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# Bacterial burden and drug-resistant bacteria in healthcare workers' mobile phones: a study in Puerto Rican outpatient clinics

R. Scharbaai—Vázquez*, A.N. García—Aponte, C. Huertas—Ayala, E.Y. Martínez—Monegro, G.M. Guadalupe—Ríos, J. Díaz—Portorreal, M.I. González—Torres, N.M. Fernández

*San Juan Bautista School of Medicine, Caguas, Puerto Rico*

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SUMMARY

***Background:*** Mobile phones used by healthcare workers (HCWs) in hospitals are significant reservoirs of drug-resistant bacteria responsible for hospital-acquired infections (HAIs).
***Aim:*** The objective of this study was to assess the level of contamination with such bacteria in outpatient clinics.
***Methods:*** Swabs from 83 HCWs' mobile phones were processed using standard biochemical and enzymatic procedures to identify pathogenic bacteria. β-Lactamase tests, antimicrobial susceptibility tests, screening for extended-spectrum β-lactamase (ESBL), and carbapenemase production were performed according to CLSI guidelines. Molecular detection of multi-drug-resistant genes (*mecA* in *Staphylococcus aureus* and *kpc*/*ndm* carbapenemases in *Klebsiella pneumoniae* and *Acinetobacter* spp.) was performed using multiplex real-time polymerase chain reaction.
***Findings:*** The overall prevalence of mobile phone contamination with one or more bacteria was 100%. A total of 51 Gram-positive and 44 Gram-negative isolates, including 20 coagulase-negative staphylococci (CoNS), 20 *S. aureus* (0 meticillin-resistant *S. aureus*), 11 *Acinetobacter* spp. and 10 *K. pneumoniae* were isolated. β-Lactamase production was detected in 45% of CoNS and 30% of *S. aureus*. Panton—Valentine Leukocidin (PVL) toxin gene in *S. aureus* was found in 20% (4/20) of the isolates. Twenty (20%) and 13% of the *Acinetobacter* spp. and *K. pneumoniae* isolates, respectively, were ESBL but not carbapenemase producers.
***Conclusions:*** The presence of HAI-causing organisms on mobile phones used by HCWs in outpatient clinics necessitates the implementation of infection control measures to mitigate the risk of cross-contamination in critical healthcare settings.



* Corresponding author. Address: San Juan Bautista School of Medicine, Caguas 00727, Puerto Rico.
*E-mail address:* Rscharbaai@sanjuanbautista.edu (R. Scharbaai—Vázquez).

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## Introduction

In recent years, there has been a significant shift in healthcare delivery from the acute-care hospital setting (inpatient) to a variety of outpatient clinics and community-based settings (ambulatory care) [1]. The increasing demand for care, severity of treated conditions, and complexity of procedures performed in outpatient settings are associated with a growing risk of transmission of infections [2,3]. Outpatient facilities often do not have the same rigorous infection-prevention infrastructure, resources, and tracking systems as hospitals, further increasing infection risks. Moreover, inadequate hand hygiene practice after coming into contact with a contaminated fomite (inanimate object) by outpatient staff and patients is a well-known risk factor for acquiring a hospital-acquired infection (HAI) [3]. HAIs caused by antimicrobial-resistant pathogens are a serious issue in the healthcare environment, leading to severe illnesses, prolonged hospital admissions, increased healthcare costs, higher expenses for second-line drugs, and treatment failures [4].

Among the fomites, mobile phone use among healthcare workers (HCWs) in hospitals has been extensively studied due to the potential of these devices to spread clinically relevant and antimicrobial-resistant bacteria that could be involved in HAIs [5—11]. Results from healthcare staff working in critical areas, including paediatric intensive care units (PICUs), intensive care units (ICUs), as well as dentistry and veterinary medicine, were considered. Many studies have found that phones are frequently contaminated with Enterobacterales, coagulase-negative staphylococci (CoNS), *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter* spp., with contamination rates varying by ward, hospital and region. In 2022, metagenomic microbial profiling of mobile phones owned by hospital staff working in paediatric areas (both general and intensive care) revealed the presence of ESKAPE bacteria [12]. ESKAPE pathogens (an acronym for *Enterococcus faecium*, *S. aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa*, and enterobacter species) are the most frequent drug-resistant bacteria causing HAIs [13]. They are notorious for their ability to 'escape' most conventional antimicrobials, leading to increased morbidity, mortality and healthcare costs [14]. Among the ESKAPE pathogens, *S. aureus*, *K. pneumoniae* and *A. baumannii* are considered urgent public health threats and priorities for intervention by the Centers for Disease Control due to their virulence, high levels of antibiotic resistance, and prevalence on medical equipment and in hospital environments [15—17].

