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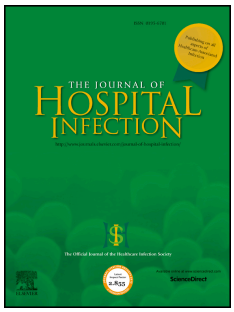

Mobile phones as fomites for potential pathogens in hospitals: microbiome analysis reveals hidden contaminants

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Mobile phones as fomites for potential pathogens in hospitals: microbiome analysis reveals hidden contaminants

Running Title: Mobile phones as fomites in hospitals

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Abbreviations

A&E Accident and Emergency; **C** Control ; **CFUs** Colony Forming Units; **CLSI** Clinical Laboratory Standards Institute; **CoNS** Coagulase Negative *Staphylococcus;* **H** Hospital ; **HAI** Hospital Acquired Infection; **ITU** Intensive Care Unit ; **MALDI-TOF MS** Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry; **MAU** Medical Assessment Unit ; **MRSA** Meticillin Resistant *Staphylococcus aureus;* **PBS** Phosphate Buffered Saline **PCR** Polymerase Chain Reaction **VRE** Vancomycin Resistant *Enterococcus*

Structured Summary

Background: Smartphones used in clinical settings harbour potentially pathogenic bacteria, and this may pose an infection risk. Previous studies have relied on culture-based methods.

Aim: To characterize the quantity and diversity of microbial contamination of hospital staff smartphones using culture-dependent and culture-independent methods. To determine the prevalence of antibiotic resistant potential pathogens. To compare microbial communities of hospital staff and control group phones.

Methods: Smartphones of 250 hospital staff and 191 control group participants were swabbed. The antibiotic resistance profile of *Staphylococcus aureus* and enterococcus isolates was determined. Swabs were pooled into groups according to the hospital area staff worked in, and DNA was extracted. The microbial community of the phone was characterised using an Illumina MiSeq metabarcoding pipeline.

Findings: Almost all (99.2%) of hospital staff smartphones were contaminated with potential pathogens, and bacterial colony forming units (CFUs) were significantly higher on hospital phones than control group. Meticillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) were only detected on hospital mobile phones. Metabarcoding revealed a far greater abundance of Gram-negative contaminants, and much greater diversity, than culture-based methods. *Bacillus* species were significantly more abundant in the hospital group.

Conclusion: This study reinforces the need to consider infection control policies to mitigate the potential risks associated with the increased use of smartphones in clinical environments, and highlights the limitations of culture-based methods for environmental swabbing.

Keywords

Mobile phones; antibiotic resistance; *Staphylococcus aureus*; *Bacillus*; *Pseudomonas*; fomites

Introduction

Within the last decade, smartphones have revolutionized the way people communicate and access information. The medical profession has rapidly integrated smartphone technology to form an important part of professional practice. Enhancing clinician communication and providing instant access to unlimited resources at point of contact, they have improved patient safety [1]

The mobile phone has become an extension of its owner and shares some of their microbiome [2]. Moving constantly with their user into new surroundings, phones come into contact with bacteria from different environments and may feasibly be responsible for the transmission of bacteria from place to place, or person to person. The average person touches their mobile phone up to 200 times a day [3], providing colonising bacteria with constant nutrition in the form of amino acids and minerals from shed skin cells and sweat [4]. Combined with the heat generated by the device and the crevices of cracked screens and phone covers, smartphones provide an excellent habitat for bacteria to colonise.

In clinical settings, phones are often used during and between patient contact periods without handwashing and, as the devices are rarely cleaned [5], this creates opportunities for cross contamination between the mobile phone and the hands of its users, which may compromise the effectiveness of hand hygiene protocols.

This potential for cross contamination between the users, device and patient may pose a threat to patient safety. Immunocompromised patients have an increased susceptibility to acquiring additional infections. If the infection is resistant to antibiotics, limited effective therapies make it harder and sometimes impossible to eliminate. This in turn increases morbidity, mortality and financial burdens [6]

Many studies have shown that smartphones in clinical settings are contaminated with an array of microorganisms, including antibiotic resistant bacteria known to be associated with hospital acquired infections (HAIs) [5,7–10]. However, previous research has been limited in its scope by a reliance on culture-dependent methods. The exact methodology used will create unintentional bias, with the type of swab, transport time and choice of culture media all affecting results [11]. The aim of this study was to overcome these limitations by using a combination of culture-dependent and culture-independent methods to characterise the quantity and diversity of microbial contamination of hospital staff smartphones. Antibiotic resistance profiles of potential pathogens were also determined. A further aim was to determine whether contaminants found on the phones of hospital staff were significantly different than those found on the phones of the control group, and whether phones from staff working in different areas of the hospital might harbour different contaminants.

