the cells. Subsequently, 200 μl of CD2 (supplied) was added to the thawed lysate and extraction was performed according to the manufacturer's protocol as per Qiagen DNeasy Powersoil Pro Kit (cat #47016). Final elution volume was 50 μl.

DNA sequencing

Library preparation

Libraries were prepared according to the manufacturer's protocol using Illumina DNA Prep Library Preparation Kit (Illumina # 20060059). The protocol as outlined was modified by reducing the total reaction volume for processing in 96 well plate format. Library preparation was run on the Mantis Liquid Handler (Formulatrix). This covers "Tagment Genomic DNA" to "Amplify DNA" in the protocol (Mantis-Nextera DNA Flex library prep protocol). Resulting amplified libraries were cleaned as per the "Clean Up Libraries" section in the manufacturer's protocol. On completion of the library prep protocol, each library was quantified, and quality control was performed using the Quant-iT™ dsDNA HS Assay Kit (Invitrogen) and Agilent D1000 HS tapes (#5067–5582) on the TapeStation 4200 (Agilent #G2991AA) as per the manufacturer's protocol.

Library pooling, quality control, loading and sequencing

Illumina DNA Prep libraries were pooled at equimolar amounts of two nM per library to create a sequencing pool.

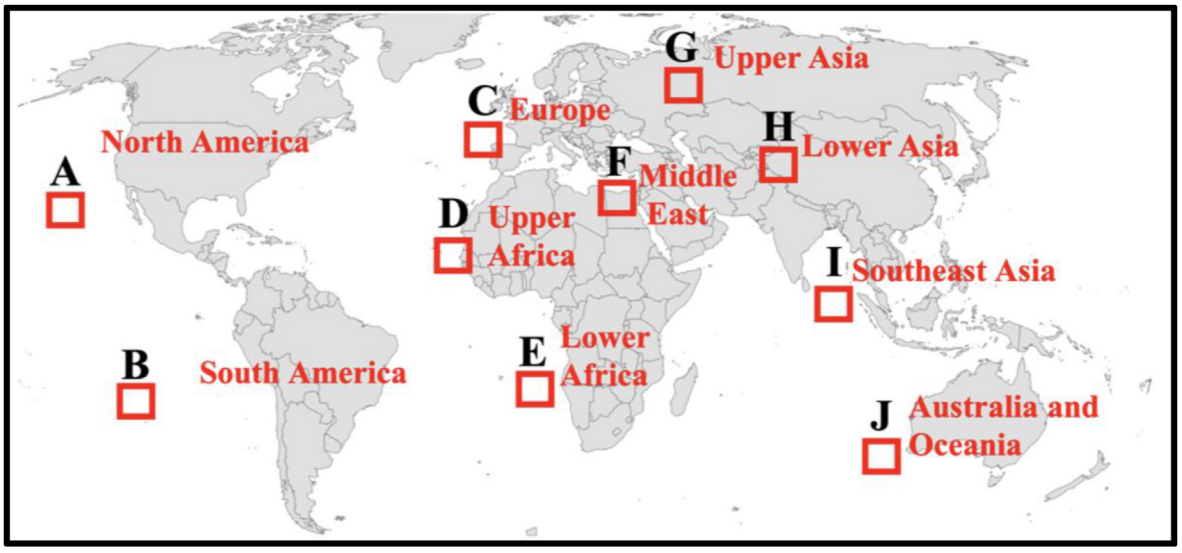

Fig. 1 World map of 10 geographical zones. Attendees had to indicate port of origin and have their mobile phone swabbed for downstream DNA sequencing process.

Table 1 DNA sequencing reads of pooled mobile phones swabs per worldwide zone.

Region	Pools	Number of swabs	Number of sequencing reads
North America	A	1	996,132
Central/South America	B	3	19,958,082
Europe	C	6	32,280,196
Northern Africa	D	3	23,401,110
Southern Africa	E	1	6,974,954
Middle East	F	2	18,652,914
Southern Asia	H	1	16,168,856
South-East Asia	I	2	9,840,594
Oceania	J	1	13,851,248

The library pool was quantified in triplicates using the Qubit™ dsDNA HS Assay Kit (Invitrogen). Library quality control is performed using the Agilent D1000 HS tapes (#5067—5582) on the TapeStation 4200 (Agilent #G2991AA) as per the manufacturer's protocol. The libraries were sequenced at a coverage of 10Gbp per sample, on a v1.5 Illumina NovaSeq6000 SP 2 x 150bp flow cell.

Bioinformatics and statistics

The post-sequencing derived raw data were retained and transferred into Illumina base space platform (https://basespace.illumina.com). Following the sequencing runs, data as demultiplexed FASTQ files were uploaded into the CosmosID platform (https://www.cosmosid.com/). Raw datasets Fastq files were analyzed using the CosmosID software to identify bacteria, fungi, virulence factor genes and antibiotic resistance genes. The datasets were then analyzed with proper mining bio-informatic analytic tools using high performance data-mining k-mer algorithm and highly dynamic comparator databases (GenBook®). The overall database is derived from curated GenBook® Databases comprising over 150,000 bacteria, viruses, fungi, and protists genomes and gene sequences from both private and public sources such as NCBI/RefSeq/WGS/SRA/nr, PATRIC, M5NR, IMG, ENA, DDBJ. Resultant data was filtered using a multi-kingdom resolutive taxonomic identification analysis built into CosmosID. This filtering was based on internal statistical scores from CosmosID, which enabled listing of results without further validation to determine their presence in each sample.