*S. aureus* is a major human pathogen that can be found as a commensal on the skin and nasal flora. *S. aureus* causes a wide range of clinical infections, from localized wound, soft tissue, and device-related infections to invasive colonization leading to bacteraemia, osteomyelitis, septic arthritis, pleuropulmonary infections, and infective endocarditis [17]. *S. aureus,* in particular meticillin-resistant *S. aureus* (MRSA) variants carrying the *mecA* gene, is the pathogen of greatest concern in the clinical setting due to its potential to confer resistance to all commonly prescribed β-lactam drugs such as penicillins, cephalosporins and carbapenems [18]. While MRSA is identified as a nosocomial pathogen in hospital settings, strains of community-associated MRSA and meticillin-sensitive *S. aureus* (MSSA) are known to produce the Panton—Valentine Leukocidin (PVL) toxin, which is associated with more severe and invasive skin and soft tissue infections, poor prognosis, and bacteraemia [19].

*K. pneumoniae and Acinetobacter* spp., in particular, the extended-spectrum β-lactamase-producing (ESBL) variants are major contributors to HAIs such as urinary tract infection (UTI), bacteraemia, meningitis, pneumonia, and burn infections [14—16]. These variants are able to inactivate β-lactam antibiotics such as ceftazidime, ceftriaxone, cefotaxime and oxyiminomonobactam. Treatment options for ESBLs consists of carbapenems and cephamycine, considered the last-resort antibiotics to treat infections caused by multi-drug-resistant Gram-negative bacteria [15]. However, carbapenem resistance due to *K. pneumoniae* carbapenemase (KPC)-producing and KPC-producing *Acinetobacter* variants has emerged and is now a global healthcare concern [20].

While this scoping review provided valuable insights, it did not encompass microbial characterization or the identification of clinically relevant and antimicrobial-resistant bacteria among outpatient clinical staff in hospitals. Moreover, no specific studies have addressed the prevalence of such pathogens on mobile phones used by HCWs in outpatient clinics. The constant handling of mobile phones by HCWs as well as patients and visitors in outpatient clinics makes it a suitable vehicle for transmission of HAIs.

The objective of this work was to describe the bacteriological profiles, antimicrobial resistance, and virulence traits of bacteria found on mobile phones frequently used by HCWs in outpatient clinics, with emphasis on *S. aureus*, *K. pneumoniae* and *Acinetobacter* spp. Understanding the distribution of these strains is vital for planning interventions.

## Methods

### *Study setting*

This cross-sectional study was conducted from August to October 2019 at six outpatient clinics of a large private non-profit healthcare centre serving the Eastern-Central region of Puerto Rico. Each clinic has multi-disciplinary departments that offer a variety of medical (general and family medicine, paediatric, obstetrics/gynaecology), dental, pharmacy, mental health, and bio-social (nutrition, health education) services. The clinics provide services to a vulnerable population of Puerto Rico, those who do not have insurance and are not qualified to receive state insurance. The majority of healthcare personnel who work in these outpatient clinics also provide services in hospitals.

### *Participant recruitment and sample processing*

All physicians, nurses, pharmacists, dentists, social workers, residents in training and professional and technical personnel were invited to participate. The mobile phones were conveniently sampled from HCWs who verbally consented to participate in the study. For the purpose of this study, samples were collected in the morning without prior notification, as soon as the HCWs arrived at their respective settings. Samples were aseptically obtained by swiping the entire surface of the phones with a sterile cotton swab moistened with 0.9% sterile normal saline. Swabs samples were immediately placed in a

properly tagged tube containing Trypticase soy broth, and samples were incubated aerobically at 37°C overnight.

### Culture and identification of bacteria

For the isolation and identification of pathogenic bacteria, each swab sample was cultured on selective media (MacConkey agar for Gram-negative bacteria and Mannitol Salt agar for isolation of *S. aureus*; Thermo Fisher Scientific, USA) and incubated aerobically at 37°C for 24 h. For *S. aureus*, final identification from Mannitol Salt agar colonies was performed using Gram staining and conventional methods for Gram-positive bacteria, including a catalase test (BD) and a positive coagulase test (BD). Final identification from Gram-negative purified colonies was performed by commercial rapid ID enzymatic system (Remel Rapid NF plus System; Thermo Fisher Scientific, USA).