Methods

Ethics, Consent and Recruitment of participants

Following institutional and NHS ethical approval (REC reference 17/WA/0413), participants were recruited from January 2018 over a six month period. A total of 250 hospital staff members were approached during their working day. An additional 191 members of the public within the same geographical area and who had not attended a hospital three months prior to participation were recruited to form the control group. Potential participants were given an information sheet and an opportunity to ask questions or decline. Willing participants then gave written consent. A questionnaire was used to record the cleaning habits and phone use details of participants.

Sample processing

A sterile cotton swab was rolled over the front, back and lateral side of the mobile device, placed in M40 aimes transport media (Sterilin) and transported to the laboratory. All samples were processed within four hours. Swab tips were removed, added to 1 ml of phosphate buffered saline (PBS), vortexed for ten seconds, and 100 µl used to inoculate each of the following agar plates: 5% blood agar, mannitol salt agar, bile esculin azide agar and eosin methylene blue agar. Plates were incubated for 48 hours at 37°C. Swab tips were stored at -80°C in PBS until DNA extraction. Total colony forming units (CFUs) present on each mobile phone were calculated by counting the number of discrete colonies on blood agar plates and eosin methylene blue agar plates and multiplying by ten.

Identification of isolates

Bile esculin azide and mannitol salt agar plates were used to isolate *Enterococcus* and *Staphylococcus* species. *S. aureus* was identified by colony morphology

(cream/golden colonies on mannitol salt agar plates), the fermentation of mannitol salt agar and a positive catalase and coagulase slide test. Enterococci were identified by colony morphology (small pin colonies on bile esculin azide agar plates) fermentation of esculin, a negative catalase test and positive mannitol fermentation. Gram-negative isolates were collectively identified following growth on eosin methylene blue agar plates and Gram staining. All isolates were confirmed to genus level using polymerase chain reaction (PCR) amplification of the V4 region of the 16S rRNA gene using published primers and reaction conditions [12]. A selection of VRE and MRSA isolates were also confirmed to species level using Matrix-Assisted Laser Desorption/Ionization-Time Of Flight (MALDI-TOF) mass spectrometry (MS).

Antibiotic susceptibility testing

All antibiotic susceptibility testing was carried out and interpreted according to Clinical Laboratory Standards Institute (CLSI) dis susceptibility testing guidelines (CLSI, Pennsylvania, USA). *S. aureus* isolates were tested for resistance to cefoxitin, erythromycin, clindamycin, tetracycline, trimethoprim, penicillin, and gentamicin. Enterococci were tested for resistance to vancomycin, teicoplanin, tetracycline, erythromycin and ampicillin. For the purposes of this study isolates showing Intermediate susceptibility were classed as resistant.

DNA extraction

Individual swabs were defrosted and centrifuged at 14 000 rpm for five minutes in their PBS solution. Swabs were removed, and the sample vortexed to resuspend cells. Hospital (H) staff phone samples were pooled into the following groups; Surgical (H1 and H6), Paediatrics (H2), Intensive care unit (ICU) (H3), Radiology (H4), Pharmacy (H5), Accident and Emergency (A&E) (H7), Medical assessment unit (H8), Mobile staff (H9). Control (C) group

samples were pooled randomly, making up six control groups (C1 to C6). Each pooled sample was then centrifuged at 14 000 rpm for five minutes, the supernatant removed and the pellet resuspended into DNA extraction buffer. DNA was extracted using the QIAamp DNA Mini Kit (Qiagen) protocol as per the manufacturer's guidelines.

Microbiome analysis

After DNA extraction, 30 µl of each sample was sent for microbiome analysis. The 16S rRNA gene V4 variable region PCR primers 515/806 (with barcode on the forward primer) were used in a 30 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94°C for three minutes, followed by 30-35 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for one minute, and a final elongation step at 72°C for five minutes. After amplification, PCR products were checked in 2% agarose gel to determine the success of amplification and the relative intensity of bands. PCR products were purified using calibrated Ampure XP beads, pooled, and used to prepare an Illumina DNA library. Sequencing was performed at MR DNA (Shallowater, Texas, USA) on a MiSeq following the manufacturer's guidelines. Sequence data was processed using MR DNA analysis pipeline (MR DNA, Shallowater, Texas, USA). In summary, sequences were joined, depleted of barcodes then sequences <150bp removed, and sequences with ambiguous base calls removed. Sequences were denoised, Operational Taxonomic Units (OTUs) generated and chimeras removed. OTUs were defined by clustering at 3% divergence (97% similarity). Final OTUs were taxonomically classified using BLASTn against a curated database derived from RDPII and NCBI (www.ncbi.nlm.nih.gov, http://rdp.cme.msu.edu). Any OTU/genus with an abundance of <10 in any sample was removed from further analysis.

