Universidade de Aveiro
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Departamento de Biologia

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Detection of antibiotic resistant bacteria on hands and mobile phones

Deteção de bactérias resistentes a antibióticos nas mãos e telemóveis

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Universidade de Aveiro
Ano 2014

Departamento de Biologia

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Microbiologia, realizada sob a orientação científica da Doutora Maria Helena de Sousa Barroso, Professora Associada do Laboratório de Microbiologia Aplicada Egas Moniz no Instituto Superior de Ciências da Saúde Egas Moniz. Adicionalmente realizada sob a coorientação científica da Doutora Sónia Alexandra Leite Velho Mendo Barroso, Professora Auxiliar com Agregação do Departamento de Biologia da Universidade de Aveiro.

To my mom, dad and brother for their unconditional love and support.

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palavras-chave

Telemóvel; reservatório de bactérias; bactérias resistentes a antibióticos; bactérias multirresistentes; MRSA.

resumo

Atualmente os telemóveis são utilizados diariamente e de modo frequente. Não se observa uma consciencialização por parte da comunidade geral para o seu potencial como reservatório para bactérias específicas. O uso de telemóveis com ecrãs tácteis encontra-se em crescimento exponencial, e os surtos hospitalares em que se verifica que o ecrã táctil é uma fonte de contaminação estão a ser registados com maior frequência.

Os ecrãs tácteis não são encarados como um meio de transmissão de bactérias potencialmente patogénicas e bactérias resistentes a antibióticos, podendo ser assim um risco para a saúde pública devido à negligência em termos de desinfeção apropriada em ambientes hospitalares.

As bactérias estão a adquirir várias resistências a antibióticos, tornando-se multirresistentes tal como o HÁ-MRSA. Isto representa um risco para a saúde pública quando confrontados com a possibilidade destas bactérias aderirem e permanecerem nos telemóveis durante um longo período de tempo. Estes dispositivos podem servir como vetor na transmissão de bactérias presentes para o seu utilizador e a terceiros. Sendo ainda mais preocupante quando os indivíduos são profissionais de saúde.

Este estudo teve como objetivo identificar e quantificar as bactérias presentes nos telemóveis e nas mãos dos seus utilizadores. As bactérias foram analisadas em termos de resistência a antibióticos e MRSA foram selecionados e geneticamente caracterizados, e o elemento SCC*mec* tipificado.

Bacillus spp. foi detetado em 7.5% dos indivíduos e em 28% dos telemóveis, bactérias hemolíticas foram detetadas em 82% dos indivíduos, *Staphylococcus* spp. em 96.5%, *S. aureus* em 82%, *Enterobacteriaceae* em 1% e MRSA em 6%. A resistência à Eritromicina por staphylococci foi 44.7% em geral. A resistência à Oxacilina e Clindamicina foi de 12.5% e 9.8%, respetivamente. 0.8% das bactérias submetidas a antibiograma apresentaram resistência a múltiplas classes de antibióticos, e 3.3% dos participantes apresentaram bactérias multirresistentes nas mãos.

Quatro amostras foram identificadas como sendo MRSA, todas multirresistentes, e destas, duas foram presuntivamente identificadas como sendo SCC*mec* tipo II e SCC*mec* tipo III, ambas HÁ-MRSA.

Indivíduos do sexo masculino têm as mãos mais "sujas" do que as estudantes do sexo feminino, isto em termos de bactérias potencialmente patogénicas. Vários fatores como, tipo de teclado, higiene das mãos e telemóvel, tamanho das unhas, tipo e presença de manicure, levar o telemóvel para a casa de banho, ter animais de estimação e lavar as mãos influenciam o número de CFU nas mãos. Os telemóveis podem servir de reservatório para bactérias específicas que podem ser patogénicas e multirresistentes a antibióticos, por isso devem ser reconhecidos publicamente como uma possível fonte de contaminação.

keywords

Mobile phones; bacterial reservoirs, bacteria resistant to antibiotics; multiresistant bacteria; MRSA.

abstract

Mobile phones are daily used and in a frequent manner. There is no awareness in the general public of their potential to be a reservoir of specific bacteria. The use of touch screen mobile phones is exponentially growing and the hospital outbreaks with touch screens as contamination source is more frequently being registered.

Touch screens are not perceived as a method of transmission of potentially pathogenic and antibiotic resistant bacteria, thus posing as a health risk due to being overlooked in terms of disinfection standards in healthcare settings.

Bacteria are acquiring resistance to various antibiotics, possibly becoming multiresistant such as HA-MRSA. This poses a public health risk when faced with the possibility that these bacteria can adhere and remain on mobile phones over a great length of time. These devices may serve as vector of transmitting bacteria to their owners and third parties. This is even more preoccupying when individuals are healthcare professionals.

This study aimed to identify and quantify the bacteria present on mobile phones and the hands of their users. The bacteria were submitted to antibiotic screening and MRSA were selected and genotypically characterized, and the SCC*mec* element typified.

Bacillus spp. was detected in 7.5% of the individuals and in 28% of the mobile phones, hemolytic bacteria were detected in 82% of the individuals, *Staphylococcus* spp. in 96.5%, *S. aureus* in 82%, *Enterobacteriaceae* in 1% and MRSA in 6%. Erythromycin resistance in staphylococci was verified to be 44.7% in general. Oxacillin and Clindamycin resistance was 12.5% and 9.8%, respectively. 0.8% of the screened bacteria were multiresistant, and 3.3% of the individuals presented multiresistant bacteria on their hands.

Four samples were identified as being MRSA, all multiresistant and from those, two samples were presumptively identified as SCC*mec* type II and SCC*mec* type III, both HA-MRSA.

Male individuals have "dirtier" hands than female students in terms of potential pathogenic bacteria. Various factors such as, keyboard type, hand and mobile phone hygiene, nail length, manicure type and presence, taking device to the bathroom, owning pets and hand washing have influence on the bacterial count of the hands. Mobile phones can serve as reservoirs of specific bacterial that may be pathogenic and multiresistant to antibiotics, and should be publically perceived as a possible contamination source.

Table of Contents

Named jury members……5

Acknowledgements……6

Resumo……7

Abstract……8

Table of Contents……9

Figure Index……14

Table Index……16

List of Acronyms and Abbreviations……..20

1 – Introduction……25

1.1 – Antibiotic resistance……..28

1.2 – Mobile phones……29

1.2.1 – Touchscreens……..29

1.2.2 – Resistive touchscreen……31

1.2.3 – Capacitive touchscreen……..31

1.3 – Bacterial colonization……32

1.3.1 – Fimbrial and afimbrial adhesins……..33

1.3.2 – Biofilms……..34

1.3.3 – Biotic and abiotic surfaces……..36

1.4 – Host-Bacteria interactions……37

1.4.1 – Mutualism and commensalism……..37

1.4.2 – Parasitism……..38

1.5 – Commensal microflora……..38

1.6 – Gram-positive and Gram-negative bacteria……..39

1.6.1 – Internal structures……41

1.7 – Pathogenic flora……41

1.7.1 – Pathogenicity……43

1.8 – Bacterial reservoirs……43

1.8.1 – Healthcare settings……..44

1.8.2 – Bacterial transmission……..46

1.9 – *Staphylococcus aureus*……48

1.9.1 – *Staphylococcus aureus* pathology……49

1.9.2 – Methicillin-resistant *Staphylococcus aureus* (MRSA)……50

1.9.3 – Staphylococcal cassette chromosome (SCC)……54

1.9.4 – SCC*mec*……54

1.9.5 – Community-associated MRSA and Healthcare-associated MRSA……..57

2 – Objectives……61

3 – Materials and Methods……..63

3.1 – Sample collection……..63

3.2 – Culture media……64

3.2.1 – Trypto-casein soy agar……64

3.2.2 – Columbia blood agar……64

3.2.3 – Mannitol salt agar……64

3.2.4 – Drigalski agar……65

3.3 – Sample inoculation……65

3.3.1 – ChromID MRSA and chromID VRE inoculation……..66

3.4 – Microbiological and quantitative analysis……..66

3.4.1 – Drigalski agar……66

3.4.2 – Columbia blood agar and Mannitol salt agar……67

3.5 – Antibiotic screening……...68

3.6 – Resistant bacteria conservation……..68

3.7 – *mecA* gene detection in presumptive Methicillin-resistant *Staphylococcus aureus*……69