### β-Lactamase detection and antibiotic susceptibility testing

For the β-lactamase test, each Gram-positive isolate was streaked on to a pre-moistened Nitrocefin® commercial disc (BD). A colour change from yellow to pink within 60 min was considered a positive reaction. *S. aureus* ATCC 29213 and *S. aureus* ATCC 25923 were used as positive and negative controls, respectively.

Phenotypic detection of MRSA strains was performed using the cefoxitin disc diffusion method on Mueller—Hinton agar with a 30-μg cefoxitin (BD) disc, following the Clinical and Laboratory Standards Institute (CLSI) guidelines [21]. All colonies with an inhibition zone of ≤21 mm, based on CLSI interpretive criteria, were considered MRSA and subjected to genotypic confirmatory tests.

### Antibiotic susceptibility testing for K. pneumoniae and Acinetobacter spp.

Antimicrobial susceptibility testing of the isolated Gram-negative bacteria was carried out using the Kirby Bauer disc diffusion method according to the CLSI guidelines [21]. Each isolate was tested against eight broad-spectrum standard antibiotic discs (Thermo Scientific Oxoid, USA): tobramycin (10 μg), cefotaxime (30 μg), ciprofloxacin (5 μg), ceftriaxone (30 μg), imipenem (10 μg), gentamicin (10 μg), ceftazidime (30 μg) and aztreonam (30 μg). Bacterial suspensions were adjusted to 0.5 McFarland and swabbed on to Mueller—Hinton agar (Thermo Fisher Scientific, USA).

Screening for ESBL-producing *K. pneumoniae* and *Acinetobacter* spp. was carried out by measuring the diameters of zone inhibitions produced by cefotaxime, ceftriaxone or ceftazidime according to the CSLI recommendations [21]. The CLSI resistance breakpoints criteria that indicated suspicion of an ESBL-producing isolate are shown in Table I.

All *K. pneumoniae* and *Acinetobacter* spp. that showed resistance in at least one of the cephalosporins tested were suspected of being an ESBL producer and considered a candidate for phenotypic confirmatory tests.

Phenotypic confirmation of ESBL-producing *K. pneumoniae* and *Acinetobacter* spp. was performed using the double-disc synergy diffusion method according to CSLI standards [21].

**Table I**
Clinical and Laboratory Standards Institute (CSLI) resistance breakpoints criteria

| *Klebsiella pneumoniae* | Cefotaxime | Ceftriaxone | Ceftazidime |
|---|---|---|---|
| | ≤22 mm | ≤19 mm | ≤17 mm |
| *Acinetobacter* spp. | ≤14 mm | ≤13 mm | ≤14mm |

Ceftazidime (30 μg) and cefotaxime (30 μg) discs were placed at a distance of 25 mm from ceftazidime-clavulanate (30/10 μg; Oxoid; Thermo Scientific) and cefotaxime-clavulanate (30/10 μg; Oxoid; Thermo Scientific) discs on a Mueller—Hinton agar plate inoculated with 0.5 McFarland-adjusted bacterial suspension. The test was considered positive when a ≥5-mm increase in zone diameter for either the ceftazidime-clavulanate or cefotaxime-clavulanate disc was measured versus the zone diameter of the respective cephalosporin discs tested alone. *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as positive and negative controls, respectively, in the ESBL screening tests.

### Carbapenemase screening in K. pneumoniae and Acinetobacter spp.

Phenotypic detection of carbapenemase production in *K. pneumoniae* and *Acinetobacter* spp. isolates was carried out by measuring the thickness of the zone of inhibition produced by imipenem as described previously [22]. The criteria for imipenem that indicated suspicion of a carbapenemase-producing isolate was ≤19 mm.

### Molecular detection of carbapenemase and virulence genes in K. pneumoniae, Acinetobacter spp. and S. aureus

Genomic DNA was extracted from one single colony of each isolate according to the manufacturers' specifications for preparation of Gram-negative and -positive bacterial cell lysate (PureLink®, Thermo Fisher Scientific, USA). The presence of antimicrobial resistance and virulence genes was assessed with a multiplex real-time polymerase chain reaction (PCR) approach and using the amplification mixture TaqMan Master kit (Thermo Fisher Scientific, USA). The protocol of Smiljanic *et al.* [23] was followed for the detection of the two most common global carbapenemases worldwide (bla $_{NDM-1}$ and bla $_{KPC}$) in *K. pneumoniae and Acinetobacter* spp. Amplification of the *mecA* (associated with meticillin resistance) and PVL (virulence factor) genes in *S. aureus* was performed using the protocol described by Fosheim *et al.* [24]. Real-time PCR primers, probes, and reference strains are listed in Table II.