Statistical analysis

The biological communities of samples were quantified using multivariate analyses in PRIMER v6.0. Phone samples were factorised as either control or hospital, or 'high risk of infection' (H1, H3, H6) or 'low risk of infection' (H2, H4, H5, H7, H8, H9) areas, and sample counts pre-treated with a square root transformation to down-weight the influence of the most abundant taxonomic units. Similarity matrices were constructed for genera and OTU datasets using the Bray-Curtis coefficient. Non-metric multidimensional scaling (nMDS) was used to ordinate all pairwise sample similarities along two axes. Analyses of similarities (ANOSIM) were used to test for community differences between control and hospital samples, or between samples from low and high risk areas within the hospital. A similarity of percentages analysis (SIMPER) was used to identify which genera or OTUs contributed most to potential differences in communities across groups. The abundances of key contributing taxonomic units were analysed using a Mann-Whitney test. A Bonferroni correction was used to adjust alpha values (α) by the number of comparisons (*k*), minimising the likelihood of Type I errors.

The relationships between the percentage of phones harbouring each contaminant isolated from hospital and community samples, and the prevalence of antibiotic resistant *S. aureus* and *Enterococcus* in hospital and community samples were tested using Pearson's chi-square test. An independent *t*-test was used to compare the mean contaminant CFUs per phone between: phones cleaned daily, and never; and hospital and control phones cleaned daily, and never. As before, a Bonferroni correction was applied to alpha values. All these analyses were carried out using SPSS v25.0 (IBM)

Results

Extent of contamination of hospital and control mobile phones: Culture dependent methods

Culturable bacteria were isolated from 99.2% of hospital staff phones, and 96.9% of the phones in the control group. The most commonly isolated bacteria were coagulase negative *Staphylococcus* (CoNS) (82.0% of hospital phones and 86.4% of control phones), *S. aureus* (32.4% of hospital phones and 22.0% of control phones ($P = 0.016$)), and *Enterococcus* spp. (9.6% of hospital phones and 6.3% of control phones ($P = 0.207$)). Gram negative bacteria were far less frequently isolated (*Acinetobacter* spp. 3.2% of hospital phones and 0.5% of control group phones ($P = 0.049$); *Pseudomonas* spp. 2.4% of hospital phones and 2.1% of control group phones, and *Enterobacter* spp. 0.4% of hospital phones and 1.6% of control group phones ($P = 0.199$)).

Extent of contamination of hospital and control mobile phones: Culture independent methods

Microbiome analysis at genus level revealed 197 genera across all samples, with 163 and 186 genera detected in control and hospital samples, respectively. Of these, 152 genera were detected in both groups, while 11 were unique to the control group and 34 were only detected in the hospital group. Figure 1 shows the relative abundance of individual bacterial genera contributing more than 5% of contamination on hospital phones in comparison to the control group. In the hospital group, the most abundant genus was *Pseudomonas*, making up 17.8% of contamination overall. Supplementary table 1 shows the prevalence of every genus detected across each sample.

At the OTU level, 485 OTUs were detected across all samples, with 355 and 450 OTUs

190 detected in control and hospital samples, respectively. Of these, 320 were detected in both
191 groups, while 35 were unique to the control group and 130 unique to the hospital group.
192 OTU richness was significantly higher in the hospital group (P = 0.005), while diversity was
193 similar across the two groups (P = 0.480).

194 **Comparison of hospital and control phone microbial communities**

195 Community compositions of genera of pooled samples from hospital or control groups were
196 at least 48% similar to each other (Figure 2). Radiology (H4) and Accident and Emergency
197 (H7) were the most dissimilar communities. There was no significant difference between the
198 genera-level compositions of control and hospital samples (P = 0.126) or between 'low risk
199 of infection' and 'high risk of infection' hospital samples (P = 0.060). However, the
200 abundance of *Bacillus* was significantly higher in the hospital group than the control group
201 (P = 0.036).

202 OTU community compositions were at least 45% similar to each other (Figure 3). Accident
203 and Emergency (H7) and C6 of the control group were the most dissimilar OTU
204 communities. There was no significant difference between OTU community compositions of
205 control and hospital samples (P = 0.073). However, the OTU community composition of
206 hospital staff phones in 'high risk of infection' and 'low risk of infection' areas was
207 significantly different (P = 0.048).

208 **Characterising the antibiotic resistance profile of Gram-positive isolates**

209 Figures 4 and 5 show the prevalence of antibiotic resistances in *S. aureus* and enterococci,
210 respectively. 27 of 81 (33.3%) of *S. aureus* isolates from hospital phones were meticillin-
211 resistant; no MRSA were detected in control group phones (P< 0.001). Likewise, vancomycin
212 resistance in enterococci was found uniquely on hospital phones.