3.7.1 – DNA extraction……..69

3.7.2 – Polymerase chain reaction (PCR)……..69

3.7.3 – DNA amplification……69

3.7.4 – Gel electrophoresis……..70

3.8 – SCC*mec* typification……..71

3.8.1 – Multiplex PCR……71

3.8.2 – DNA amplification……71

3.8.3 – Gel electrophoresis……..73

4 – Results and Discussion……75

4.1 – Questionnaire analysis……75

4.2 – Microbiological analysis……85

4.2.1 – Columbia blood agar and Mannitol salt agar medium……87

4.2.2 – Drigalski agar médium……89

4.2.3 – chromID MRSA medium……90

4.2.4 – chromID VRE medium……91

4.2.5 – Data analysis……...92

4.2.6 – Quantitative analysis……..95

4.2.6.1 – Quantitative analysis by gender and course……..96

4.2.6.2 – Overall quantitative analysis……..102

4.3 – Microbiological results versus individual characteristics……..103

4.3.1 – Keyboard type influence……...103

4.3.2 – Nail length influence……..105

4.3.3 – Manicure and ring influence……107

4.3.4. – Special hygiene with mobile phone influence……..110

4.3.5 – Special hygiene with hands/nails influence……...111

4.3.6 – Taking mobile phone to bathroom influence……113

4.3.7 – Biting nails and/or surrounding skin influence……..114

4.3.8 – Having pets influence……116

4.3.9 – Hand washing influence……...117

4.3.10 – Overall influence results……119

4.3.11 – *Enterobacteriaceae*……120

4.4 – Antibiotic resistance screening……..…..122

4.4.1 – Overall antibiotic screening…….....122

4.4.2 – Multiresistance to antibiotics……..…127

4.4.3 – Resistance to Erythromycin……..128

4.4.4 – Oxacillin and Cefoxitin as presumptive indication of MRSA……129

4.4.5 – Individual characteristics of the participants that presented MRSA…….132

4.5 – MRSA genetic characterization……135

4.5.1 – *mecA* gene detection……136

4.5.2 – SCC*mec* element detection and typification……...138

4.5.3 – MRSA classification……...143

5 – Conclusions and Future Perspectives……146

6 – Bibliography……..148

7 – Appendix……....161

7.1 – Informed consent form and questionnaire……..161

7.2 – Antibiotic information and inhibition zones……165

7.3 – RTP Bacteria Mini Kit DNA extraction protocol……..166

Figure Index

Figure 1: Resistive touchscreen composition and capacitive touchscreen structure. ……...32

Figure 2: Adhesion process (adhesin-receptor). ……..33

Figure 3: Adhesion by fimbrial adhesins and adhesion by afimbrial adhesins. …….34

Figure 4: Biofilm development. ……... 36

Figure 5: Gram-positive bacteria versus Gram-negative bacteria. …… 41

Figure 6: The percentage of Methicillin-resistant *Staphylococcus aureus* in invasive isolates presented by the European Antimicrobial Resistance Surveillance Network's (EARS-Net) annual report of 2012, by country of the European Union (EU)/European Economic Area (EEA). ……52

Figure 7: *Staphylococcus aureus* trends of invasive isolates resistant to Methicillin (MRSA) in percentages, by country, EU/EEA countries, 2009 to 2012. …..53

Figure 8: Schematic representation of attachment sites for SCC*mec* integration. …...54

Figure 9: Horizontal and vertical gene/MGE transfer. ……..56

Figure 10: Structural classes of *mec* operon. ……56

Figure 11: Comparison of SCC*mec* types. ……57

Figure 12: Kovac's indole reaction. ……..…….67

Figure 13: Various TSA media analyzed with different results. ……...86

Figure 14: Various CT media analyzed with different results. ……87

Figure 15: Mannitol salt agar medium with *S. epidermidis* colonies. ……..88

Figure 16: Mannitol salt agar medium with pure *S. aureus* colonies. ……88

Figure 17: *Enterobacteriaceae* (Gram-negative bacteria) present in the Drigaslki medium. ……...90

Figure 18: Positive indole test of the *Enterobacteriaceae* found in the Drigalski medium. ……90

Figure 19: Methicillin-resistant *S. aureus* present in chromID MRSA medium. ..……..91

Figure 20: Various samples submitted to antibiotic susceptibility testing. ……..…..122

Figure 21: Genetic marker used (25 bp DNA Step Ladder by Promega®). ……137

Figure 22: Electrophoresis gel results from the *mecA* gene amplification of the samples ♂1, ♂2, ♂7 and ♀17. ……..……..137

Figure 23: Electrophoresis gel result from the *mecA* gene amplification of the samples ♂6 (2nd sampling) and ♂7 (2nd sampling). ……..……137

Figure 24: Genetic marker used (GeneRuler Low Range DNA Ladder, Ready-to-Use 25 to 700 bp by Thermo Scientific®). ……139

Figure 25: Electrophoresis gel result from the amplification of the different SCC*mec* types of the samples ♂6 (2nd sampling), ♂7 (2nd sampling), ♀17 and ♂22. ……..……139

Table Index

Table 1: The main characteristics of HA-MRSA and CA-MRSA strains. ……58

Table 2: Primers used for the amplification of the *mecA* gene. ……...70

Table 3: Amplification program applied. ……70

Table 4: Primers used in the multiplex PCR assay applied for typifying different SCC*mec* types. ……..…...….72

Table 5: Percentage of participants, by gender, in the first sampling that used touchscreen devices and mobile phones with a keypad. ……..….76

Table 6: Percentage of participants, by gender, in the second sampling that used touchscreen devices and mobile phones with a keypad. ……77

Table 7: Percentage of participants, by gender that presented short, medium and long nails. ……78

Table 8: Percentage of participants, by gender that presented and did not present manicured nails. ……..…79

Table 9: Percentage of participants, by gender, that presented and did not present rings on their examined hand. ……..…….79

Table 10: Percentage of participants, by gender, that paid and did not pay any special attention to the hygiene of their hands and/or nails. ……..….80

Table 11: Percentage of participants, by gender, that paid and did not pay any special attention to the hygiene of their mobile phones. ……..……81

Table 12: Percentage of participants, by gender, that washed and did not wash their hands after the use of the bathroom. ……..……..81

Table 13: Percentage of participants, by gender, that brought and did not bring their mobile phones with them when using the bathroom. ……..……82

Table 14: Percentage of participants, by gender, that bit and did not bite their nails and/or surrounding skin. ……..……..83

Table 15: Percentage of participants, by gender, that presented and did not present the presence of pets in their household. ……..…84

Table 16: Percentage of participants, by gender, that washed their hands less than 5 times, between 5 and 10 times, between 10 and 15 times and more than 15 times a day. ……..85

Table 17: Percentage of participants, by gender and by course, that presented colonies of *Bacillus* spp., hemolytic bacteria, *Staphylococcus* spp., *S. aureus*, *Enterobacteriaceae* and Methicillin-resistant *S. aureus* on their hands. …..95

Table 18: Number of CFU presented by the male participants, by course and sampling, overall and *Bacillus* spp. on the mobile phones, and overall, *Bacillus* spp., hemolytic bacteria, *Staphylococcus* spp. and *S. aureus* on the sampled hands. ……..…..……...….98

Table 19: Number of CFU presented by the female participants, by course and sampling, overall and *Bacillus* spp. on the mobile phones, and overall, *Bacillus* spp., hemolytic bacteria, *Staphylococcus* spp. and *S. aureus* on the sampled hands. ……..……..…….101

Table 20: Number of CFU presented by the participants, by gender, overall and *Bacillus* spp. on the mobile phones, and overall, *Bacillus* spp., hemolytic bacteria, *Staphylococcus* spp. and *S. aureus* on the sampled hands. …...103

Table 21: Keyboard influence on the bacterial count of the mobile phones of the 1st sampling, overall count and *Bacillus* spp. ...……...104

Table 22: Keyboard influence on the bacterial count of the mobile phones of the 2nd sampling, overall count and *Bacillus* spp. ……..……...105

Table 23: Bacterial count of the sampled hands of the participants of both genders in correspondence to nail size. ……..……..……..……106

Table 24: Bacterial count of the sampled hands of the participants of both genders in correspondence to manicure presented. ……..……..…108