Through this process, the raw data of millions of short reads can be distinctively aligned against sequences of microbial genomes and genes (CosmosID Metagenomics Cloud). Sequences of antibiotic resistance genes assigned to specific species are compiled in CosmosID's overall reference database; a reference built by the assimilation of sequences collected from open-source databases (including NCBI, CARD, ResFinder, ARDB, ARG-ANNOT, SEEC) and from published scientific literature with resistant genes. Operational taxonomic units (OTUs) and genes were not subject to quantitative testing. The higher the read number (indicative of DNA sequencing depth), the higher the taxonomic resolution to detect and identify with accuracy distinct microbes and genes. Sub-analysis included richness (total number of individual OTUs or genes), and cumulative hits (count of OTUs from all geographical zones without removal of replicate OTUs). The number of strictly distinct microbes identified across all sampled phones corresponds to richness. In the other hand, each distinct microbe, named by taxonomic identity (e.g. strain), may be present in multiple different samples (20 phones were sampled) and therefore 'hits' is a measure of the number of the repeated retrievals of such microbe from phones to phones. For example, if only two distinct identified microbes were found in 20 phones, and present in 8 and 16 phones respectively, the Richness would therefore be reported as 2 and hits as 8 and 16 for each of these two respective microbes.

Alpha diversity to account for the different and diverse underlying microbiome compositions in between samples was run. Particular attention was focused on comparing Oceania derived mobile phone metagenomic input metrics with the datasets derived from the rest of the other world geographical regions.

Results

Twenty mobile phone swabs were subject to metagenomic DNA analysis to determine by high taxonomical resolution with the identification the levels of the genus, species and strains of microorganisms present on mobile phones belonging to council members of the international WONCA 2023 conference. Participants, and their mobile phones, represented nine different geographical zones from around the world (Fig. 1).

Sequencing reads and depth

Following DNA extraction and shotgun sequencing of raw sequencing data from each corresponding geographical zone, high depth sequencing reads were generated for each pooled sample (Table 1). The average amount of sequencing reads per pool was approximately 16.78 million reads. Sample E: Southern Africa (NVS074-05) contained the lowest (6.97 million) number of reads whilst sample C: Europe (NVS074-03) had the highest (32.28 million).

Metagenomic profiling

The metagenomic richness profiling of all 20 mobile phone swabs was 837 distinct microorganisms and 151 distinct antibiotic resistance and virulence factor genes (65 and 86 respectively). Across the 20 mobiles phones, the number of microbial hits totaled 2204 (with 882 bacteria, 1229 viruses, 88 fungi and 5 protozoa) and 366 antibiotic resistance and virulence factor genes (142 and 224 genes respectively) (Fig. 2). Most viruses identified were bacteriophages with hits and richness of 1156 and 303 respectively, while other types of viruses accounted for 73 hits of a richness of 30 distinct viruses. Eleven different strains of *human papilloma* viruses were found with a total hit number of 21 and included *HPV* strains 4, 5, 9, 10, 49, 50, 96, 112, 129, 134 and 163. Three viruses of the family of Herpesviridae were found and included the *herpes simplex type 1*, the *betaherpesvirus 7* and the *Epstein Barr virus* but accounted to four hits only. *Merkel cell polyomavirus* was found with a total number of six hits and were found on phones sourced from North America, Central/South America, Europe, Northern Africa, Southern Africa and Middle East zones. *Human adenovirus 2* and *Mastadenovirus C* were retrieved with a few hits of three and five respectively. Plant and animal pathogens were profiled such as the *Tobacco vein clearing virus* (*Nicotania* host), *Sewage-associated gemycircular virus 4*, *Caribou faeces-associated gemycircularvirus*, *Cotton leaf curl Multan virus satellite U36-1*, *HCBI9.212 virus* (*Bos taurus* host) and *Cotton leaf curl M. virus satellite U36-1*.

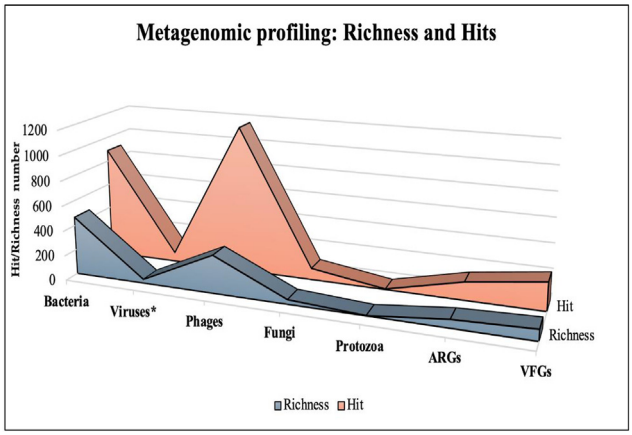

Fig. 2 Metagenomic profiling of 20 attendee's mobile phones of the WONCA 2023 international conference in Sydney. Both richness and hit counts found on these 20 phones are reported (including bacteria, viruses, phages, fungi, protozoa, antibiotic resistance genes and virulence factor genes).