Mobile phone usage and cleaning behaviour

In total, 91.6% of hospital staff admitted to using their device while at work. Less than 10% of hospital staff said they cleaned their device daily, 28.4% said they cleaned their phone weekly and 62.0% had never cleaned their device. Within the control group, 5.8% cleaned their device daily, 13.2% weekly and 81.0% had never cleaned it. The mean number of bacterial CFUs on devices that were never cleaned was significantly higher in the hospital group than the control ($P < 0.001$; $mean_{hospital}$ 1,431.2 ± 107.3 SE, $mean_{control}$ 405.1 ± 53.0 SE. A similar difference was observed between hospital and control phones cleaned daily ($P = 0.043$). Daily cleaning of phones significantly reduced contamination load ($P < 0.001$; $mean_{daily}$ 72.3 ± 11.8 SE, $mean_{never}$ 918.1 ± 66.5 SE).

Discussion

Extent and diversity of contamination of mobile phones: Culture dependent methods

Nearly all mobile phones tested (98.2%) were contaminated with at least one species of bacteria, reinforcing the hypothesis that these devices are potential fomites [13]. CoNS and *S. aureus* were the most commonly isolated bacteria within both groups. Along with the isolation of enterococci and the low numbers of Gram-negative bacteria, these finding corroborate with other studies globally [5,8,10,14]. *Staphylococcus* spp. are prevalent members of the human microbiome, and therefore their presence was expected. However, they are also opportunistic pathogens capable of causing a wide range of diseases in immunocompromised individuals [15], so their presence on staff mobile phones is also potential cause for concern. Enterococci are normally found in the intestines, therefore their presence on mobile phones might suggest poor hand hygiene [16]. It is estimated that 75% of the population use their mobile devices whilst in the bathroom [17], which may explain their presence on participant's mobile phones. Additionally, *Enterococci* are known to survive for several weeks on dry surfaces [18].

Extent and diversity of contamination of mobile phones: Culture independent methods

This study offers the first insight into the microbiome of mobile phones in a clinical environment. Microbiome analysis revealed the true extent and diversity of device contamination and highlighted the potential limitations of traditional culture-based methods in infection control procedures. Gram-negative contamination was particularly under-represented using a culture-based approach, with microbiome analysis revealing that *Acinetobacter* spp. and *Pseudomonas* spp. were at least as abundant as *Staphylococcus* spp., yet they were rarely detected by culture. Previous studies have shown the ability of these,

and other Gram-negative bacteria, to persist on inanimate surfaces for several months [19]. The high prevalence of Gram-negatives, and the detection of 197 different genera, would suggest that culture-based methods are only a biased and selective representation of true contamination. The limitations of the swabbing method to detect mobile phones has previously been highlighted [20]. However, microbiome analysis cannot distinguish between viable and non-viable cells, and the technique is still relatively expensive. Therefore, the best approach might be a combination of culture-dependent and culture-independent methods.

Comparison of hospital and control phone microbial communities

Spore-forming *Bacillus* spp. Were significantly more abundant in hospital than control samples, but the reasons for this difference are not clear. *Bacillus* spores are resistant to many forms of disinfectants used in healthcare, and some disinfectants may even encourage sporulation [21]; possibly the stringent disinfection protocols of hospitals encourage a greater abundance of spore formers.

Overall, hospital and control phone microbiomes were not significantly different at genus level. However, analysis of communities at the OTU level did reveal significant differences between hospital departments classed as 'high risk of infection' and 'low risk of infection'. OTU richness was also significantly higher on hospital phones, indicating a larger number of species. Again, the reasons for these differences are unclear, but this and the higher prevalence of resistant isolates on hospital staff phones suggests that mobile phone microbiomes do not just mirror the microbiome of their owner [2] but also potentially the environment their owner is in.

Presence of antibiotic-resistant potential pathogens on hospital staff mobile phones

The overall prevalences of MRSA (10.8%) and VRE (2.4%) in this study corroborate with other similar studies [9,22]. Both of these (largely nosocomial) pathogens were only detected on hospital staff phones. As hospital staff are often using personal mobile phones at work, then there is the potential for phones to facilitate the transmission of these drug resistant potential pathogens between the hospital and the community [23], although evidence supporting this is limited [24].

One limitation of this study was that only representative isolates of each colony type, and not every isolate from every phone, were characterised, so some contaminants may have been overlooked. Another limitation was that culture-independent analysis was from pooled samples, so no information about the microbiome of individual phones was collected.

Conclusion

Mobile phones of hospital staff are heavily contaminated with potentially pathogenic and drug resistant bacteria. With 92% of patient-facing staff in this study using their device at work, there is clear opportunity for cross contamination between phones, hands and patients. The role of the environment in the transmission of HAIs is increasingly being recognised, and the ubiquity of mobile devices in that environment warrants consideration of their role in infection transmission [11,13]. Recently, whole genome sequencing methods have been used to characterise potential pathogen transmission routes in hospitals, but only one study has included mobile phone contaminants to date [25]. No attempt was made to determine relatedness of phone isolates to infection isolates in this study, and further research in this area is needed to accurately quantify the risks. Phones cleaned on a daily basis were significantly less contaminated, so raising awareness amongst staff to encourage

regular cleaning of phones could be an effective intervention to mitigate any potential risks, although further research is needed to determine the best method of doing so. This study has also highlighted the limitations of using swabs to characterise microbial contamination of the hospital environment.

