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RESEARCH ARTICLE



Contamination by antimicrobial-resistant enterobacteria isolated from cell phones and hands in a veterinary hospital

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ABSTRACT

Hospital infections are of great relevance in human and animal health, and fomites are important in the spread of pathogens in hospital units. The aim of this study was to investigate the frequency of enterobacteria in the operating room of a veterinary hospital, the potential cross-contamination of samples, and to characterise the susceptibility profile of the isolates to antimicrobials. Sixty-five samples were collected from five different surgical procedures. These samples came from the hands and cell phones of the surgical team and pet owners, operating tables, and patients. Species detection was performed through polymerase chain reaction, genetic diversity by pulsed-field gel electrophoresis (PFGE), and susceptibility to antimicrobials through an antibiogram. *Escherichia coli* and *Proteus mirabilis* isolates were obtained from eight samples, from the hands of the anaesthesiologist, the pet owner, and the surgeon; the surgeon's, the nurse's and the anaesthesiologist's cell phones, and two surgical tables. Furthermore, PFGE showed high genetic diversity among the isolates, which showed multidrug resistance. The identification of multidrug-resistant *E. coli* and *P. mirabilis* on cell phones of the surgical team is a major concern and, although no direct correlation was found, the isolation of these bacteria inside the clean area of the operating room shows the possibility of nosocomial transmission from cell phones to susceptible patients.

KEYWORDS

pulsed field gel electrophoresis, nosocomial infection, fomites, small animals, surgery centre

INTRODUCTION

Nosocomial infections represent a global issue, given the high levels of associated morbidity. Secondly, there is an increase in the length of hospital stay for patients, thus increasing treatment costs (Kollef et al., 2021). Mortality appears as a final consequence, and has been alarmingly high in human and veterinary patients (Sprague, 2009; Willemsen et al., 2019).

Surgical site infections account for a quarter of all nosocomial infections, and are the most common cause of infections in human surgical patients (Cheadle, 2006). In veterinary medicine, the rate of surgical site infection in small animals varies from 3 to 10% among clean and clean-contaminated procedures. As in human medicine, the infection of these animals results in increased morbidity, mortality, prolonged hospitalisation, and increased

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treatment cost, in addition to the emotional tension between the pet owner and the veterinarian (Turk et al., 2015).

The biological agents that cause nosocomial infections can be divided into endogenous pathogens, i.e. those that inhabit the microbiota or are present in pre-existing foci of infection of the patient, and exogenous pathogens, which are carried by medical equipment, accessories and hands of surgeons and other professionals (Burgess, 2019). In this context, cell phones are the target of studies about their potential to harbour pathogenic bacteria as reported by Brady et al. (2006), who found that 89.7% of cell phones belonging to health professionals were contaminated with bacteria. In addition, 15% of these bacteria were potentially nosocomial, such as *Escherichia coli* and *Proteus mirabilis*. Antimicrobial-resistant bacteria have been isolated from cell phones in other studies as well (Ramesh et al., 2008; Tagoe et al., 2011; Julian et al., 2012). More recently, Morvai and Szabó (2015) reviewed the literature and noted that the contamination rates of cell phones over the years increased from 40% to 100%, highlighting a great concern about the potential for the spread of pathogenic bacteria from cell phones, since these are one of the most often touched objects within the hospital environment (CDC, 2019).

The bacteria *E. coli*, *P. mirabilis*, and *Klebsiella pneumoniae* have become especially problematic in veterinary medicine due to the large number of infections caused by them over the past few years and the development of resistance to antimicrobials used in pets (Normand et al., 2000). Multidrug-resistant bacteria were considered endemic for the veterinary hospital environment (Sanchez et al., 2002) and represent a significant challenge due to the ease of their dissemination in that environment (Morley, 2004). Multidrug resistance to antimicrobials has become a public health problem, which leads to a heated debate about the use of these drugs. The implementation of infection control practices and strategies in veterinary medicine is still very limited, and the bond between humans and animals becomes ever closer, even allowing the exchange of multidrug-resistant bacteria between species (Perez et al., 2007).