Bacterial composition and world map geographical zones

A total of 882 bacterial hits were found on the surface of the 20 mobile phones (Fig. 3). Pools from Europe followed by North America geographical zones were the highest in bacterial number with 172 and 141 bacteria respectively. The lowest number of bacteria found corresponded to the Southeast Asia's sample. The most abundant species corresponded to *Corynebacteria* (86 hits), *Prevotella* species (68 hits), *Coagulase negative staphylococci* (42 hits). ESKAPE type of bacteria were found with *Enterobacter* spp. (2 hits) and *Escherichia coli* (2 hits), *Staphylococcus aureus* (9 hits), *Klebsiella* spp. (2 hits) including one *Klebsiella pneumoniae*, *Acinetobacter* spp. (19 hits) including 3 hits for *Acinetobacter Baumannii*, *Pseudomonas* spp. (8 hits)

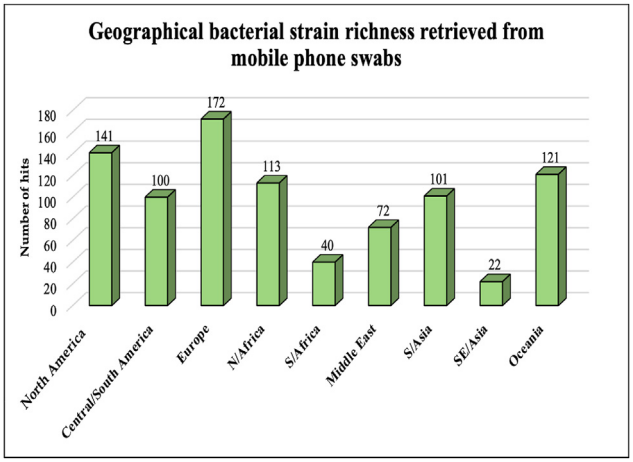

Fig. 3 Geographical bacterial hits for each specific geographical zone that were retrieved from mobile phones swabbed at the WONCA 2024 conference. Of note, the total number of hits is reported per geographical pooled sampling.

including one *Pseudomonas aeruginosa*. No *Enterococcus* spp. were found.

Bacteria relating to four of the five species within the HACEK group were identified. Specifically, these represented *Heamophilus* spp. (26 hits) including *Haemophilus parainfluenza*, *Aggregatibacter spp* (8 hits) including *aggregatibacter aphrophilus*, *Cardiobacterium* spp. (2 hits) including *Cardiobacterium hominis*, *Kingella spp* (4 hits) including *Kingella denitrificans*. However, *Eikenella corrodens* was not found. Of note, *Neisseria* spp. counted for 14 hits, *Anaerococci* spp. For 10 hits, *Campylobacter* spp. for 9 hits, *Cutibacterium spp*. for 10 hits and *Fusobacterium* spp. for 12 hits.

Antibiotic resistance genes (ARGs)

A total of 144 antibiotic resistance genes were found on 20 mobile phones (Fig. 4). The highest number of antibiotic resistance genes targeted bacterial cell protein synthesis (112/144; 77.8%) and included macrolide resistance (59/144; 41%), tetracycline resistance (23/144; 16%), aminoglycoside resistance (21/144; 14.6%), fusidic acid resistance (5/144; 3.5%), phenicol resistance (2/144; 1.4%) and pseudomonic acid resistance (2/144; 1.4%). Antibiotic resistance genes targeting bacterial cell wall directed antibiotics accounted for 18.8% (27/144) of all ARGs and consisted of beta-lactamases (20/144; 13.9%) and fosfomycin genes *Fos A* and *Fos B* (7/144; 4.9%). Resistance to antibiotics acting on the metabolism of bacteria consisted of mainly the chromosome-encoded dihydrofolate reductase gene *dfrC* (conferring trimethoprim resistance).

Genes conferring resistance to macrolides, tetracyclines, aminoglycosides and beta-lactams accounted for 85.4% (126/144) of the global resistome found on swabbed mobile phones. The distribution across the different zones of these main four antibiotic resistance genes showed a higher percentage of these genes from the Northern zone of Africa with 20.6% (26/126) followed by Central/South America with 15.9% (20/126) and Europe with 13.5% (17/126) (Fig. 5). Percentages of these genes in the Middle East, Oceania, Southern Asia, North America, Southern Africa and South-East Asia accounted for 11.1% (14/126), 9.5% (12/126), 9.5% (12/126), 8.7% (11/126), 7.9% (10/126), 3.2% (4/126) respectively (Fig. 5).