Conflicts of interest

No conflicts of interest reported.

Funding

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401

402 **Figure Legends**

403 **Figure 1.** Relative abundance of major genera in hospital and control phone communities as
404 determined using 16S rRNA microbiome analysis. All genera contributing >5% relative
405 abundance in hospital or control samples are included.

406 **Figure 2.** nMDS ordination of genera from pooled samples with 40% and 60% similarity
407 contours. Strength and direction vectors for key genera are displayed: *Acin*. = *Acinetobacter*;
408 *Arthro*. = *Arthrobacter*; *Bac*. = *Bacillus*; *Entero*. = *Enterobacter*; *Pseudo*. = *Pseudomonas*; and
409 *Staph*. = *Staphylococcus*. Samples are labelled by hospital (H) department: H1 = Surgical; H2
410 = Paediatrics; H3 = Intensive care unit; H4 = Radiology; H5 = Pharmacy; H6 = Surgical; H7 =
411 Medical assessment unit; H8 = Accident and Emergency; and H9 = Mobile staff. Control (C)
412 samples are randomly pooled and labelled as C1-C6.

413 **Figure 3.** nMDS ordination of OTU samples with 40% and 60% similarity contours, and
414 strength and direction vectors for key OTUs displayed. Samples are labelled by hospital (H)
415 department: H1 = Surgical; H2 = Pediatrics; H3 = Intensive care unit; H4 = Radiology; H5 =
416 Pharmacy; H6 = Surgical; H7 = Accident and Emergency; H8 = Medical assessment unit; and
417 H9 = Mobile staff. Control (C) samples are randomly pooled and labelled as C1-C6.

418 **Figure 4.** Comparison of antibiotic resistance prevalence between *S. aureus* isolates from
419 hospital and control phones. Significant relationships between resistance and phone type
420 frequencies are indicated by * (adjusted $P = 0.006$; $k = 9$).

421 **Figure 5**. Comparison of antibiotic resistance prevalence between *E. faecalis* isolates from
422 hospital and control phones.

Supplementary Table 1. Abundance of every detected genus across all samples. Table shows total counts for every detected genus across all samples. Each count is one copy of a 16S rRNA gene DNA sequence matching taxonomically to that particular genus.

426 **Figures**

427 **Figure 1.**

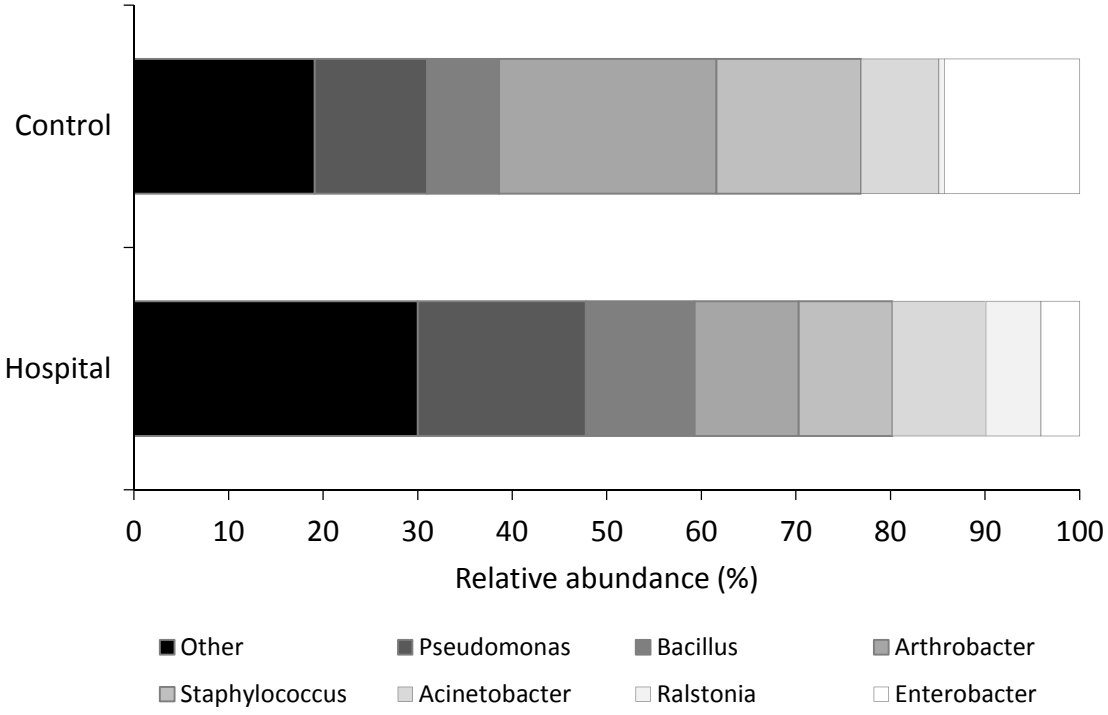

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Figure 2.