## Results

### Socio-demographic characteristics of study participants and bacterial isolates

A total of 83 HCWs participated in the study, each providing one swab sample from their mobile phones. The distribution of samples across the six outpatient clinics was uneven, influenced by variations in healthcare services provided and the

**Table II**
Primers, probes and polymerase chain reaction reference strains used in this study

| Gene | primer pairs (5′—3′) | Fluorophore-probe | Ref. |
|---|---|---|---|
| blaKPC | *for*-GCGATACCACGTTCCGTCTG<br>*rev*-CGGTCGTGTTTCCCTTTAGC | 6FAM-AGCGGCAGCAGTTTGTTGATTG-BBQ | [15] |
| blaNDM | *for*-TTTGGCGATCTGGTTTTCCG<br>*rev*-ATCAAACCGTTGGAAGCGAC | VIC-AGACATTCGGTGCGAGCTGGC-BBQ | [15] |
| mecA | *for*-AAAGAACCTCTGCTCAACAAGT<br>*rev*-TGTTATTTAACCCAATCATTGCTGTT | VIC-CCAGATTACAACTTCACCAGGTTCAACT | [16] |
| pvl | *for*-AATGAAATGTTTTTAGGCTCAAGACA<br>*rev*-TGGATAACACTGGCATTTTGTGA | SYBGreen-AGCAACTTAAATGCTGGACAAAACTTCTTGGAA | [16] |
| ATCC reference strains | | | |
| *Klebsiella pneumoniae* ATCC BAA-1705 | | Carbapenemase bla $_{KPC}$ +, bla $_{NDM}$ - | N/A |
| *K. pneumoniae* ATCC BAA-2473 | | Carbapenemase bla $_{KPC}$ -, bla $_{NDM}$ + | N/A |
| *Staphylococcus aureus* ATCC BAA-1717 | | MecA +, PVL + | N/A |

ATCC, American Type Culture Collection, USA.

availability of clinic facilities and physician offices (Table III). As shown in Table IV, 82% (68/83) of the phone samples were from the patient care team, consisting primarily of nurses (17/68, 38%), physicians (26/68, 25%), pharmacists (9/68, 13%) and social workers (7/68, 10.3%). Fifteen individuals (18%, 15/83) were from the facilities and support services team (Table IV). Overall, 72 (87%) of the samples were from female participants, and 11 (13%) were from male participants. In total, 95 isolates were obtained with overall cell phone contamination registered at 100%. Of the 95 isolates, 53.7% (51/95) were Gram-positive bacteria, whereas 46.3% (44/95) were Gram negative (Table IV).

## *Distribution of gram-positive and gram-negative bacteria*

CoNS and *S. aureus* were the most frequently isolated Gram-positive bacteria, each comprising 39.2% (20/51) of the isolates, followed by *Streptococcus* spp. at 13.7% (7/51) and *Enterococcus* spp. at 7.8% (4/51) (Table IV). The proportion of β-lactamase-producing CoNS, *S. aureus*, *Streptococcus* spp., and *Enterococcus* spp. was 45% (9/20), 30% (6/20), 43% (3/7) and 50% (2/4), respectively (Figure 1).

As shown in Table IV, *Acinetobacter* spp. was the most prevalent Gram-negative bacteria identified (25%, 11/44), followed by *K. pneumoniae* (22.7%, 10/44).

**Table III**
Characteristics of the 83 healthcare workers enrolled

| Characteristic | Total number of phones included in the study (%) |
|---|---|
| Outpatient hospital | |
| 1 | 18 (22) |
| 2 | 5 (6) |
| 3 | 19 (23) |
| 4 | 10 (12) |
| 5 | 11 (13) |
| 6 | 20 (24) |
| Total | 83 (100) |

## *Demographics associated with clinically relevant bacterial contamination on HCWs' mobile phones*

The distribution of cellular phones contaminated with clinically relevant bacteria was evaluated according to the owner's demographics, including gender, profession and department. Nursing staff in the paediatric and adult medicine departments represented the highest proportion of clinically relevant Gram-positive and Gram-negative bacteria, at 84.6% (22/26), including *S. aureus* (5/26) and *Acinetobacter* spp. (4/26) (Table IV). Eighty-five per cent (17/20) of the *S. aureus* isolates were recovered from cell phones belonging to female users. *S. aureus* was isolated from 30% (6/20) of the cell phones belonging to the facilities and support services team (Table IV). Mobile phones from pharmacy staff represented the largest source of clinically relevant Gram-negative bacteria, with a total rate of 100% (9/9), including *Acinetobacter* spp. (2/9, 22.2%) and *K. pneumoniae* (4/9, 44.4%). The majority of *Acinetobacter* spp. isolates (90.9%, 10/11) were derived from the mobile phones of female owners, and all *K. pneumoniae* isolates were exclusively obtained from females.