Table 25: Bacterial count of the sampled hands of the female participants in correspondence to the type of manicure they were wearing. ……..……...108

Table 26: Bacterial count of the sampled hands of the participants of both genders in correspondence to ring presence. …..……..……..…109

Table 27: Bacterial count of the mobile phones and sampled hands of the participants of both genders in correspondence to special mobile phone hygiene. …..111

Table 28: Bacterial count of the sampled hands of the participants of both genders in correspondence to special hand/nail hygiene. ……..……112

Table 29: Bacterial count of the mobile phones and the sampled hands of the participants of both genders in correspondence to taking mobile phone to the bathroom. ……..……..……114

Table 30: Bacterial count of the sampled hands of the participants of both genders in correspondence to biting nails and/or surrounding skin. ……..……...115

Table 31: Bacterial count of the sampled hands of the participants of both genders in correspondence to owning pets. ……..……...…117

Table 32: Bacterial count of the sampled hands of the participants of both genders in correspondence to hand washing times a day. ……..……..……118

Table 33: Percentage of participants, by gender, that presented bacteria resistant to the specific antibiotics used. ……..…..……125

Table 34: Antibiotic susceptibility results of the only two *Enterobacteriaceae* colonies obtained. ……..……..127

Table 35: Percentage of screened samples and participants, by gender, that presented bacteria with multiresistance to antibiotics. ……..…..…...128

Table 36: Antibiotic susceptibility patterns of the samples that presented resistance to the antibiotic Oxacillin. ……..……...130

Table 37: Antibiotic susceptibility patterns of the bacteria that presented growth in the chromID medium. ……..……...132

Table 38: Individual characteristics, attained by the questionnaire, of the participants that presented MRSA growth. ……..…….……...135

Table 39: Characterization of the different SCC*mec* types when resorting to multiplex PCR strategy. ……..…….……140

Table 40: Characteristics of the eight types of the SCC*mec* elements. ……...141

Table 41: Characteristics of the three recently found types of SCC*mec* elements. ...142

Table 42: Differences between healthcare-associated MRSA and community-associated MRSA. ……..……..143

Table 43: Main differences between health care-associated MRSA and community-associated MRSA. ……..……..……144

List of Acronyms and Abbreviations

1st – First sampling

2nd – Second sampling

AMC – Amoxicillin + Clavulanic Acid

BHI – Brain Heart infusion broth

bp – Base pair

CA-MRSA – Community-associated Methicillin-resistant *Staphylococcus aureus*

CAZ – Ceftazidime

CES - Consumer Electronics Show

CFU – Colony-forming units

CHAP – Mannitol salt agar

CIP – Ciprofloxacin

CLSI – Clinical and Laboratory Standards Institute

CN – Gentamicin

COH – Columbia blood agar + 5% horse blood

CoNS – Coagulase-negative staphylococci

CT – Count-tact agar

CTX – Cefotaxime

DA – Clindamycin

DNA - Deoxyribonucleic acid

dNTPs – Deoxynucleotide triphosphates

DRIG – Drigalski agar

dsDNA – Double-stranded deoxyribonucleic acid

E – Erythromycin

E. coli – *Escherichia coli*

EARS-Net – European Antimicrobial Resistance Surveillance Network

EEA – European Economic Area

EPS – extracellular polymeric substance

ESBL – Extended-spectrum β-lactamase

EU – European Union

F – Forward primer

FOX – Cefoxitin

HAI – Hospital-acquired infection

HA-MRSA – Healthcare-associated Methicillin-resistant *Staphylococcus aureus*

i.e. – *id est* ("that is")

Inc. - Incorporated

IPM – Imipenem

ISCSEM – Instituto Superior de Ciências da Saúde Egas Moniz

ITO- Indium thin oxide

IV – Intravenous

J regions – Joining regions

K. pneumoniae – *Klebsiella pneumoniae*

kb – Kilo-base

LA-MRSA – Livestock-associated Methicillin-resistant *Staphylococcus aureus*

LCD – Liquid-crystal display

LPS – Lipopolysaccharides

M – Genetic marker

$MgCl_2$ – Magnesium chloride

MGE – Mobile genetic element

MH2 – Mueller-Hinton 2 agar

MHS – Mueller-Hinton + 5% sheep blood

MRSA – Methicillin-resistant *Staphylococcus aureus*

N/a – Not applicable

NaCl – Sodium chloride

NC – Negative control

ORF – Open reading frame

OX – Oxacillin

PBP – Penicillin binding protein

PCR – Polymerase chain reaction

PET – Polyethylene terephthalate

PVL – Panton-Valentine leukocidin

R – Reverse primer

RNA – Ribonucleic acid

RPM – Rounds per minute

S. aureus – *Staphylococcus aureus*

S. epidermidis – *Staphylococcus epidermidis*

S. pyogenes – *Streptococcus pyogenes*

S. sciuri – *Staphylococcus sciuri*

SBPW – Sterile buffered peptone water

SCC – Staphylococcal cassette chromosome

SCC*mec* - Staphylococcal cassette chromosome *mec*

SDW – Sterile distilled water

SFD – Staphylococcal foodborne disease

SSS – Scalded skin syndrome

TAE - Tris-acetate-EDTA

TSA – Trypto-casein soy agar

TSS – Toxic shock syndrome

U.S. – United States

UV – Ultraviolet

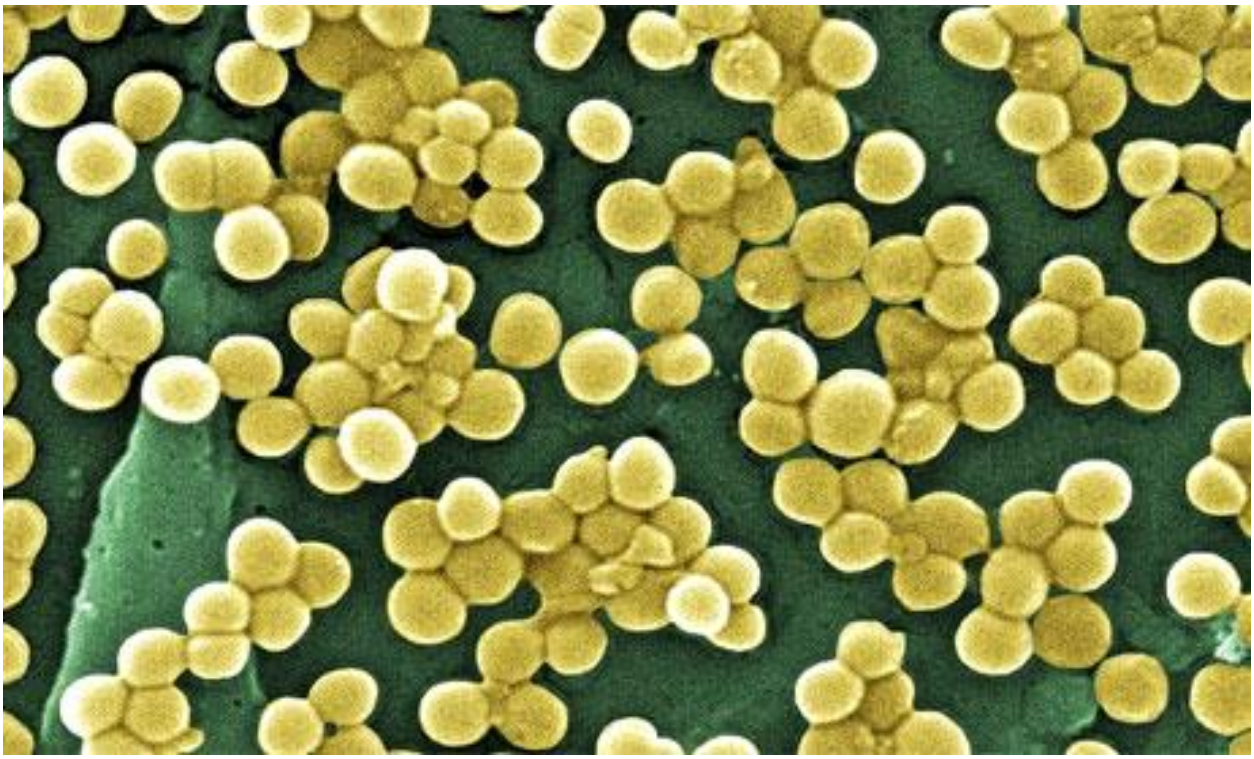
V – Volt

VRE – Vancomycin-resistant enterococci

Vs. – Versus

WHO – World Health Organization

I – INTRODUCTION



(http://www.theguardian.com/news/datablog/2012/aug/22/mrsa-related-deaths-fall-but-poor-still-worst-affected)

1 - Introduction

Nowadays we are faced with the broad expansion of technology on a global scale. It can securely be said that in our time it is a rare occurrence when an individual does not carry his mobile phone with him. The use of these electronic devices, laptops, computers, tablets and others, is daily, so it is natural to presume that they have the potential to carry a wide range of bacteria. It becomes a greater concern when faced with the possibility that those bacteria could be potential pathogens and resistant to antibiotics, as people can become ill and no therapeutics will be effective.