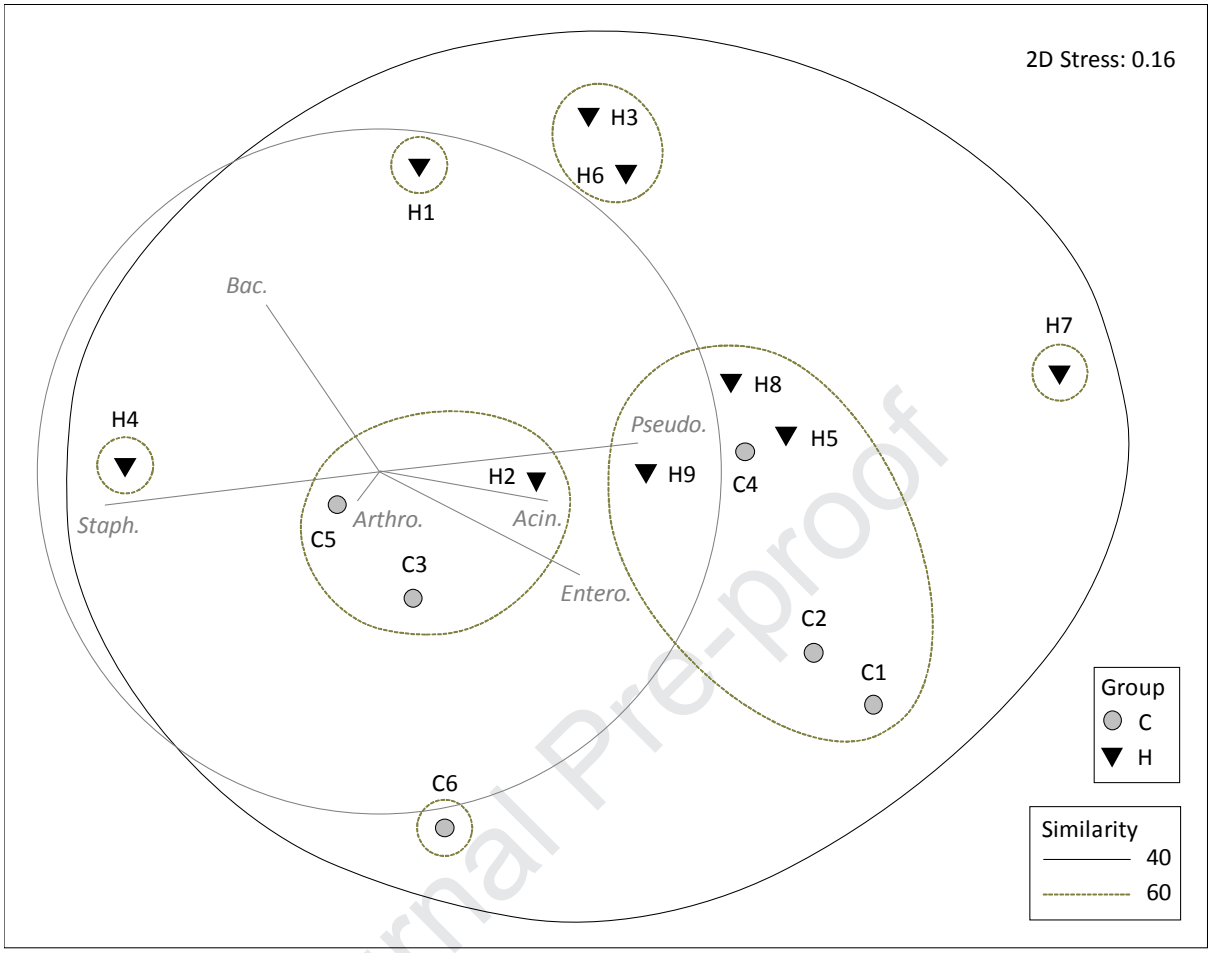

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Figure 3.