## *Analysis of antibiotic resistance and virulence factors in S. aureus*

Phenotypic detection of meticillin resistance in *S. aureus* using the cefoxitin disc diffusion test, along with genotypic detection of the *mecA* gene via multiplex real-time PCR, yielded negative results (0/20). However, PCR analysis indicated the presence of the virulence-associated *pvl* gene in 20% of the *S. aureus* isolates (4/20). *pvl*-Positive *S. aureus* strains were isolated from one medical technologist and three nurses, including one from the triage department (1/20) and two from the paediatrics department (2/20).

## *Phenotypic detection of antibiotic resistance in Acinetobacter spp. and K. pneumoniae*

The antibiotics resistance pattern of *K. pneumoniae* and *Acinetobacter* spp. isolated in mobile phones from HCWs against eight antibiotics is shown in Table V. *Acinetobacter* spp. showed the highest resistance to the broad-spectrum cephalosporins cefotaxime (3; 30%) and ceftazidime (4; 40%).

**Table IV**
Occupational factors associated with clinically relevant bacterial contamination on mobile phones in outpatient clinics

| Healthcare personnel | Gram positive bacteria ($N = 51$) | | | |
|---|---|---|---|---|
| | CoNS | *Staphylococcus aureus* | *Streptococcus* spp. | *Enterococcus* spp. |
| Patient care team ($N = 68$) | | | | |
| Physicians ($N = 17$) | 3 | 3 | 2 | 2 |
| Nurses ($N = 26$) | 3 | 5 | 3 | 0 |
| Dentists ($N = 2$) | 0 | 2 | 0 | 1 |
| Pharmacists ($N = 9$) | 3 | 0 | 0 | 0 |
| Social workers ($N = 7$) | 3 | 2 | 0 | 0 |
| Optometrists ($N = 2$) | 0 | 0 | 1 | 0 |
| Psychologists ($N = 2$) | 0 | 1 | 0 | 0 |
| Medical technologists ($N = 1$) | 1 | 1 | 0 | 0 |
| Case management specialist ($N = 2$) | 1 | 0 | 0 | 1 |
| Facilities and support services team ($N = 15$) | 6 | 6 | 1 | 0 |
| Total, *N* (%) | 20 (39.2) | 20 (39.2) | 7 (13.7) | 4 (7.8) |

| Healthcare personnel | Gram negative bacteria ($N = 44$) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Citro | *E. coli* | Steno | Shig | Enterob | Serrat | Haf | Burkh | Panto | Acinet | Pseudo | Kp |
| Patient care team ($N = 68$) | | | | | | | | | | | | |
| Physicians ($N = 17$) | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 1 | 1 |
| Nurses ($N = 26$) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 4 | 2 | 1 |
| Dentists ($N = 2$) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 |
| Pharmacists ($N = 9$) | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 2 | 0 | 4 |
| Social workers ($N = 7$) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 |
| Optometrists ($N = 2$) | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Psychologists ($N = 2$) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Medical technologists ($N = 1$) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Case management specialist ($N = 2$) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 |
| Facilities and support services team ($N = 15$) | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 0 |
| Total, *N* (%) | 1 (2.3) | 1 (2.3) | 1 (2.3) | 1 (2.3) | 1 (2.3) | 1 (2.3) | 1 (2.3) | 6 (13.6) | 6 (13.6) | 11 (25) | 4 (9.1) | 10 (22.7) |

Acinet, *Acinetobacter* spp.; Burkh, *Burkholderia* spp.; Citro, *Citrobacter* spp.; CoNS, coagulase-negative *Staphylococcus*; E. coli, *Escherichia coli*; Enterob, *Enterobacter* spp.; Haf, *Hafnia* spp.; Kp, *Klebsiella pneumoniae*; Panto, *Pantoea* spp.; Pseudo, Pseudomonas spp; Serrat, *Serratia* spp.; Shig, *Shigella* spp.; Steno, *Stenotrophomonas* spp.