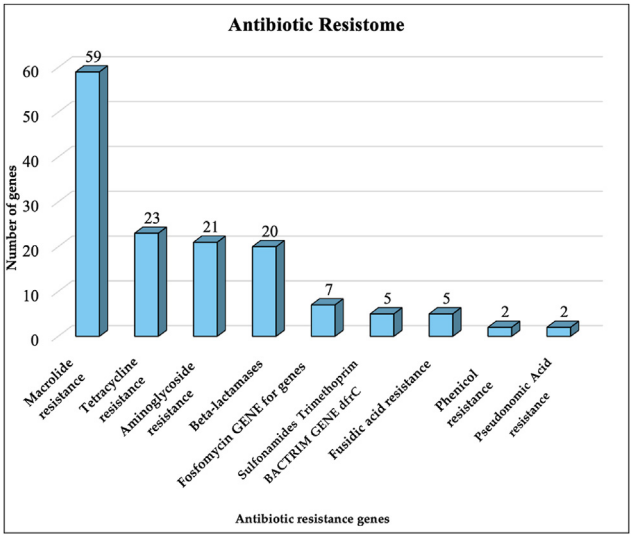

Fig. 4 Antibiotic classes targeted by ARGs or specific ARGs found on mobile phones. A total of 144 antibiotic resistance genes were identified on 20 mobile phone-derived swab samples. The largest number of genes resistant to antibiotics were those against macrolides (39), whilst the lowest were against phenicol (2) and against pseudomonic acid (2).

Virulome

Virulent factors genes identified from metagenomic profiling, showed the presence of aminoglycoside genes related specifically to certain bacteria. The *AACA-APHD*, *aadE* and *aphaA7* genes of *S. aureus*, *Enterococcus feacium* and *E. coli* respectively were found. *S. aureus* presented with additional specific genes and including *blaI*, *blaR1* and the *blaZ* gene responsible for regulating beta-lactamase and PBP-2a protein production. Other genes belonging to macrolide resistance were found from *Streptococcus pyogenes*, *Staphylococcus lentus* and *Enterococcus faecium* with genes such as *msrD*, *ermC* and *ermB* respectively. The *sat4* gene from *E. faecium* was additionally found. Resistance genes to tetracyclines consisted of *tetB*, *tetK*, *tetL* and *tetQ* and originated from *Heamophilus parasuis*, *S. lentus*, *E. faecium* and *Bacteroides fragilis* respectively. The virulence factor gene *psaA* from *Streptococcus pneumoniae*, coding for pneumococcal surface adhesion A and responsible for the colonization of the bacteria in human hosts was found. Additionally, important virulence factors were found including the *pilT* from *P. aeruginosa* and the *orf6* from *Klebsiella pneumoniae*. Of note, genes conferring resistance to quaternary ammonium compounds were found. For example, the *S. aureus qacC* gene which belongs to the protein family of small multi-resistant proteins and *qacEdelta1* genes from *Pseudomonas putida* and *E. coli*

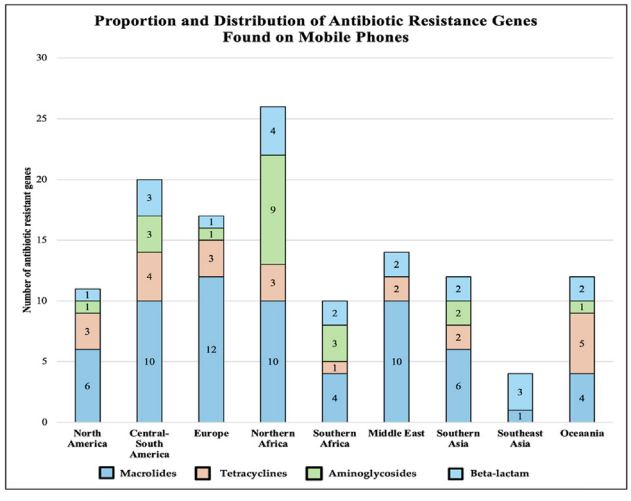

Fig. 5 Distribution and proportion of antibiotic resistance genes from mobile phone-derived swab samples, grouped per worldwide geographical zone. The antibiotic resistance genes targeted mainly four antibiotic categories which included Macrolides, Tetracyclines, Aminoglycosides and Beta-lactams.

were detected. The virulence factor genes found in this study presented a large array of DNA mobile elements such as transposons and plasmids as well as integrons, with their respective genes identified as *ntl1*, *mobA*, *mobB*, *repB*, *repC*, *repL*, *retB*, *tnpA* and *tnpR*.

Fungi

A total number of 92 hits with 36 distinctive fungi (richness) were retrieved from 20 phones. At the phylum taxonomic rank, the *Ascomycota* and *Basidiomycota* richness consisted of 22 and 14 respectively, while hits of these two phyla accounted for 45 and 47 respectively. Fungi were identified in all zones of the world and consisted of *Candida parapsilosis*, *Malassezia restricta* and *M. globosa*. Additionally, *Balansia obtecta* was found in all geographical zones except for the Southern Asia zone. Under the *Ascomycota* phylum, the species of aspergillus's hit count was 10 and included *Aspergillus chevalieri*, *A. glaucus* and *A. ruber*. The most abundant fungi of the *Basidiomycota* phylum were species of Malassezia, with a total number of 37 hits across all samples including *Malassezia japonica*, *M. obtusa*, *M. restricta*, *M. furfur*, *M. equina* and *M. sympodialis*. Four other fungi belonging to the *Basidiomycota* phylum included *Melampsora pinitorqua*, *Moesziomyces aphidis*, *Puccinia arachidis*, *Trichosporon asahii* and *Wallemia mellicola*.