In light of all the problems regarding nosocomial infections and their epidemiological outcome, as well as the scarcity of research aimed at identifying cell devices as fomites in veterinary medicine, the aim of our study was to investigate the incidence of *E. coli*, *P. mirabilis* and *K. pneumoniae* on cell phones of the surgical team and pet owners in a veterinary hospital, and also to evaluate the potential cross-contamination and to characterise the antimicrobial susceptibility profile of the isolates obtained.

MATERIALS AND METHODS

This study was carried out in accordance with the international standards on animal welfare after approval by the Ethics Committee on the Use of Animals of the Faculty of Agricultural and Veterinary Sciences of São Paulo State University (UNESP), Jaboticabal, SP (protocol number: 08283/19).

The Clinical Research Committee and the Human Institutional Review Committee approved the methodology for members of the surgical team and animal owners (protocol number 17281019.6.0000.5420).

Case selection

Five surgical procedures performed on patients of the canine species, selected from the surgical routine of the Veterinary University Hospital of São Paulo State University, Jaboticabal Campus, were chosen. The study enrolled animals whose surgical procedure was considered clean or clean-contaminated, according to the Centers for Disease Control and Prevention 2017 classification (Boyle et al., 2018), which did not have pre-existing signs of infection and which had not undergone antimicrobial therapy within 72 h prior to the procedure. The surgical team, composed of the main surgeon, auxiliary surgeon, anaesthesiologist, and veterinary nurse, as well as the patient's owner, were only admitted to the study if they had not been using antimicrobials or had not used them in less than 72 h before the sample collections.

The surgical and anaesthesiology team is composed of professors and residents who take turns in the routine of the veterinary hospital; therefore, in the selected procedures, there was no repetition of any professional during the collection of the samples.

Surgical preparation

All surgical procedures followed the same anaesthetic and preparation protocols for aseptic surgery. Perioperative antimicrobial protocol was used with the use of cefazolin (25 mg kg⁻¹ intravenously), administered 30 min before surgery and reapplied every 90 min until the end of the procedure.

The hair of all dogs was clipped using a #40 clipper blade, in an anteroom located outside the clean area of the operating room, approximately 30–45 min before the induction of anaesthesia. The patient was then transferred to the operating room, where a veterinary nurse wearing non-sterile gloves performed the initial cleaning of the patient's skin in a uniform manner with sterile gauze, in circular movements from the centre to the periphery, using 2% chlorhexidine and 70% isopropyl alcohol. In the operating room, definitive skin antisepsis was performed using a sterile technique with 0.5% alcoholic chlorhexidine solution, also applied with sterile gauzes in circular movements from the centre of the operating field to the periphery. After the patient's antisepsis was completed, the surgical procedure was continued by the placement of sterile surgical drapes in such a way that only the region to be manipulated would be exposed.

Sampling

A total of 65 samples from the five surgical procedures were collected using a dry sterile swab, being: from both hands of the main surgeons, before and after hand antisepsis (10

samples), the auxiliary surgeons after hand antisepsis (5 samples), the veterinary anaesthesiologists after the procedure (5 samples), the veterinary nurses in charge of the operating room after the procedure (5 samples), and the pet owners (5 samples). Samples from their respective cell phones (25 samples), samples from the subcutaneous region prior to skin suturing (5 samples), and from the surface of the operating table after the end of the procedure (5 samples) were also collected.

All collections were performed by a single professional in an aseptic manner, with this professional being appropriately dressed, wearing a cap, mask, and sterile gloves. Samples from the surgical team were collected inside the operating room, while those from the pet owners were collected inside an office attached to the surgical block.

To obtain the samples, the swabs were rubbed with rotating movements, repeating the procedure three times in each region. From the hands, samples were taken from the wrists to the fingertips (back and palm). In the case of cell phones, samples were collected from the front, side, and back surfaces of the devices, the wound was harvested in all extent of subcutaneous exposure, and the operating tables along their entire length. After collection, each swab was transferred to a test tube containing 3 mL of Brain Heart Infusion (BHI) broth previously prepared and sterilised.