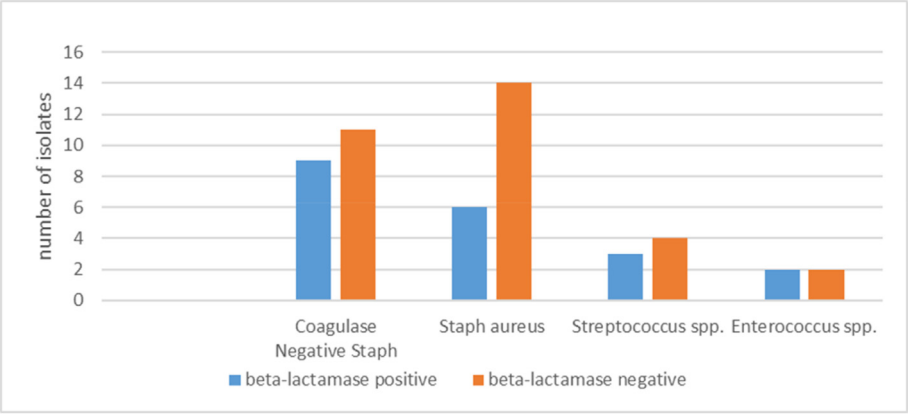


**Figure 1.** Proportion of drug-resistant Gram-positive species among healthcare workers at outpatient clinics ($N = 51$).

**Table V**
Antimicrobial resistance profiles of *Klebsiella pneumoniae* and *Acinetobacter* spp. against broad-spectrum antibiotic isolated from healthcare workers' mobile phones at outpatient clinics

| Isolate (n) | Antimicrobial resistance profile | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | TOB *N* (%) | CTX *N* (%) | CIP *N* (%) | CRO *N* (%) | IPM *N* (%) | GM *N* (%) | CAZ *N* (%) | ATM *N* (%) |
| *K. pneumoniae* (8) | 0 (0.0) | 1 (13) | 0 (0.0) | 1 (13) | 0 (0.0) | 0 (0.0) | 1 (13) | 0 (0.0) |
| *Acinetobacter* spp. (10) | 0 (0.0) | 3 (30) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 4 (40) | ND |

ATM, aztreonam; CAZ, ceftazidime; CIP, ciprofloxacin; CRO, ceftriaxone; CTX, cefotaxime; GM, gentamycin; IPM, imipenem; ND, not determined by Clinical and Laboratory Standards Institute (CLSI); TOB, tobramycin.

### *Prevalence of ESBL-producing K. pneumoniae and Acinetobacter spp.*

Overall, five (28%) of the isolates were positive for the double-disc diffusion screening test of ESBL production (Table VI, Figure 2). No isolate was a carbapenemase producer as measured with the imipenem zone of inhibition.

### *Genotypic detection of carbapenemase genes KPC and NDM in K. pneumoniae and Acinetobacter spp.*

The eight *K. pneumoniae* and 10 *Acinetobacter* spp. were analysed for the presence of bla KPC and bla NDM-1 genes using the multiplex real-time PCR. The PCR results were negative for KPC and NDM-1-type carbapenemases in all the *K. pneumoniae* and *Acinetobacter* spp. isolates tested.

**Table VI**
Frequency of extended-spectrum β-lactamase (ESBL)- and carbapenemase-producing *Klebsiella pneumoniae* and *Acinetobacter* spp.

| Isolate | Total tested | ESBL producer, *N* (%) | | Imipenem resistance, *N* (%) | |
|---|---|---|---|---|---|
| | | Positive | Negative | Positive | Negative |
| *K. pneumoniae* | 8 | 2 (25) | 7 (0.0) | 0 (0.0) | 0 (0.0) |
| *Acinetobacter* spp. | 10 | 3 (30) | 8 (80) | 0 (0.0) | 0 (0.0) |

## Discussion

To our knowledge, the present study is the first to examine HCWs' mobile phone bacterial contamination among HCWs at outpatient clinics. Most studies on HCWs' mobile phones have been conducted in inpatient hospital environments, revealing a prevalence of contamination with nosocomial and antimicrobial-resistant pathogens ranging from 10% to 100% (reviewed in [11]). In our study, the percentage of contaminated mobile phones with at least one bacterial pathogen was 100%.