Alpha diversity analyses

Boxplot alpha diversity (Fig. 6) shows the differences found in between the geographical zone Oceania versus all the other regions of the world. Alpha diversity representations were run to assess the data distribution of collated multi-kingdom abundance (combined abundance data of all bacteria, viruses inc phages, protozoa and fungi found in the metagenome), virulome and resistome (Fig. 6A, B, 6C).

Discussion

The current study aimed to undertake the metagenomic DNA profiling of microbes present on 20 mobile phones from international and national attendees of the international conference WONCA 2023 held in Sydney, Australia. These 20 mobile phone swabs resulted in the identification of 2204 microbes including 882 bacteria, 1229 viruses, 88 fungi and 5 protists. Of particular interest, a single mobile phone was swabbed from an attendee from Australia which harbored 11.5% (252/2204) of all microbes retrieved in this experiment and included 121 bacteria, 120 viruses, 10 fungi and 1 protozoan. The remaining 19 mobile phones belonging to international attendees harbored 88.5% (1952/2204) of all microbes identified in this study. More precisely these 1952 microbes consisted of 761 bacteria, 1109 viruses, 78 fungi and 4 protists accounting for 86.3%, 90.2%, 88.6% and 80% respectively. Alpha diversity distribution of sample microbial composition outside Oceania, has shown a large diversity of microorganisms when compared to Oceania derived sampling (Fig. 6A).

The WONCA international conference in Sydney, Australia occurred in October 2023 with over 4000 attendees. According to Tourism Australia, between January to October 2023 there were a total of 6.8 million arrivals to Australia [20]. Extrapolating from the results from this metagenomic DNA profiling study on 20 phones, an estimated 749.2 million microbial organisms may have been introduced into Australia in the year 2022 by the means of mobile phones alone (299.8 million bacteria, 417.8 million viruses, 29.9 million fungi and 1.7 million protists), a portion of which may potentially pose a risk to the Australian biosecurity and public health (Fig. 7).

The presence on mobile phones of microorganisms possibly impacting plants and agriculture raise biosecurity concerns. *Pantoea stewartii*, a plant bacterial enteric pathogen was found on the surface of mobile phones originating from lower Africa (Zone E). This pathogen causes disease in a variety of agricultural crops [21—25] but has also been reported to infect endangered western parrots in Australia [26]. Other bacterial plant pathogens species were retrieved from phones originating in Europe, North America, South-East Asia and Africa (both upper and lower zones). These plant pathogens included *Pectobacterium carotovorum* (found on phones from Europe and the lower zone of Africa) and *Xanthomonas campestris* (found on phones from North America, South-East Asia and the upper zone of Africa) which are responsible for bacterial crop soft rot diseases and black rot diseases [27—29]. The first report of the presence of *P. carotovorum* and *X. campestris* in Australia was in 1944 and 2019 respectively. While already introduced into Australia, these plant pathogens have since evolved into new strains around the world that may, if re-introduced into Australia, have more pathogenicity than existing endemic strains [30]. If re-introduced these pathogens could have a significant impact on Australia's agricultural industry as they have the potential to contribute to the loss of a variety of crops including potatoes, carrots, capsicum, tomato, cabbage, chili, eggplant, melon, beans and pumpkin.

Other important biosecurity concerns were associated with the presence of fungal microorganisms, including plant pathogens found on mobile phones flown into Sydney, Australia. Pathogens, present on phones of participants of the South/Central America zone consisted of *M. aphidis*, *Hanseniaspora uvarum*, *Fusarium solani*, *M. aphidis* and *P. arachidis*. *P. arachidis* was also retrieved from swabs from the Southern zone of Africa which also possessed *Aspergillus chevalieri*. Mobile phones from Europe harbored *Oidium heveae* (also present on the single Oceania mobile phone) and *Melamspora pinitorqua* (also found on mobile phones from Africa's North zone). Finally, *Xeromyces bisporus* and *Microcyclospora tardicrescens* were found on mobile phones originating from the Middle East and Lower Asia respectively.

Of further consideration, the 20 WONCA tested mobile phones contained 142 antibiotic resistance and 224 virulence factor genes. With 6.8 million visitors to Australia between January to October 2023 [20], the extrapolated estimation may suggest the introduction of 48.2 million ARGs and 76.1 million VFGs into Australia within a short period of 10 months. Along with streptococci and staphylococci found on the surface of WONCA mobile phones, the presence of microbes belonging to public health notorious health-care-associated pathogens like ESKAPE and HACEK