Identification of bacteria

After the collections, the samples were transferred to the microbiology laboratory, where they remained incubated in an oven at 37 °C for 18 h in BHI broth. After incubation, the samples were sown on MacConkey agar, selective for enterobacteria, and again incubated at 37 °C for 24 h. After this period, the samples with colony growth suggestive of *E. coli* and *K. pneumoniae* (pink because they are lactose positive) and suggestive of *P. mirabilis* (colourless because they are lactose negative) were selected separately, enriched in BHI broth and then sent for species confirmation by polymerase chain reaction (PCR). For this, DNA extraction was performed by modifying the technique proposed by Keskimäki et al. (2001). In this technique, 1 mL of the culture was transferred to an Eppendorf tube and centrifuged at 15,000 rpm for 2 min to precipitate the cells and discard the supernatant. The precipitated cells were resuspended in 1 mL of phosphate buffered saline (PBS) and vortexed for 30 s. The bacterial culture was again precipitated by centrifugation and the washing process repeated, this time with 500 µL of sterile ultrapure Milli-Q water (Millipore Corporation, USA). After washing, the Eppendorf tube containing the culture was placed in boiling water for 10 min. After that period, the cells were precipitated by centrifugation at 14,000 rpm for 2 min, and a 300-µL aliquot of the supernatant (DNA) was removed and transferred to another Eppendorf tube. This lysed material was stored in a freezer at –80 °C. The product from this preparation was used to perform the PCR analysis to detect the bacterial species.

After extraction, the primers and PCR protocols for the detection of *E. coli*, *P. mirabilis* and *K. pneumoniae* were

performed according to Maheux et al. (2009), Zhang et al. (2013) and Li et al. (2019), respectively.

Antimicrobial susceptibility test

After confirming the positive samples, the evaluated isolates were seeded in a tube containing 3 mL of BHI broth and incubated at 37 °C until they reached the 0.5 MacFarland standard. After incubation, the culture was sown with the aid of a sterile cotton swab on Mueller-Hinton agar and, after approximately 3 min, the antimicrobial discs (DME®) were applied. After 24 h of incubation, the diameter of each inhibition zone was measured.

The antimicrobials were chosen from the main antimicrobial drugs used in clinical and surgical routine, and were the following: ampicillin (10 µg), ampicillin with sulbactam (20 µg), amoxicillin (20 µg), amoxicillin with clavulanic acid (10 µg), gentamicin (10 µg), amikacin (30 µg), enrofloxacin (5 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), levofloxacin (5 µg), cephalexin (30 µg), cefazolin (30 µg), cefoxitin (30 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), tetracycline (30 µg), sulphamethoxazole with trimethoprim (25 µg), azithromycin (15 µg), imipenem (10 µg), ceftazidime (30 µg), cefotaxime (30 µg), cefepime (30 µg) and piperacillin with tazobactam (110 µg). The disc diameters followed the guidelines proposed by CLSI (2018).

Pulsed-field gel electrophoresis (PFGE)

The isolates were characterised by the PulseNet standard PFGE protocol, as described by Ribot et al. (2006) with minor modifications in relation to the number of washes in the phase preceding the enzymatic lysis and the electrophoretic running time: there were three washes with buffer and one with water, and the run was carried out for 21 h. The system used was the CHEF DR-III (Bio-Rad, USA), with the chromosomal DNA being digested with the XbaI enzyme (Invitrogen, USA) and the electrophoresis performed on a Pulsifield Certified 1% agarose gel (Bio-Rad, USA), with a voltage of 6 V cm⁻¹, at an angle of 120°, with an initial polarity reversal time of 2.2 s and a final polarity reversal time of 54.2 s. The *Salmonella* Braenderup (H9812) strain was used as a standard, and the gels were submitted to electrophoresis for 21 h at a temperature of 14 °C. Fragment similarities were compared using the Dice coefficient at 1% tolerance and 0.5% optimisation. The dendrogram was calculated using the UPGMA grouping method, using BioNumerics Software, version 7.1 (Applied Mathematics, Sint-Martens-Latem, Belgium).