Several studies have shown that nosocomial pathogenic and resistant bacteria are also present in outpatient clinics [25—27]. For instance, ESBL-producing *E. coli* has been isolated from outpatient urinary samples, blood, wounds, and the respiratory tract [25]. Hefzy *et al.* [27] found that contamination of reusable medical equipment such as stethoscopes and ultrasound transducers before disinfection intervention was 100.0%. However, there is no clear evidence of whether the bacterial burden generated at the outpatient clinics could cross-contaminate the cell phones of HCWs nor whether contaminated cell phones from outpatient clinics introduce potentially pathogenic bacteria to hospitals. Additional comparison studies between outpatient and hospital groups are needed to confirm the hypothesis of this relationship.

In this study, *S. aureus* and CoNS were the two most prevalent Gram-positive bacteria isolated from HCWs' mobile phones at outpatient clinics. Similar patterns were observed in some studies conducted in hospitals [5—11]. Analysis of virulent and antibiotic-resistant variants of *S. aureus* (PVL-encoding

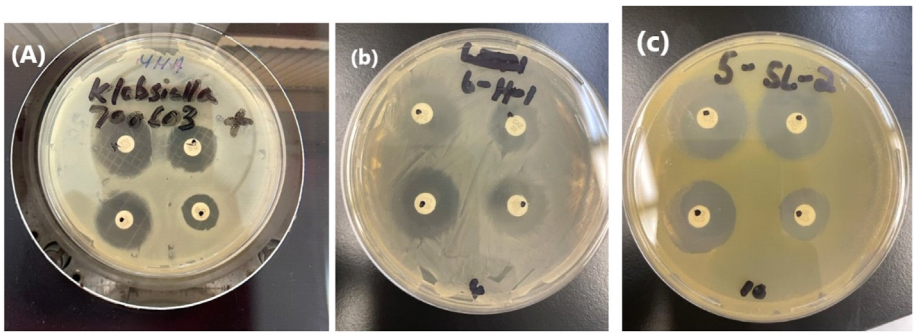


**Figure 2.** Double-disc synergy test between cefotaxime or ceftazidime and cefotaxime-clavulanate or ceftazidime-clavulanate. (a) Positive control *Klebsiella pneumoniae* 700603. (b), (c) Extended-spectrum β-lactamase producers *Acinetobacter* strain 6-H-1 and *Acinetobacter* strain 5-SL-2.

and MRSA, respectively) were of particular interest because of their emergence as major pathogens in the community and hospitals, respectively [18,19,28—31]. In the current study, 20% (4/20) of the *S. aureus* isolated from HCWs' mobile phones carry the PVL gene. Low occurrence of PVL was also reported in clinical *S. aureus* isolates [19,28].

In our study, all isolated *S. aureus* strains were negative for the mecA gene. This finding contrasts with some studies that report the presence of MRSA on the cell phones of healthcare personnel caring for critically ill patients in the hospital [11,28,30,32]. Although MRSA has been identified as a causative agent of nosocomial infections [18], and HCWs have been described as carriers of MRSA in nares, hands, or cell phones [30], there is currently no evidence of direct transmission from cell phones to hospitalized patients. The absence of MRSA in our study, however, does not exempt *S. aureus* from being pathogenic. MSSA commonly circulates in the community and is associated with worse outcomes than *Streptococcus pneumoniae* community-acquired pneumonia [31]. Infection control practices among HCWs in outpatient clinics should be extended to include monitoring cell phones for clinically relevant *S. aureus* to identify epidemiologic trends and risk factors for the transmission of these strains to the hospital.

β-Lactamase activity was found in all four groups of Gram-positive bacteria isolated from HCWs' mobile phones, as previously described by other studies [12]. The mechanism of β-lactam resistance in *S. aureus* and the majority of gram-positive bacteria is the production of a narrow-spectrum β-lactamase (BlaZ) that inactivates penicillin by hydrolyzing the β-lactam ring of the drug. This type of β-lactamase, however, is of little clinical concern as the addition of a ß-lactamase inhibitor is sufficient to restore the antimicrobial efficacy.