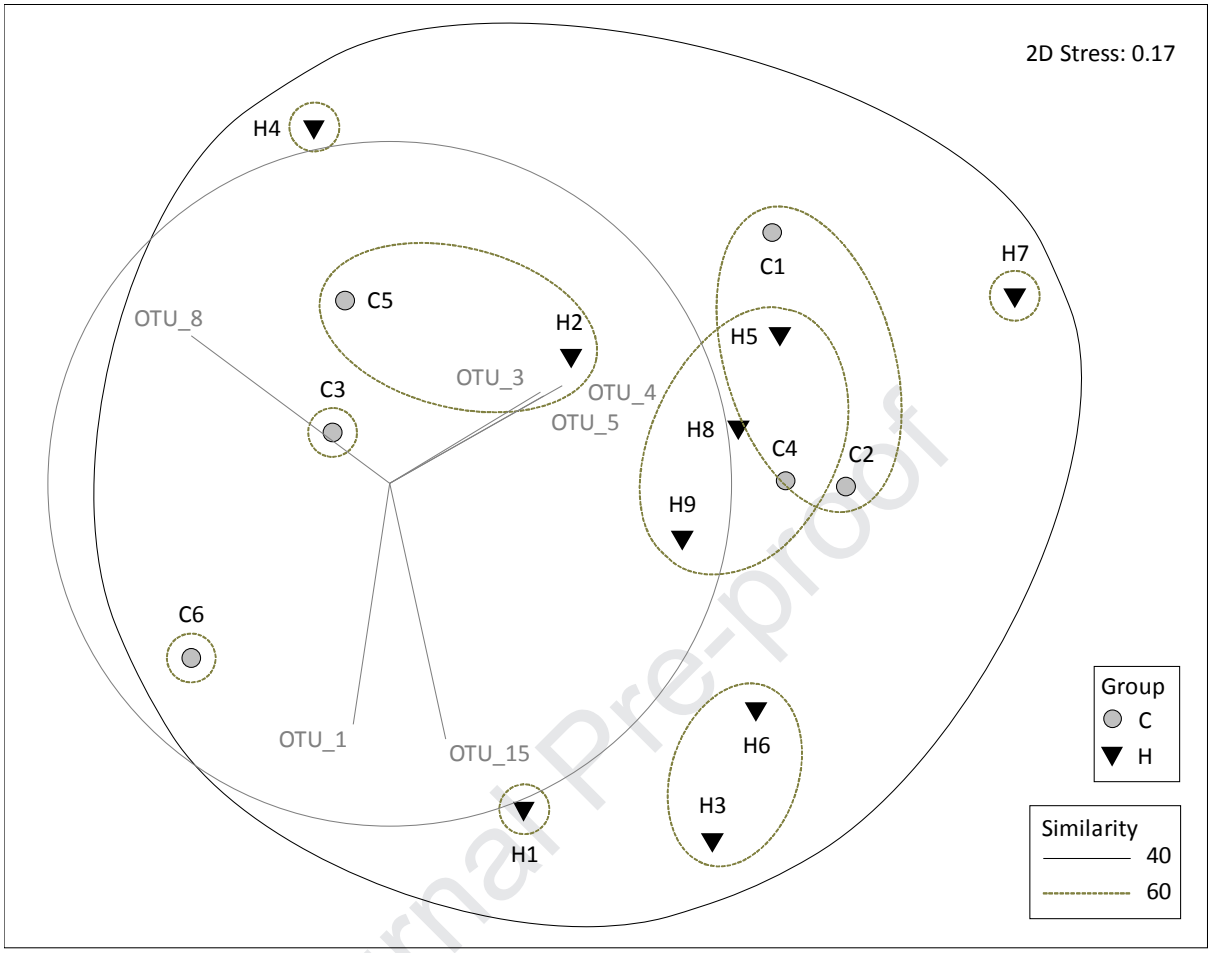


Figure 3. nMDS ordination of OTU samples with 40% and 60% similarity contours, and strength and direction vectors for key OTUs displayed. Samples are labelled by hospital (H) department: H1 = Surgical; H2 = Pediatrics; H3 = Intensive care unit; H4 = Radiology; H5 = Pharmacy; H6 = Surgical; H7 = Accident and Emergency; H8 = Medical assessment unit; and H9 = Mobile staff. Control (C) samples are randomly pooled and labelled as C1-C6.

Figure 4.