Case monitoring

All dogs were followed up for a minimum period of four weeks. The skin sutures were removed two weeks after the surgery, and the surgical wounds were evaluated for hyperaemia, oedema, painful sensitivity, drainage of serous and/or purulent secretion, suture dehiscence, abscess, and fistulas. When three or more of the signs mentioned above were noted or in presence of purulent

secretion, the wound was submitted to bacteriological culture (Eugster et al., 2004).

RESULTS

Eight microorganisms were identified by PCR. Seven samples were positive for *E. coli* (hand of the anaesthesiologist responsible for the first surgery; hand of the owner of the animal of the first surgery; hand of the surgeon responsible for the second surgery, before antisepsis; cell phone of the surgeon responsible for the third surgery; cell phone of the nurse responsible for the fifth surgery and two operating tables, corresponding to the first and the third surgery). One sample was positive for *P. mirabilis* (cell phone of the anaesthesiologist responsible for the fourth surgery). No sample was positive for *K. pneumoniae*.

Pulsed-field gel electrophoresis was performed only in the case of the *E. coli* isolates, because only one *P. mirabilis* isolate was obtained, and therefore it was not possible to carry out a comparative analysis with it. The PFGE showed that the seven *E. coli* isolates were heterogeneous, with a similarity of less than 70%, which shows that there was no cross-contamination between the samples analysed (Fig. 1).

In the antimicrobial susceptibility test, it was found that 7 of the 8 isolates were multidrug resistant, being thus classified as having resistance to three or more classes of antimicrobials, according to the consensus proposed by the European Center for Disease Control and Prevention (ECDC) in association with the Center for Disease Control and Prevention (CDC) (Magiorakos et al., 2012). Still, it is worth mentioning that samples classified as intermediately susceptible were considered resistant, as they should not be used in the clinic (Table 1).

All samples were sensitive to amikacin and piperacillin with tazobactam and all samples were resistant to amoxicillin, ampicillin and cefazolin.

The samples of *E. coli* from the hand of the surgeon before antisepsis and the operating table corresponding to the first surgical procedure, as well as the sample of *P. mirabilis* from the anaesthesiologist's cell phone were those in which there was a higher incidence of resistance to the antimicrobials tested. In addition, the three cell phone isolates were also resistant to antimicrobials important in

the clinical hospital routine, such as ceftazidime and cefotaxime.

There were no signs of surgical infection in the patients' wounds during the 4-week follow-up.

DISCUSSION

The major challenge in nosocomial infections is the interruption of the transmission bridge between the source and the susceptible host (Detels et al., 2015). In this context, health professionals represent the most important source (Calfee, 2012). Despite hand hygiene being a major subject of studies on the transmission and prevention of nosocomial infections in the last decades (Fung and Cairncross, 2007; Rutala and Weber, 2016), personal fomites for everyday use, especially electronic portable devices, have been gaining prominence.

These objects represent an important route for carrying microorganisms between the external environment and the hospital (Collins, 2020). It is believed that 5–21% of cell phones belonging to healthcare professionals are reservoirs of bacteria highly suitable for causing nosocomial infection (Brady et al., 2006; Jeske et al., 2007; Brady et al., 2009a, 2009b; Ulger et al., 2009; Sadat-Ali et al., 2010). The vast majority of studies focusing on understanding the role of cell phones in nosocomial infection were carried out in human hospitals with a direct focus on human health (Brady et al., 2006; Ramesh et al., 2008; Tagoe et al., 2011). Inspired by the importance of understanding such dynamics in veterinary medicine and transposing a focus on animal health, we developed this research.