In this study, *K. pneumoniae* and *Acinetobacter* spp. were the two most commonly isolated Gram-negative bacteria from HCWs' mobile phones in outpatient clinics. Similar results were found in a review study from 2005—2013 in hospitals [11]. The multi-drug-resistant variants found in both genera, particularly the ESBLs are of greater concern, as the emergence and spread of such strains are often responsible for the failure of antibiotic treatment in hospital settings [32]. ESBLs confer resistance to most β-lactam antibiotics, including penicillins, cephalosporins, and the monobactam aztreonam [32]. ESBLs are associated with increased morbidity and mortality within the intensive care setting, and the use of carbapenems which are considered antibiotics of last resort. In our study, the rate of ESBL-producing *K. pneumoniae* was 25%. This frequency is lower than from a study in hospitals in Ethiopia and Peru (28%, and 30.8%, respectively) [8,33]. Conversely, the frequency of ESBL-producing *Acinetobacter* spp. isolates in our study (30%) is significantly higher than other studies in hospitals from Ethiopia and Israel (14.6% and 20%, respectively) [8,9]. The high frequency of *Acinetobacter* spp. may be attributable to the fact that our sampling was conducted during the summer, and this pathogen has a higher prevalence of skin carriage in the summer than in the winter [34]. This result is also in agreement with another study in a country with a hot and humid climate similar to that of Puerto Rico, such as India [10]. Indoor temperature and humidity conditions in the outpatient clinics, along with the frequent portability of phones and poor hygiene, may facilitate the transmission of such pathogens.

Among the ESBLs, the carbapenemase-producing strains are resistant to virtually all β-lactam antibiotics. In our study, no KPC or NDM genes were detected in the *K. pneumoniae* or *Acinetobacter* spp. tested, as measured by molecular methods. This was consistent with the study of cell phones from Peruvian hospitals [33]. The spread of carbapenemase-producing strains poses a significant threat to clinical patient care and public health; therefore, continuous monitoring of carbapenemase production in outpatient clinics is an essential first step in combating this problem.

In our study, female sex and employment in the pharmacy and nursing departments were found to be factors associated with healthcare professionals' mobile phones and bacterial contamination. Although the majority of the participants in the study were female (87%), previous studies have described a higher frequency of bacterial contamination on the cell phones of women compared with men [19]. Contamination of women's mobile phones with Gram-negative bacteria, particularly *K. pneumoniae* and *Acinetobacter* spp., is highly indicative of faecal contamination [35,36].

Due to the exploratory nature of this research, the findings may not be generalizable, as the study sample size was limited by funding constraints. Furthermore, the majority of the study isolates were obtained from female participants, which may limit the representativeness of the sample. Additionally, the isolates were collected from six outpatient clinics in the Eastern Central Region of Puerto Rico, which may not reflect conditions across the entire country. These factors probably contributed to the emphasis on descriptive statistics in the analysis. However, it offers real-world relevance, as this study

provides insights into how cell phone hygiene is practised in everyday settings. This study was conducted prior to the COVID-19 pandemic, a period characterized by less stringent cleaning and disinfection protocols. It serves as a baseline for future research to assess mobile phone hygiene levels in outpatient settings across Puerto Rico, particularly during and after the COVID-19 pandemic, when heightened infection control measures were implemented. Such studies would provide a more comprehensive understanding of mobile phone hygiene in these settings under varying disinfection practices.

Based on this study, we concluded that the contamination of HCWs' mobile phones in outpatient facilities with potentially pathogenic and drug-resistant bacteria is significant. Furthermore, a relationship in bacterial contamination may exist between the cell phones of outpatient HCWs and those of hospital-based HCWs. This hypothesis is supported by our observation that the cell phones sampled in this study showed no evidence of prior sanitization before entering outpatient clinics and exhibited bacterial profiles similar to those reported in previous hospital-based studies. Because many physicians and HCWs divide their time between outpatient and inpatient services, they may become potential vectors for the transfer of such bacteria from non-critical to critical settings, increasing the risk of an HAI. In addition, the bacteria isolated from mobile devices in this study pose a threat to the elderly and vulnerable patient population visiting the ambulatory clinics. Therefore, the rigorous clinical hygiene standards and practices and mobile phone usage policies already present in hospital settings need to be implemented in outpatient facilities. Active screening for drug-resistant bacteria in outpatient facilities is recommended to reduce the burden of pathogenic bacteria to levels comparable to those observed in critical healthcare settings.

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## Author contributions

R.S.—V., A.N.G.—A., C.H.—A., E.Y.M.—M., G.M.G.—R., J.D.—P., M.I.G.—T., N.M.F.: conceptualization. A.N.G.—A., C.H.—A., E.Y.M.—M., G.M.G.—R., J.D.—P., M.G.—T., N.M.F.: investigation. A.N.G.—A., G.M.G.—R., E.Y.M.—M.: data curation. R.S.—V., N.M.F.: writing — original draft preparation. R.S.—V.: supervision, writing — reviewing and editing.

## Ethics statement

This study was revised and approved by the Institutional Review Board of the San Juan Bautista School of Medicine (Study Protocol IRB # EMSJBIRB-10-2019).

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## Conflict of interest statement

The authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

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