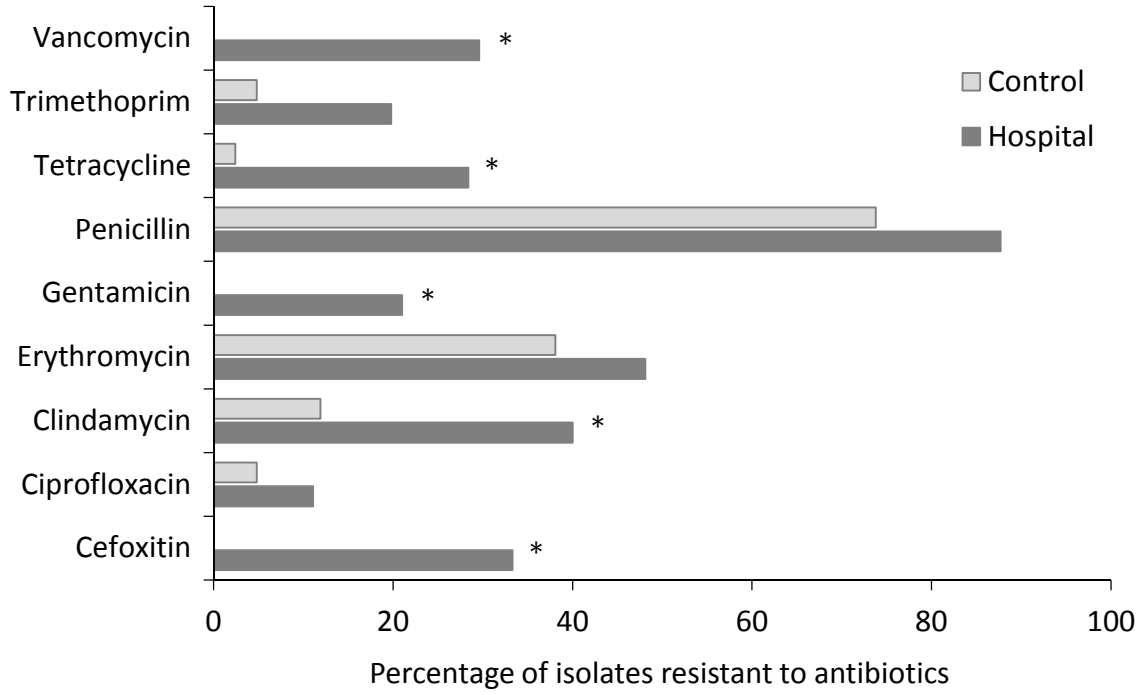
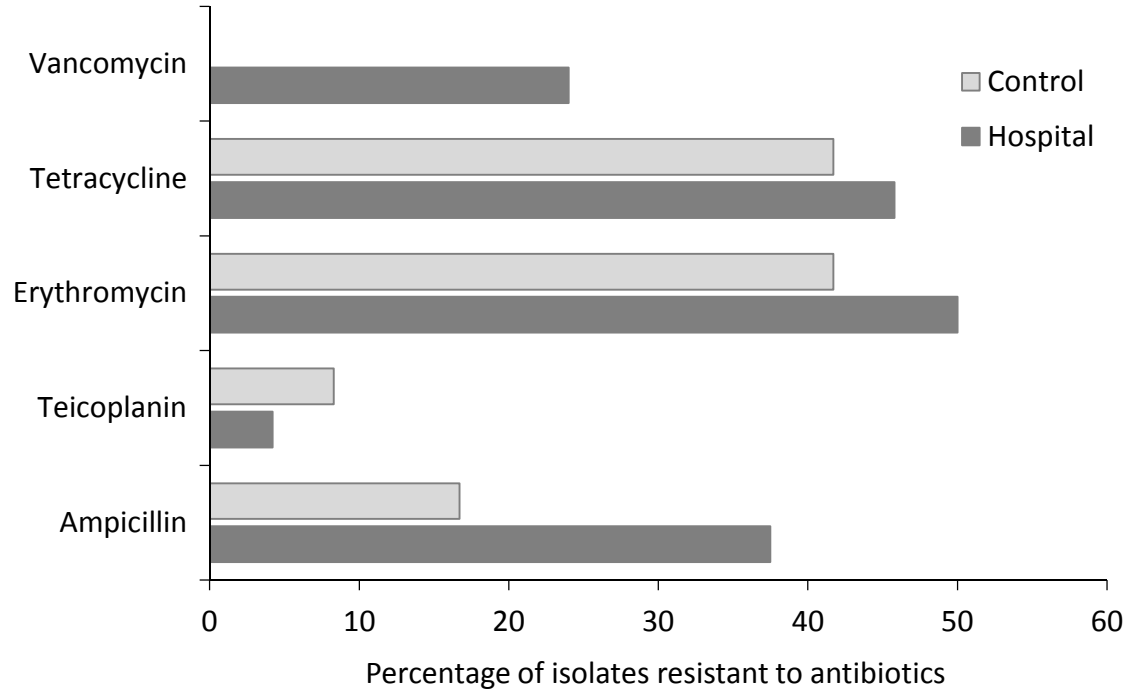


Figure 4. Comparison of antibiotic resistance prevalence between *S. aureus* isolates from hospital and control phones. Significant relationships between resistance and phone type frequencies are indicated by * (adjusted $P = 0.006$; $k = 9$).

474 **Figure 5.**



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476 **Figure 5**. Comparison of antibiotic resistance prevalence between *Enterococcus* isolates

477 from hospital and control phones.

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