All health professionals who were present in the procedures had a cell phone device and carried the device with them inside and outside the surgical environment, in contact with other patients and with the hospital environment. Previous studies have already shown that 98% of health workers have a cell phone, and 84.5% bring it to the professional environment every day; moreover, these devices are already located in critical places in the hospital environment, such as the operating room (Brady et al., 2006). Gunasekara et al. (2015) stated that 95% of health professionals come into direct contact with a cell phone in the operating room and 78% use it at least once during the surgical procedure,

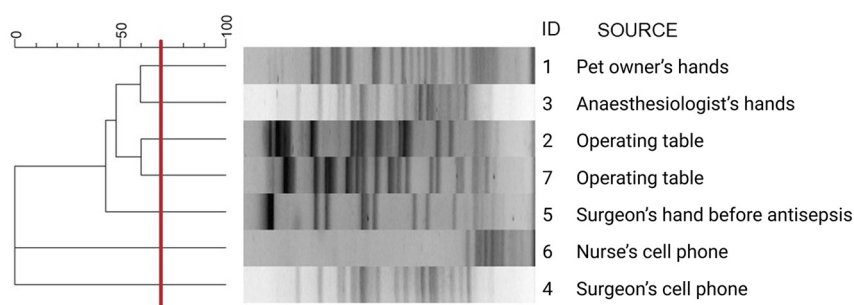


Fig. 1. Pulsed-field gel electrophoresis dendrogram of *Escherichia coli* isolates

Table 1. Analysis of resistance to antimicrobial classes based on CLSI (2018)

Samples	Isolated	Aminoglycoside			Beta-lactam										Macrolide		Quinolone				Amphenicols CLO	Tetracyclines TET	Sulphonamides SUT		
		AMI	GEN	AMO	AMC	AMP	APS	CFE	CFZ	CPM	CTX	CFO	CAZ	CRO	IPM	PIT	AZI	CIP	ENO	LEV				NOR	
1	<i>E. coli</i>			R		R	R	R	R	R						R			R	R					
2	<i>E. coli</i>			R	R	R	R	R	R	R															R
3	<i>E. coli</i>			R		R	R	R	R	R	R		R												R
4	<i>E. coli</i>			R	R	R												R		R					R
5	<i>E. coli</i>			R	R	R					R	R	R	R											R
6	<i>E. coli</i>			R		R		R	R	R	R	R	R	R											R
7	<i>E. coli</i>			R	R	R		R	R	R	R	R	R	R											R
	<i>Proteus mirabilis</i>		R	R	R	R	R	R	R		R	R	R	R						R					R

R: resistant; empty frame: sensitive; **sample 1**: anaesthesiologist's hand (Surgery 1); **sample 2**: pet owner's hand (Surgery 1); **sample 3**: surgeon's hand before antiseptics (Surgery 2); **sample 4** and **sample 5**: surgical tables (Surgeries 1 and 3); **sample 6**: surgeon's cell phone (Surgery 3); **sample 7**: nurse's cell phone (Surgery 5); **sample 8**: anaesthesiologist's cell phone (Surgery 4); AMI: amikacin; GEN: gentamicin; AMO: amoxicillin; AMC: amoxicillin with clavulanate; AMP: ampicillin; APS: ampicillin with sulbactam; CFE: cephalaxin; CFZ: cefazolin; CPM: cefepime; CTX: cefotaxime; CFO: ceftazidime; CRO: ceftriaxone; IPM: imipenem; PIT: piperacillin with tazobactam; AZI: azithromycin; CIP: ciprofloxacin; ENO: enrofloxacin; LEV: levofloxacin; NOR: norfloxacin; CLO: chloramphenicol; TEI: tetracycline; SUT: sulphamethoxazole with trimethoprim.

which is of great concern in view of the possibility of this object behaving as a fomite for nosocomial infections. On the other hand, one should not ignore the benefits that cell phones have brought from the technical point of view to the advancement of medicine.