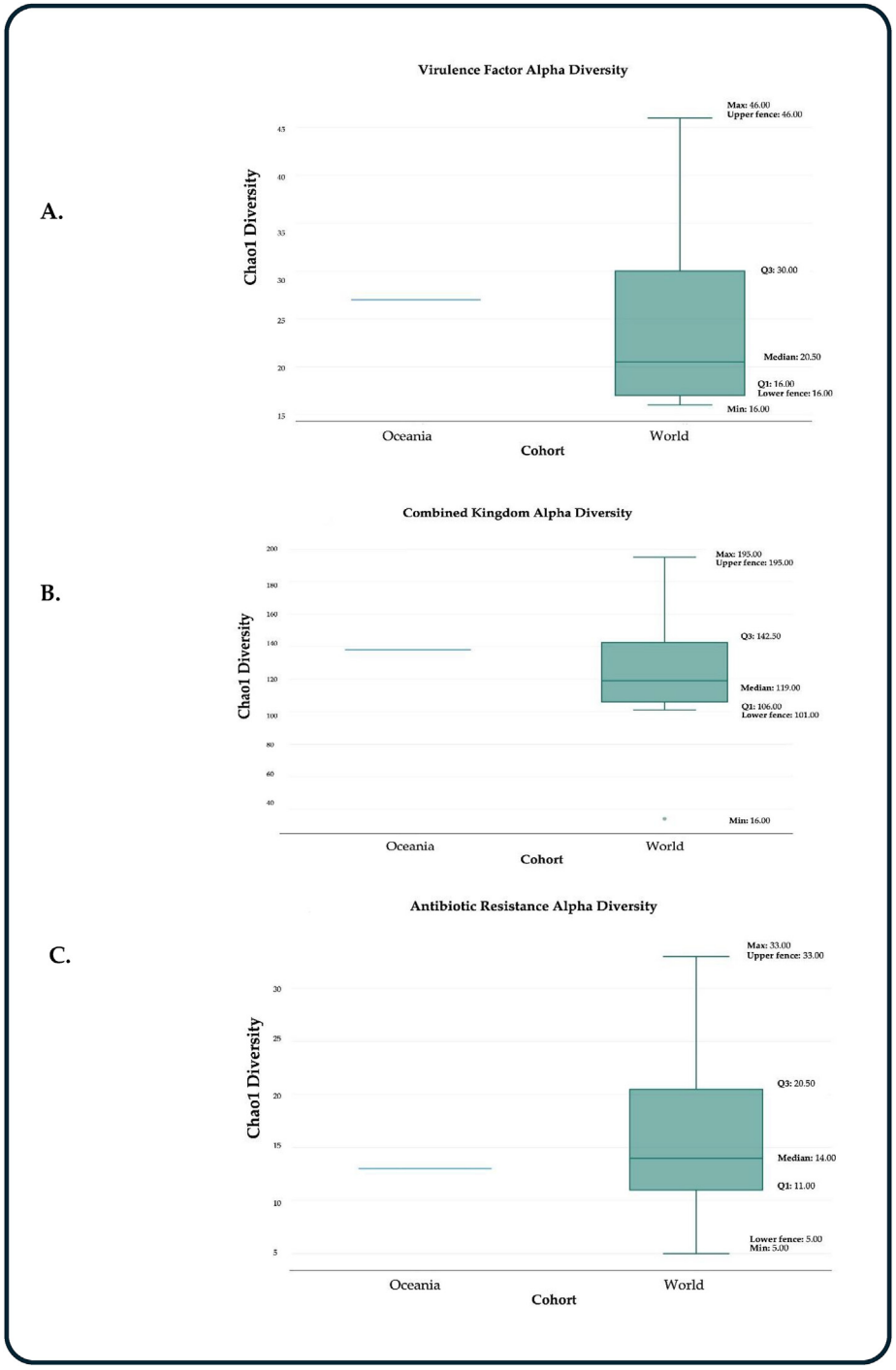

Fig. 6 Oceania samples are compared to the rest of the world geographical zones. CHAO1 Alpha diversity data distribution of the Virulome (**A**), multi-kingdom (**B**) and Resistome (**C**).

bacterial groups warrants significant concern, especially since these mobile phones belonged to health care staff visiting Australia. Our previous work showed that staff in hospitals have mobile phones contaminated with viable microbes responsible for healthcare associated infections, community-acquired infections, nosocomial infections and that their phones harbored a large amount of ARGs and VFGs [16,18].

The presence of microorganisms with drug resistance attributes on mobile phones cannot be overlooked as such personal fomites are poorly cleaned and are crossing borders, unchecked, by means of modern transport. Together, these pose numerous concerns to both public health and biosecurity since novel pathogens with mutable genomes and newly acquired resistance have the potential to travel fast and spread worldwide. No study to date has drawn attention to the potential consequences travellers' mobile phones pose to biosecurity and public health. But our study is without appeal, mobile phones of travellers carry antibiotic resistance genes. The United Nations (UN) wellbeing