The bacteria present on these gadgets vary from subject to subject. Each individual has his own particular routines in terms of hygiene, and it must be taken into consideration his personal and professional characteristics. Since people's hands are in frequent contact with their mobile phones throughout the day, it can be assumed that the bacteria that reside on their phones are the same that are found on their hands. There is the possibility that individuals, either as their mobile phones, serve as a reservoir of certain antibiotic resistant bacteria. This can lead to the dissemination of these resistant bacteria, transmitting these same bacteria to third parties and potentially bringing about illness. This cross contamination can be a severe public health issue if not controlled appropriately.

A study elaborated by Al-Ghamdi and co-workers, focused on the bacterial contamination of computer keyboards and mice, elevator buttons and shopping carts, revealed that 95.5% of the collected samples presented bacterial growth [1]. The computer keyboards and mice presented 100% contamination, and although their samples were collected from public cafes, these objects can be compared to mobile phones as they are used on a daily basis and in a frequent manner. Pathogenic bacteria were isolated, such as *Staphylococcus aureus*, *Pseudomonas* spp. and Gram-negative bacilli, and with the addition of the presence of commensal bacteria, it was concluded that these objects might act as reservoirs and contamination source of potentially pathogenic bacteria [1].

Another study carried out by Andrej and co-workers researched if mobile phones can act as a potential microbiological threat, and confirmed that they can be a factor of cross contamination [2]. The most frequently found bacteria were *Staphylococcus* spp. and *Enterobacteriaceae*, which are potentially pathogenic. It additionally showed that ethanol (70%) is not the most effective manner of disinfecting the devices (75 to 100%), as antibacterial putty reduces the CFU (colony-forming units) count between 94 to 100% [2]. This study also referred that only one fingertip may present a CFU count that ranges from 0 to 300 CFU, when sampled with agar contact methods [2]. It can be verified by this study that colonization can be significantly reduced by inexpensive methods, which enable the prevention of the transmission of bacteria.

A research done by Tagoe about the bacterial contamination of mobile phones and concluded that 100% were in fact colonized by bacteria. Eleven bacterial species were isolated, where 81.8% of all isolates were pathogenic bacteria and presented resistance to antibiotics [3]. It was concluded that mobile phones are heavily colonized ($9.915x10^7$ CFU/mL), being that a very high percentage are pathogenic bacteria. Therefore they are considered potential vehicles for transmission of disease [3].

This was also verified by a study conducted by Shahaby and co-workers which studied the potential of mobile phones for being reservoirs for bacterial pathogens. The study was elaborated with different participants, such as university staff and healthcare personnel. They found the predominant growth of *Staphylococcus* spp. and *Bacillus* spp. It was additionally found that 61.5% of the mobile phones of the healthcare professionals presented contamination, thus working as a potential source of nosocomial infections [4].

Focusing on the professional aspect it seems logic that individuals working in healthcare facilities would present a higher risk of carrying pathogenic and possibly antibiotic resistant bacteria. Accordingly to the literature, it was found that touchscreens that are operated in a health care environment are most of the time forgotten or not viewed as a source if possible contamination. This means that they are not cleaned

properly and/or the professionals that uses the medical device, do not consider it necessary to decontaminate the screen after treating a patient.

Narciso and co-workers described a case that occurred in a hospital in Lisbon, in which 4 hospitalized individuals became infected with the same nosocomial *K. pneumoniae* bacteria. These bacteria resided on the touchscreen of the ventilator that was used on the patients, and as there was no special care or attention dispended to the decontamination of the screen, it served as a reservoir of the bacteria. It was then transmitted to the patients through the healthcare professional [5]. These situations in healthcare environments pose a substantial concern, especially if one thinks that those antibiotic resistant nosocomial bacteria could be brought to the general population through these breaches in hospital vigilance.

A research conducted by Walia and co-workers confirms the previously stated by Andrej et al. and by Tagoe, where he was able to conclude that mobile phones can act as reservoirs and thus transmit hospital-acquired infections (HAI) in the dental setting [6].

Another study by Bhat and co-workers, about the spread of nosocomial pathogens due to the function of mobile phones as reservoirs, revealed that 99% of the healthcare workers mobile phones presented bacterial contamination. It was also verified that 64.8% exhibited growth of pathogenic bacteria, where 37.9% were multiresistant to antibiotics [7]. Some of the pathogenic bacteria isolated were Methicillin-resistant *S. aureus* (MRSA), *Escherichia coli*, *Klebsiella pneumoniae* and *Enterococcus faecalis*. Out of all of the healthcare professionals that participated in the study, 40% admitted to use their phones between examinations and only 6% used disinfectants to clean their mobile phones [7]. This only proves that mobile phones are frequently used and during patient contact by healthcare professionals, which increases the health risk as these devices serve as a potential source of nosocomial pathogens.

The Copper Development Association Inc. has researched about the antimicrobial potential of copper alloys. Their developed paper named "Reducing the Risk of Healthcare Associated Infections: The Role of Antimicrobial Copper Touch Surfaces." evidences that various studies have been conducted that imply the antimicrobial potential of copper and other heavy metals, due to inhibiting the growth of microorganisms [8]. The incorporation of copper alloys in touchscreen devices in healthcare settings significantly decreases their bacterial contamination, and therefore the transmission of these bacteria throughout the environment and third parties. However, it is not a substitute for the implemented hygiene standards for these devices, as they continuously should be cleaned and disinfected accordingly [8].

These developments are important to battle the transmission of antibiotic resistant bacteria in healthcare settings. It is known that antibiotic resistance is a serious threat to public health and these kinds of innovations may subdue its further development [9]. However, resistance to copper and other heavy metals have already been documented [10][11].

1.1 – Antibiotic resistance

Every day it is becoming more apparent that antibiotic resistant bacteria can be a real threat on a global scale, and that studying them and their sources is an important way to understand and fight against them [12]. A bacteria that is resistant or even multiresistant to antibiotics can be very dangerous and a health risk on a global scale, for it increases considerably the possibility of developing disease in individuals and it increases the chances of the disease to persist.

The mass utilization of the mobile phone is, as stated previously, a possible way to spread numerous bacteria, including pathogenic and antibiotic resistant bacteria. These devices can facilitate the transmission of bacteria, and subsequent development of illness.

1.2 – Mobile phones

Mobile phones with touchscreen are the most frequently used nowadays [13]. Although they are being so widely used, the degree of affinity of bacteria to the different touchscreen surfaces is not yet known. To gain an insight on this matter, a comparison between the mobile phones that have a touchscreen and those who function through a keypad was made. This examination can evidence which mobile phone surface, touchscreen or keypad, presents a greater risk in terms of contamination by bacteria, more specifically pathogenic bacteria.

A study developed by Pal and co-workers had the objective to understand if mobile phones with a keypad pose an increased risk of microbial contamination in comparison to touchscreen mobile phones. The research was associated to healthcare workers and the results were that touchscreen phones presented a median CFU count of 0.09 CFU per cm^2, whilst keypad mobile phones exhibited 0.77 CFU per cm^2 [14]. Additionally it was verified that touchscreen mobile phones do not exhibit a high presence of Methicillin-resistant *S. aureus* (MRSA) or Vancomycin-resistant enterococci (VRE) in comparison to keypad devices, 3% and 24% respectively. This concludes that mobile phones with a keypad are more prone to bacterial adherence and to harboring pathogenic bacteria, due to the makeup of their surface. However these bacterial counts may vary due to hand contamination of each individual, which is influenced by hygiene and sanitary practices [15].