In our study, *E. coli* could be isolated from the cell phone of the surgeon and veterinary nurse, and *P. mirabilis* could be isolated from the cell phone of the anaesthesiologist, in addition to *E. coli* being isolated from the hands of the anaesthesiologist and the surgeon. These bacteria belong to the Enterobacteriaceae family and are colonisers of the gastrointestinal tract (Donaldson et al., 2016). Therefore, their presence on cell phones and hands indicates environmental contamination. This is further aggravated by the fact that such bacteria have been isolated inside the operating room and on surgical-related objects, and that they are associated with nosocomial infections that are difficult to treat in veterinary medicine (Normand et al., 2000).

Although this study did not correlate the isolates from the hands of the pet owners with those of their cell phones, previous studies have reported such a correlation (Meadow et al., 2014), and their results converged on the need for improvements in the hygiene of both. We can state that, despite the methodological differences and the results, our research supports the same recommendation. On the other hand, the rate of compliance with hand hygiene by human health professionals remains below 50% (McGuckin et al., 2009) and there is no evidence to suggest better adherence by veterinarians. Compared to hand hygiene, the cleaning of cell phones shows even more alarming numbers, with regular cleaning being carried out by about 8%–29% of the health professionals (Singh et al., 2010; Brady et al., 2012).

A second fact aggravating the isolation of these bacteria in this research was that they were also resistant to multiple drugs. Antimicrobial resistance has been a worldwide topic of discussion in recent decades, due to its high impact on public health, and portrays a huge concern regarding the maintenance of these bacteria in fomites inside surgical centres, as well as their eventual spread to geriatric or oncology patients with chronic diseases or to immunocompromised patients (Cheadle, 2006; Turk et al., 2015), mainly during surgery, when there is the manipulation of deep tissues, peripheral and central venous accesses, and the surgical wound itself.

Among the multidrug-resistant bacteria, the extended spectrum beta-lactamase (ESBL-) producing enterobacteria are of great concern to the medical community, since their existence is suspected when strains belonging to the Enterobacteriaceae family show resistance to at least one of the third-generation cephalosporins recommended for the demonstration of ESBL (cefepodoxime, ceftazidime, and cefotaxime) and its confirmation occurs through the identification of specific genes by PCR (Jessen et al., 2019). In our study, the three isolates from cell phones were resistant to ceftazidime and cefotaxime, raising high suspicion that they were ESBL-producers, bacteria of difficult clinical treatment with the potential for cross-infection and outbreaks of serious hospital infections.

Cefazolin is a cephalosporin that has been recommended as an optimal prophylactic drug in dogs and cats undergoing surgery (Gonzalez et al., 2017). We could observe bacterial resistance to this antimicrobial in all samples isolated in our study, which is a worrying fact, since prophylactic therapy is an essential part in reducing the microbial load and the risk of intraoperative contamination. Therefore, other factors may have come together for not having isolated any bacteria in the surgical wound or that none of our patients developed signs of infection during the postoperative follow-up period, such as the correct antisepsis performed. Although we isolated *E. coli* from the hands of a surgeon prior to antisepsis, it did not remain there. In addition, the patient's immune status and surgical time can also contribute to the absence of contamination and infection in patients (Cheadle, 2006; Burgess, 2019).

Unfortunately, most of the multidrug-resistant bacteria found in veterinary hospitals are also transmissible to humans and can be subsequently transferred to the community through colonised people, animals, and objects (King et al., 2018). China is already dealing with serious problems, with antibiotic resistance genes widely distributed in surface waters, sewage treatment plant effluents, soil, and animal waste (Qiao et al., 2018), demonstrating the need to mitigate the spread of bacterial resistance in order to avoid a public health crisis bigger than the one we have been facing in the last decades.

The sample size in this study can be considered the main limitation; however, it is believed to have not interfered with the results obtained. In addition, further studies are required to verify the importance of isolates obtained from cell phones in the spread of disease among professionals and patients.

The multidrug-resistant *E. coli* and *P. mirabilis* isolates that colonise the surgical team and their cell phone devices raise concerns about the ability of these objects to transmit infection to patients, even if their implication in the spread of hospital-acquired infection remains unproven, taking into account the massive presence of cell phones in the hospital environment, especially in the surgical environment.

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