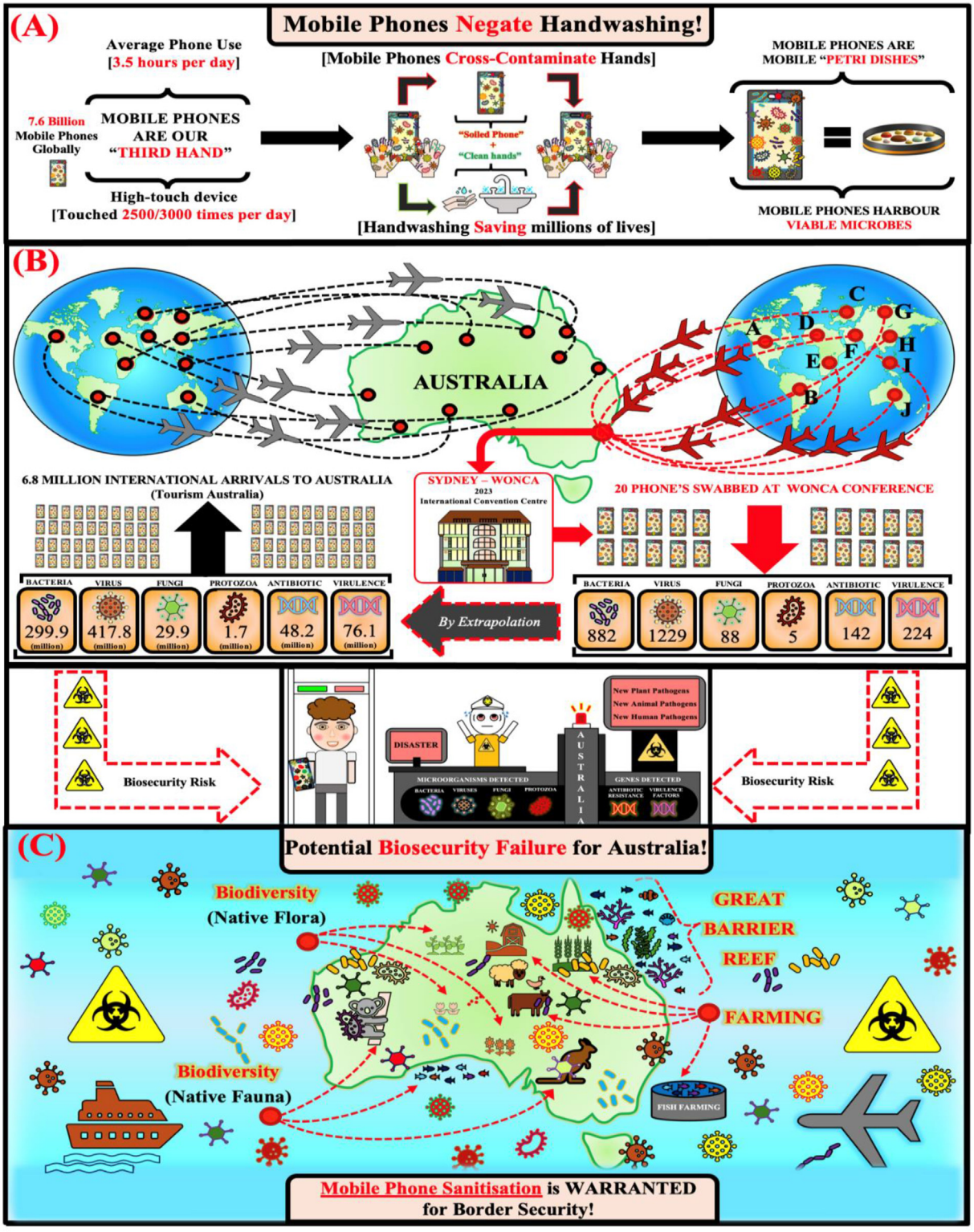

Fig. 7 (**A**) Mobile phones in billions are contaminated platforms. These devices negate the benefit of hand washing and act as mobile 'Petri dish' like platforms while in the hands of billions of people. (**B**) Metanalysis of 20 mobile phones of WONCA 2023 international conference attendees contained more than two thousand microorganisms (red arrow). By extrapolation, with millions of international visitors and phones entering Australia in 2023, the numbers of microorganisms entering the country are greater than 700 million. **(C)** Biosecurity risks posed by diverse microbial contamination of mobile phones may affect different sectors including farming and agriculture, indigenous flora and fauna biodiversity. Protocols for sanitation of phones are urgently warranted. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and good health sustainable development goal SDG #3 is aimed at fighting the development, spread and impact of antimicrobial resistance. In 2019, there was an estimated 4.95 million deaths associated with bacterial antimicrobial resistance [31] while the UN predicts that in 2050, 10 million individuals will die from antimicrobial resistant microbes. In light to these threats to humanity's wellbeing, the World Health Organisation (WHO) has implemented measures to prioritise the fight against critical multidrug resistant microbes including to name few penicillin-resistant *S. pneumoniae*, methicillin resistant *S. aureus*, carbapenem resistant *Acinetobacter baumaunii*, carbapenem resistant *P. aeruginosa*. For example, Methicillin-resistant *S. aureus* alone caused more than 100,000 deaths while 6 additional resistant microorganisms are causing between 50,000—100,000 deaths each [31]. The potential spread of superbugs by means of mobile phones must be scrutinised and further research is urgent given the existence of billions of mobile phones in circulation globally and how 'hospitable' these platforms represent for microbes to thrive and possibly breed onto such devices. As a scientific discussion, microbes with antimicrobial resistance found on mobile phones are either indirectly deposited on these fomites (e.g. hands touching other fomite, then hand touching mobile phone) or/and directly the result of horizontal genetic transfer (HGT) within the mobile phone surface they contaminate. Our current study and previous work report on thousands of organisms found on mobile phones therefore the close proximity of all these viable microbes as well as the presence of DNA of non-viable dead microbes on the surface of mobile phones may confer the ideal establishment of HGT. Horizontal gene transfer, a process by which single or double stranded DNA is transferred from one cell to another, and is being recognised for its dynamic role in contributing to the development of antimicrobial resistance globally [32]. Horizontal gene transfer allows for bacteria to develop antimicrobial resistance through three primary mechanisms; transduction, conjugation and transformation [32]. In contrast to transformation which involves the uptake of exogenous DNA segments from the environment, conjugation requires cell to cell contact to enable unidirectional transfer of DNA via a mating apparatus [32]. Despite this dependency on close proximity, conjugation is the manner by which antimicrobial resistant genes are most conveyed through horizontal gene transfer [32]. Regardless, both processes require DNA and/or bacteria with unique antimicrobial resistant genes to be located on a surface within close proximity to each other. Finally, the process of transduction involves the transfer of genetic material to bacteria via bacteriophage viruses. Previous research has confirmed the presence of a large variety of bacteriophages on mobile phones used by healthcare workers in high-risk settings such as neonatal intensive care units and emergency departments [33]. From this cross-sectional viability-metagenomic study, 734 bacteriophages were detected across 30 mobile phones with phage's most specifically targeted against *Staphylococcus* and *Salmonella*.