These results in terms of bacterial counts found on keypad and touchscreen mobile phones were also verified in the study elaborated by Andrej and co-workers, which obtained 1.51 and 1.05 CFU per cm^2, respectively on the students' mobile phones [2].

1.2.1 – Touchscreens

There are two types of touchscreens that are applied to mobile phones, the resistive and the capacitive touchscreen. The resistive type of touchscreen functions

based on pressure exerted by any object, not requiring direct human contact, therefore it can be controlled by the use of a digit (with or without gloves), nail or with a stylus, which is specific for the use on this type of touchscreen. To obtain this sort of screen, it is necessary to have a certain flexibility of the surface itself which is made of a resistive kind of material (e.g. plastic), this being one of the main differences between the two varieties.

The capacitive touchscreen function based on changes in electric charge, meaning that an external charge is necessary to operate the device. These touchscreens can only be controlled by direct human contact, or any object that can disrupt the electric charge by being an electrical conductor, just like the human body. The explanation for this is that the surface of the device's screen is made of a hard and inflexible material (e.g. glass), so applying pressure to the screen is not sufficient [16].

Corning Inc. is known for producing the glass (Gorilla Glass) used for touchscreens of various mobiles phones. Recently, this firm has announced at the Consumer Electronics Show (CES) 2014 the "first U.S. Environmental Protection Agency-registered antimicrobial glass cover" which consists in adding silver ions in their already distributed line of Gorilla Glass for tablets and smartphones [17]. This added silver ions have an antimicrobial function, inhibiting the growth of algae, mold, mildew, fungi, and bacteria, being effective for the lifetime of the devices [17].

This type of development is crucial for the evolution in combating potentially pathogenic microorganisms. Silver has been documented as enhancing antibiotic activity and to eradicate antibiotic resistant bacteria [18]. However, it may not be a long-term solution as bacteria and other microorganisms have the ability to develop and acquire resistance to the inhibitors they are exposed to. Resistance to metals and heavy metals have been documented in various studies [19][11].

1.2.2 – Resistive touchscreen

As stated previously, the resistive touchscreen requires flexibility of the screen itself to be handled and therefore this surface it composed by a plastic material made of polyethylene terephthalate, commonly known as PET. Starting by the first and outer layer of the touchscreen composition, PET is a thermoplastic polymer resin which can be used in synthetic fibers, food and beverage containers, also having thermoforming applications and also used in engineering resins in combination with fiber glass. Following the plastic outer layer, there is an indium thin oxide layer, also known as ITO, and it has the purpose of conducting the electrical current. The ITO layer is followed by an air gap, then another ITO layer, a stable base layer and lastly a liquid-crystal display, universally referred to as LCD (figure 1). The air gap formerly mentioned is essential to operate the touchscreen, since it separate the two ITO layers. If there is no pressure applied to the surface of the device there will be no reaction, on the other hand, when pressure is exerted, the two layers of ITO will establish contact with one another, creating an electric current and activating the selection on the screen [16].

1.2.3 – Capacitive touchscreen

The capacitive touchscreen, as referred to previously, functions in a different manner, since it is not operated by applying pressure but rather by creating an electrical current with an object that can act as a conductor. The outer layer of the touchscreen is normally a glass substrate chemically strengthened by a hard coat of silicon dioxide, mostly known as silica. This chemical strengthening is required on account of the thinness of the glass layer, and by putting the glass through this treatment it is less presumably to break. The types of glass generally used are soda-lime glass and aluminium silicate (alumina), inasmuch as they are considerably inexpensive, chemically stable, relatively hard and extremely workable [16][20]. Following the glass layer, there is an ITO conductor, subsequently an acrylic pressure-sensitive adhesive, referred to as PSA, another glass substrate, followed by another ITO layer and lastly

the LCD (figure 1). So it is evident that the composition of the two touchscreens vary but also have materials in common, thus perfectly adapt to their functionality.

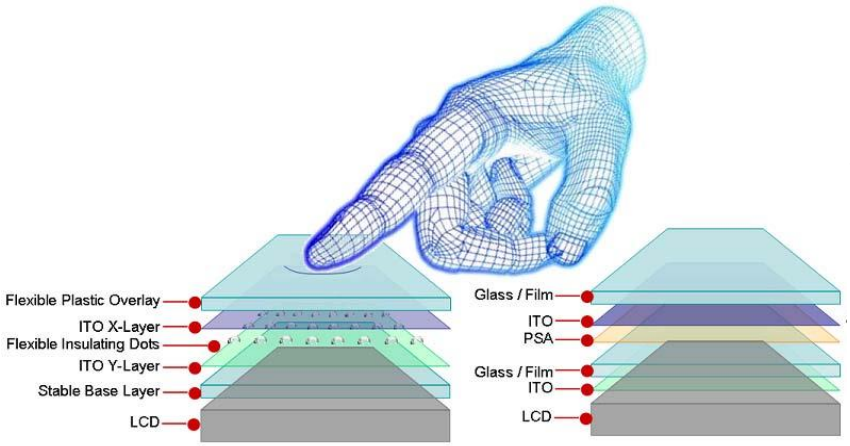


Figure1: Left - Resistive touchscreen composition; Right - Capacitive touchscreen structure, illustration adapted from [16].

The material that were relevant to the research, were the constitution of the outer layer of the touchscreen, accordingly the PET layer when referred to the resistive screen and silica when addressing the capacitive screen, additionally silicone when examining mobile phones with a keypad. The capacity of adhesion of certain bacteria to the surface of these mobile phones, touchscreen and tactile, were analyzed since their surfaces may exhibit different levels of bacterial adhesion. These bacteria can be pathogenic and even multiresistant to antibiotics, which causes great concern and it is extremely important in terms of public health.

1.3 – Bacterial colonization

For bacteria to successfully colonize a host, being an object or a living organism, it demands the contribution of both parties, host and bacteria, therefore accomplished through receptors and adhesins (ligands) respectively. This attachment process depends on various other elements such as, the bacterial species, composition of the host's surface, environmental factors and essential gene products (e.g. RNA and

proteins), referring to biochemical materials that are a product of the expression of a gene. Although a bacteria can bind to inanimate and animated hosts, the connection to each is established through different procedures, resorting to nonspecific interactions (e.g. hydrophobic) and specific molecular mechanisms (e.g. adhesins and lectins) correspondingly (figure 2) [21].

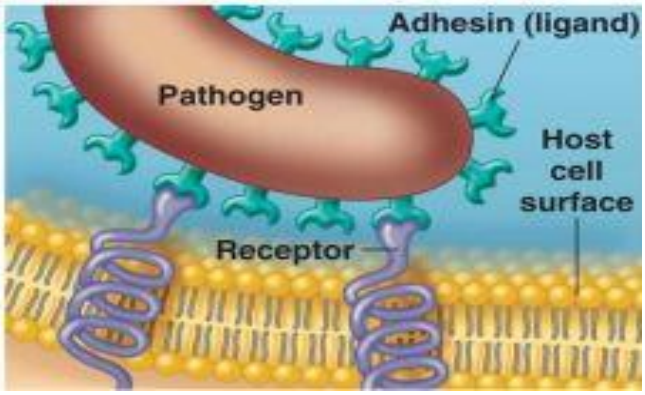


Figure 2: Adhesion process (adhesin-receptor), illustration adapted from [22].

1.3.1 – Fimbrial and afimbrial adhesins

Furthering into the multiprotein complexes that assist the adhesive bonding process, these proteins can be classified as fimbrial adhesins and afimbrial adhesins. Fimbrial adhesins are normally long and thin protrusions of the bacterial cell surface (pili), mainly present in Gram-negative bacteria, which are composed by major and minor protein subunits who hold an adhesive function. These protrusions have been evolutionarily optimized, in a way to facilitate the establishment of the initial contact between bacteria and host, accordingly assisting the adhesion process. It becomes clear that fimbriae are an excellent tool in terms of long distance contact between bacteria and host surface. The fimbrial adhesins are able to recognize carbohydrate moieties that are found in glycoproteins and glycolipids of the membrane, thus functioning as lectins (carbohydrate-binding proteins). Considering that for the adhesion to take place, the previously stated is necessary, it is consistent that there

can be interference with the process when the carbohydrates are of low-complexity. Opposed to the fimbrial adhesins, the afimbrial adhesins are either embedded into de bacterial cell membrane or attached to it, normally not resorting to bacterial protrusions. They resort to direct protein-protein interactions when adhering to a host's surface, being normally present in Gram-positive bacteria but also in a wide variety of Gram-negative bacteria [22][23].

Fimbrial adhesins are recognized as fundamental bacterial structures, who mediate the initial contact between bacteria and host. Additionally, afimbrial adhesins provide an additional arsenal to pathogenic bacteria to interact intimately with their host and trigger specific responses when in contact with the receptors (figure 3). It is becoming apparent that the binding of the bacterial adhesins to the receptors of the host have an effect that goes beyond the sole adhesion to the surface, namely modulation and formation of the infection process, which occurs after the binding process [23].

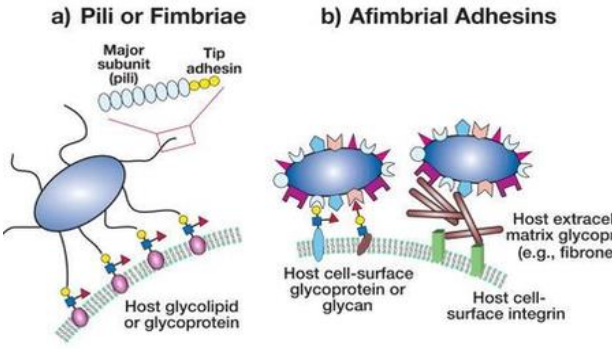


Figure 3: a) Adhesion by fimbrial adhesins; b) Adhesion by afimbrial adhesins, illustration adapted from [24].

1.3.2 – Biofilms

The attachment of bacteria to an animate and inanimate object, can lead to the colonization of it and create an aggregate of microorganisms in which bacterial cells adhere to the surface or to each other, and this community of bacterial cells is referred to as a biofilm (figure 4). These cells normally exists incorporated into a self-produced

matrix made of extracellular polymeric substance (EPS), being that this whole constitutes a community of microorganisms physically associated who are attached to a surface and among themselves, that can be encountered throughout all nature. Taking this fact in to account, biofilms can exist on all sorts of surfaces, whether animate or inanimate, in natural, industrial and hospital environments, consequently being a prevalent manner of microbial life.

For a biofilm to develop and prevail, the surface has to be capable to function as a nutrient source (e.g. cellulose in paper), because lacking such nutrients the biofilm cannot advance into long-term colonization. Standing as a biofilm entails many advantages for the microorganisms that are a part of it, such as facilitate colonization because of non-specific adhesion, communication between the bacterial cells that constitute de biofilm, nutrient reserves, protection against desiccation, alongside the host's immune response and antimicrobial agents, therefore posing a serious threat to public health.

However, these advantages can only be achieved through the cooperation and interaction among the different bacteria that compose the biofilm. It is then found that these bacteria present an organization within the structure, allowing an optimal interaction with the environment and without compromising cell survival or deplete the resources that are available to them. Living in such a community, cells do not have the need to exert all functions on their own, depending on other bacteria to take their part, permitting each bacterial cell to focus on certain roles and achieving an optimized performance for the community as a whole [21][22].

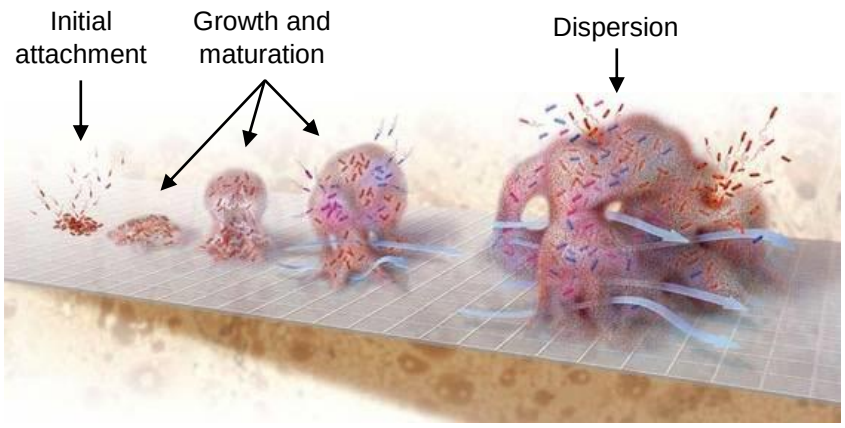


Figure 4: Biofilm development, illustration adapted from [25].

Biofilms developed by pathogenic bacteria such as Methicillin-resistant *S. aureus* can be difficult to eradicate. A study developed by Okuda and co-workers state that MRSA biofilms can present resistance to antibiotic treatments and the immune system of their host [26]. MRSA biofilms in healthcare settings can be developed on surfaces of various medical devices such as in indwelling vascular catheters, pacemakers and prosthetic joints, which has a high risk factor as they are introduced in the patients [26]. If they are not thoroughly disinfected as the hands of their handlers posteriorly, these may be transmitted to other devices and patients. Bacteria normally exist as biofilm formations in healthcare settings [27].

1.3.3 – Biotic and abiotic surfaces

As previously referred, bacterial cells have the capability to attach to animate and inanimate surfaces, properly called biotic and abiotic surfaces correspondingly. Abiotic surfaces extent numerous materials, such as glass, plastic, metal and others, whereas biotic surfaces include our skin, mucosa, alive or devitalized tissue. Bacteria can live in association with these surfaces, producing positive interactions, termed as commensalism.

It is known that people have more microorganisms on the surface of their skin than number of cells that compose the entire body. The microorganisms that are a part of an individual, that live in association with him in a consistent manner, are known as indigenous microbiota or normal bacterial. This association is known as being a symbiotic relationship between bacteria and host, whereas it can be divided in three categories of interactions, such as mutualism, commensalism and parasitism. Although, only bacteria that present a mutualism or commensalism relationships with their hosts are considered as being part the normal bacterial flora (e.g. bacteria of the intestine) [22][28].

1.4 – Host-Bacteria interactions

As formerly mentioned, bacteria live in symbiosis with their biotic host and their relationship can be of a positive or negative nature, then being defined as a two organisms that live in association with one another. The nature of the relationship is based on the quality that the bond carries for each part of the symbiotic link, verifying that when the interaction is of a mutualism or commensalism nature it is normally positive for the host. On the contrary, when the symbiotic relationship is based on parasitism, it has a negative impact on the host and can lead to severe consequences when not treated promptly [22].

1.4.1 – Mutualism and commensalism

A mutualistic relationship is observed when both parties benefit from the symbiotic association, for example the microorganisms that are a part of the normal bacterial flora of the intestine. In this kind of interaction the bacteria benefit as they have an endless nutritional source and the individual benefits according as the microorganisms aid in digestion and produce vitamin K. Commensalism, on the other hand, does not present an apparent benefit or caused harm on either parties of the link, though if analyzed thoroughly, there is always one party that is benefited or harmed.

For instance *Staphylococcus epidermidis* that take part of the indigenous microbiota of our skin, inhibits the growth of other microorganisms less tolerant to acid and possibly pathogenic because of lactic acid production. On the contrary, the other metabolites produced by it are a relevant source of unpleasant body odors, which can pose a problem on a social level.

1.4.2 - Parasitism

Finally, the parasitic relationship is the symbiotic interaction that leads to the development of illness in the biotic host, as it is pathogenic, and as such it is the most important, most dangerous and of greatest interest in terms of public health. This implicates that the parasite takes advantage of the host, potentially causing him harm and limiting the response of the immune system. Generally all parasites that are not indigenous to the microflora lead to disease when interacting with hosts that are not immunized. Opposed to that, there are parasites that are a part of the indigenous microbiota which only develop disease in an opportunistic manner [5][7].

1.5 – Commensal microflora

Commensal flora refers to the indigenous flora of an individual and is present in an abundant quantity on all body surfaces, especially in the mouth, nose, skin and the large intestine. The normal bacterial flora gives various advantages to the host, such as nutrient production (e.g. vitamin K), stimulation of the immune system, protection against infection (e.g. prevention of colonization by other bacteria), aiding in the metabolism of foods and provides essential growth factors. If this normal microbiota would be removed by the use of antibiotics, a severe infection would develop, mainly originated by microorganisms resistant to them. To understand the importance and extent of the indigenous microbiota, if it was absent, life as we know it today would be impossible [5][7].