Healthcare professionals in medical settings are exposed to antimicrobial resistant bacteria by means of fomites, patient-to-patient or colleague-to-colleague interactions (e.g. shaking hands) However, healthcare professionals do not effectively sanitise their mobile phones while important fomites. A 2023 study showed that among 46 fomites in the clinical settings, the most abundant contaminated fomites were mobile phones [34] It is therefore imperative that efficient and effective sanitation solutions for mobile phones are integrated into healthcare provider behaviours to prevent i) the negation of current hand hygiene practices, ii) the potential HGT development of antimicrobial resistance on phones and iii) the widespread of microbes by means of non-sanitized contaminated mobile phones [35].

Mobile phones, as reservoirs of antibiotic resistant microorganisms and viruses, may act as platforms propagating superbugs, pathogens and non-endemic strains to other countries. In 2020, our systematic review warned of the possibility that COVID-19 virus SARS-CoV-2 may be spread by mobile phones around the world and is a contributing vector in the spread of the pandemic [19]. In that same year, CSIRO published that SARS-CoV-2 could remain 28 days on mobile phones [36]. New strains or non-endemic biological entities that enter a country may impact national biosecurity and public health. Microbial laden mobile phones, in the hands of billions of people, serve as 'third hand' niches of microorganisms which with the assistance of modern transport may currently bypass biosecurity protocols and disperse microbial threats around the world including superbugs. In this way un-sanitized mobile phones currently act as billions of 'trojan horses' crossing borders and resulting in worldwide microbial dissemination without yet attracting the scrutiny of public and biosecurity authorities.

Study limitations

This study has potential limitations. The mobile phones investigated in this research were limited to sample size of 20 and further research with higher power is warranted. The largest number of mobile phone-derived swab samples was taken from Europe (6 swabs), whereas other geographical locations including North America, Southern Africa, Southern Asia and Oceania consisted of only one sample each. This discrepancy between the number of swabs collected across different locations was due to the limited participants consenting to having their phone sampled. More specific prominent organisms may be over-represented and limit further the microbial diversity. Our approach with metagenomic analysis (coverage of 10Gbp per sample) has however been able to profile and identify a large number of microbes entering Australia by means of mobile phones. This methodology was incorporated to enable detection of organisms which may have very low abundance, previously unreported or difficult to identify via traditional methods.

Conclusion

Thousands of microbial strains were found harboring the surface of mobile phones of attendees of an international conference in Australia. Attendees to that Sydney conference and particularly originating from overseas had microbial laden mobile phones which may pose a real risk to the Australian biosecurity and public health. Previous

scientific publications have shown that mobile phones are niches of viable pathogenic microbes resistant to antimicrobial drugs. As mobile phones act as mobile 'petri-dishes', the authors recommend measures be implemented to sanitize phones at airports and harbors national wide. Protecting our biodiversity and reducing the spread of superbugs is critical not only for the economy but for our national security.

Author's recommendations

Biosecurity and public health vulnerabilities are constant critical challenges all countries in the world are facing. Studies evaluating the benefits of sanitizing mobile phones, known to be highly microbial contaminated platforms are urgently warranted. Modern transport, urbanization and environmental conditions like climate change enable the propagation of emerging and re-emerging pathogens across the globe. Additionally, the lack of sanitation practices of these devices and the fact these are important fomites, may severely impact the World Health Organization's worldwide hand hygiene campaign simply because mobile phones negate hand washing benefits. The future of prevention and infection control must be technologically driven by smart automatic sensors and sanitizers to render such practice more efficient and systematic. For example, ultra-rapid 10-s, enclosed efficient germicidal ultra-violet C mobile phone sanitizers exist. The implementation of these devices in airports, healthcare settings, boat cruises, food retails, public restrooms and other crowded settings will lead to significant economical savings with drastic downstream positive impacts to global biosecurity and public health.

Ethics and consent

Bond University Human Research Ethics Committees (16,004) approved the study.

Authorship statement

Conceptualization was performed by LT, JL, MM. Data analysis and interpretation was performed by MO, LT, RaA., RG, GS, AS and AG. Manuscript was prepared by LT, RaA, AG, MO and ReA. Funding was obtained by RaA, SMcK, LT. Methodology: LT sampled all participants. Project administration by LT, RaA, MM, JL and SMK. All authors reviewed the manuscript.

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Declaration of competing interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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