Our normal bacterial flora is determined by numerous factors and it will vary throughout the course of our lives, therefore changes due to age, gender, diet, hormones, personal hygiene and health, amongst others. The microflora can also be altered due to external factors, such as being hospitalized, which augments significantly the exposure to various other bacteria that are possibly pathogenic, or when taking antibiotics who compromise the immune system.

The exposure to bacteria can lead to a transitional or permanent colonization or lead to developing illness, when faced with pathogenic or antibiotic resistant bacteria. Colonization differs from infection, as colonization can implicate a relationship of commensalism or mutualism, not negatively affecting its host. Hence an infection, and consequently disease, entails the invasion of the host by pathogenic bacteria who could possibly cause great harm to the host, this being the definition of parasitism.

Nowadays treating infectious disease is compromised by the increasing number of bacteria that acquire multiresistance to antibiotics [12]. A study conducted by Sommer and co-workers researched the human microflora as a potential reservoirs of antibiotic resistance genes. Since these genes are exchanged between bacteria, the commensal microflora may acquire and transfer these resistance genes to pathogenic bacteria which increases their pathogenicity, being an additional public health risk [12].

Understanding microbiology requires not only an understanding of the different classes of bacteria, but also their propensity and means of developing diseases in the host. It is known that not all bacteria have a negative impact on their hosts and the environment, since many are useful on an industrial level and in medical treatments, aside of the advantages previously revealed in terms of the indigenous microbiota [22][28].

1.6 – Gram-positive and Gram-negative bacteria

Bacteria are divided in two big groups, Gram-positive and Gram-negative. Whereas they are similar in terms of internal structures, their external composition are

very different (figure 5). The cell wall is the component that permits to make a clear distinction between these two groups, achieved through a coloration method known as Gram staining, which tinges the cell wall of either bacterial group in a different color because of the difference in structure, components and functionality. This staining method requires the utilization of dyes, such as crystal violet stain, which tinge Gram-positive bacteria with a violet color by retaining the dye. Gram-negative bacteria cannot retain the dye due to their cell wall properties and will be colored by a second dye with a pink color.

The differences in the cell wall of these two major groups of bacteria rely on their layer of peptidoglycan. In Gram-positive bacteria, the peptidoglycan layer is thick, presenting multilayers, however in Gram-negative bacteria this layer is thin and appears to be a single one. Due to the fact that Gram-positive bacteria exhibit a thick layer of peptidoglycan, their cell wall is more rigid, determining the profile of specific bacterial cells. On the contrary, Gram-negative bacterial cells are involved by an outer membrane that is composed by lipopolysaccharides, generally known as LPS, and proteins, which functions as an impermeable barrier to antibiotics and hydrophobic dyes, conferring the bacteria with resistance to them [29].

In addition to the differences formerly exposed, there are several others, including the presence of a periplasmic compartment, a high content of LPS, lipids and lipoproteins, a primary production of endotoxins and an elevated resistance to drying and physical disruption in Gram-negative bacteria. Gram-positive bacteria, contrarily, practically do not exhibit a periplasmic compartment, do not present an outer membrane, the content of LPS is practically nonexistent, the content in lipids and lipoproteins is low, they predominantly secrete exotoxins and the resistance to drying, physical disruption and antibiotics is low. Assembled all this information, it is easy to comprehend that although they may be similarly structured internally, that the external features provide enough diversification to distinguish the two groups and attribute very different characteristics, such as the interactions with the surrounding environment [22][29].

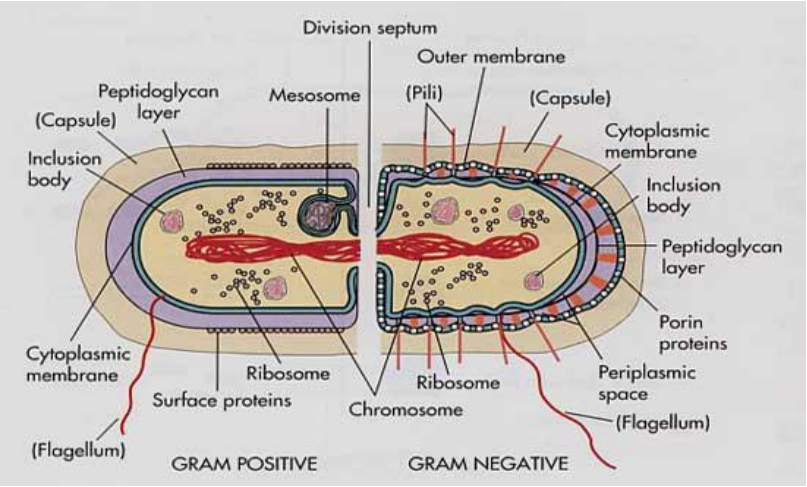


Figure 5: Left - Gram-positive bacteria; Right - Gram-negative bacteria, illustration adapted from [30].

1.6.1 – Internal bacterial structures

It is known that bacterial chromosome is a unique circle constituted by double-stranded DNA, also referred to as dsDNA, which is not enclosed in a nucleus but rather in a nucleoid. In this case, histones are not necessary to maintain the conformation of the DNA, and the DNA does not form nucleosomes. Bacteria can present plasmids, which are small structures and composed by extrachromosomal circular double-stranded DNA, most frequently found in Gram-negative bacteria. Although they are not essential to the cell's survival, they provide a very important advantage, through enhancing the resistance to antibiotics. This aspect is very important when studying multiresistant bacteria [22][29].

1.7 – Pathogenic flora

Although the commensal flora has been discussed previously, the pathogenic flora is equally important if not more, since this flora encompasses bacteria that can induce severe disease in a host and simultaneously be drug resistant. These bacteria

can either be obligatory or potentially pathogenic, or even opportunistic. The manifestation of the disease is verified when a bacterium-host relationship is established, equal to the aforementioned adhesion.

As formerly mentioned, some bacteria that are a part of the commensal flora of the individual can induce illness to him when the conditions are prone to it. Potential pathogens are considered to be bacteria that do not cause disease in a healthy individual, unless an opportunity arises, due to a depressed immune system or a weakness of the anatomical barriers, for instance. Additionally these bacteria present an advantage, as they are able to colonize or infect third parties who encounter themselves in an immunodepressive state. On the other hand, obligatory pathogens are only in association with their host to cause disease. They can however, occasionally be found as a part of the normal bacterial flora, as for example in asymptomatic carriers or carries in recovery, and even in cases where the host is not capable to eradicate the pathogens.

The opportunistic pathogens, just like the potential pathogens, induce disease in hosts that stands immunocompromised, meaning that the development would not be verified in a healthy individuals. Furthermore, the opportunistic pathogens can be a part of the normal microflora of the individual (e.g. *Staphylococcus aureus* or *Escherichia coli*) or may originate from the surrounding environment (e.g. *Pseudomonas aeruginosa*), as the opportunistic pathogens that originate from the environment derive from the air, soil, water and food. When an indigenous bacteria causes an opportunistic infection in an individual, this disease is denoted as an endogenous bacterial disease. The most common diseases induced by this type of bacteria are dental caries and periodontal disease, more specifically brought on by bacteria of the indigenous microbiota of the oral cavity [22][28][29][31].

1.7.1 - Pathogenicity

The source of contamination referrers to the site where the pathogen has originated. This can be an exogenous or endogenous source or even stem from a reservoir. When the bacterial source of contamination is exogenous, the bacteria is contracted from an environment external to the host, thus from water, food, animals, another ill or infected individual or simply a carrier of the bacteria. An endogenous bacterial source of contamination relates to bacteria that are a part of the indigenous microbiota of the individual, hence by bacteria that are potentially pathogenic, as explained earlier on. The other alternate source of contamination is the reservoir of specific bacteria [22][31].

1.8 – Bacterial reservoirs

There exists the possibility that individuals, and even their mobile phones, can be potential reservoirs of specific bacteria. This implies that certain bacteria reside in a host without causing them harm and inducing disease, but can be transmitted to third parties and even induce disease in other individuals. In case of the mobile phones serving as a reservoir of specific bacteria, there may be certain bacteria that have the ability to adhere to them and maintain that connection, and as such transmit them to individuals that handle or come in contact with the phones in question. Attending what was previously exposed, a reservoir is a site where bacteria reside persistently and in a constant manner, which can be a human being, animals, inanimate objects, surrounding environment, among others [12][4] [6].

It is known that there are individuals in the community that serve as reservoirs of specific bacteria (e.g. *Mycobacterium tuberculosis*), as they have already been formerly infected and developed the disease, however the bacteria remained in their system, and as such present the possibility of transmitting them to other individuals and causing them to develop the disease. Within a community the risk of contamination is high, especially when dealing with pathogenic and antibiotic resistant bacteria, since

many premises are vastly frequented and the elevated number of individuals provide the bacteria with numerous vehicles for transmission. The individuals that pose a higher threat in terms of propagation of pathogenic and antibiotic resistant bacteria, and thus a risk for public health in a community, are those who work in the branch of healthcare [22][12][4][6].

A study developed by Badr and co-workers researched mobile phones and nosocomial infections in terms of bacterial reservoirs. It was found that many healthcare professional present meticulous hygiene care in terms of contamination of clothing, jewelry, and hands but not to their mobile phone [27]. It was verified that many professionals take their phones with them into operating environments as there does not exist a specific policy against this behavior. This lack of policy indicates that there is little to no awareness to the potential transmission source a mobile phone may be, therefore increasing the potential of being a vehicle for transmission of infection [27]. Mobile phone can act as bacterial reservoirs, both healthcare, community and environmentally acquired. They can be transmitted into the operating environments and ICU in healthcare facilities, which is dangerous and poses a real health risk to the patients treated there [27].

1.8.1 – Healthcare settings

The healthcare professional deal, on a daily bases, with patients and their samples that are possibly contaminated with pathogenic and multidrug resistant bacteria, and this implies that they have to meticulously control their hygiene or they might contaminate samples, patients, other healthcare personnel or individuals foreign to the healthcare work place, referring to cross contamination. There has to be caution since the bacteria can leave the healthcare premises by adhering to money, mobile phone, and other personal objects of the healthcare provider. Once outside the healthcare facilities, the bacteria can be transmitted to third parties, this being a

transmission route through indirect contact, namely the individual does not contact directly with the source of contamination [27][7].

An example of transmission through indirect contact, is the case formerly presented in which a hospital outbreak of *Klebsiella pneumoniae* occurred because of its presence on a ventilator's touchscreen. This was possible because of the fact that touchscreens are not viewed as possible contamination sites or bacterial reservoirs, which makes them easily forgotten in terms of hygiene control and decontamination, and as such opens the possible transmission of bacteria from healthcare professional to patient [5]. Another route of transmission is through direct contact, where an individual to individual contact is necessary. Disease is then carried out in an individual because of the transmission of the bacteria by another, due to the establishment of direct contact.

Loftus and co-workers developed a study on hand contamination of anesthesia providers as an important risk factor for direct intraoperative bacterial transmission. The results obtained revealed that in 11.5% of the cases studied were identified as intraoperative bacterial transmission to the IV stopcock set and that 47% of these cases were of provider origin. They also studied this bacterial transmission to the anesthesia environment and was verified in 89% of the cases of which 12% were of provider origin [32]. These numbers are significant and preoccupying and the disinfecting standards both for the environments, utensils and healthcare professionals should be more strictly imposed.

Shiferaw and co-workers analyzed the bacterial contamination, bacterial profile and antimicrobial susceptibility patterns of isolate from stethoscopes, which is a utensil frequently used in various patients and potentially overlooked as a source of contamination. From all the samples collected 256 bacterial strains were isolated, from those bacteria 52% were potentially pathogenic, including *S. aureus*, *Klebsiella* spp., *Salmonella* spp., *Pseudomonas aeruginosa* and *E. coli*. In addition, all strains were resistant to multiple antibiotic classes [33]. The stethoscopes that never had been disinfected presented a 90.9% contamination rate and those who were disinfected a

week or less prior to the sampling presented 72.2% of contamination, which is still high proving that they should be regularly disinfected and taken in to consideration in the hygiene standards [33].

A study carried out by Carling and co-workers analyzed if it was possible to improve environmental hygiene in intensive care units to decrease multidrug resistant bacterial transmission. It revealed that using a structured approach which includes a highly objective surface targeting and a good communication with the environmental services personnel as to obtain the optimal hygiene standard, can diminish the bacterial transmission [34]. It was found that by improving the meticulousness environmental hygiene of the intensive care units it enhances patient safety significantly in terms of transmission of pathogens multiresistant to antibiotics [34].

1.8.2 – Bacterial transmission

There exist other methods of bacterial propagation, in which they resort to other means of transportation, such as the transmission by water, food and through air, commonly referred to as aerosol. The last being the most important when it comes to nosocomial infections, and presenting the highest risk of contamination and transmission via air-conditioners for instance. It is a widely documented fact that admitted patients in hospitals or healthcare premises, contracted pneumonia, in those cases nosocomial pneumonia with air-conditioners as contamination source [35][36][17]. Various nosocomial aerosol contaminations have been documented, such as *Legionella* aerosol contamination by Cassier and co-workers, Bioaerosol deposition in hospital rooms by King and co-workers and airborne transmission of disease in hospital by Eames and co-workers [37][38][36].

Additionally disease can be transmitted through the use of vectors, which can be animals or insects, and in case of the study, the hands were the primary "vector" in the contamination of the mobile phones. This could lead to disease development by the

owners and third parties that come in contact with the device, provided that the bacteria that reside there are pathogenic and possibly drug resistant.

It is then important and relevant to the research, to understand this type of transmission, adherence and permanency of the bacteria to the different mobile phones, and the potential risk that it could represent in terms of health hazards. The purpose is to comprehend which specific bacteria are present on the devices and if these could be potentially pathogenic and multiresistant to antibiotics, hence could lead to disease.

Since primarily our hands are the utensils that we use to operate our mobile phones, it can be assumed that the bacteria of the commensal flora of the hands, are the bacteria that are present on the devices, as there is a mutual and constant exchange of microorganisms between them. This will reveal if mobile phones and their users are effectively reservoirs of specific pathogenic and drug resistant bacteria [4][6][39]. The World Health Organization (WHO) states that the normal bacterial count for a healthcare worker's hand ranges from 3.9×10^4 to 4.6×10^6 CFU per cm^2 [40].

The individual characteristics of the participants submitted to sampling must also be considered, since these and their hygienic care may be reflected on their devices, thus influencing it in terms of being a reservoir and harboring pathogenic and resistant bacteria [41].

Tambekar and co-workers studied the role of hand washing in transmission of enteric infections among students and observed that 100% of the participants presented contamination of the hands before washing occurred. The bacteria that were mainly found were *Escherichia* spp. (27%), *Staphylococcus* spp. (17%) and *Pseudomonas* spp. (11%) [42]. Washing was assumed to be with water and soap, and in 21% of the individuals a complete bacterial removal was verified and there was an 56% overall reduction in bacterial count [42]. It can thus be verified that hand washing does reduce the overall bacterial count, and in a very significant manner.

The time expended to washing hands is relevant in terms of reducing the overall bacterial count of the hands. The WHO has revealed that by washing the hands with plain soap, i.e. non-antimicrobial, during 15 seconds it is reduced by 0.6 to 1.1 $\log_{10}$. However, if the hands are washed for 30 seconds, these bacterial counts can be reduced by 1.8 to 2.8 $\log_{10}$, which is significantly higher [40].

Another study, carried out by Borchgrevink and co-workers researched hand washing practices in a college town environment and found that many individuals do not wash their hands appropriately and they do it for a very short time (±7 seconds), only 5.3% take more than 15 seconds to wash their hands [43]. They call to the attention that proper hand washing practices should be continuously encouraged as to better learn how to wash your hands and to understand the consequences that it may have.

These studies reveal that proper hand washing is not executed and that the impact of a 7 second wash will not be sufficient to significantly reduce the overall bacterial count. This also means that probable transmission of potentially pathogenic bacterial is increased, as several studies demonstrate that plain soap fails to remove pathogens from the hands of healthcare workers [40][44].

1.9 – *Staphylococcus aureus*

When addressing the topic of pathogenic bacteria, one of the most common staphylococci and most successful human pathogenic bacteria is *Staphylococcus aureus* [45][46]. This bacteria can be found in a commensal association with humans, being that these individuals are asymptomatic, i.e. do not develop any symptoms or disease when healthy, but are still able to transmit the bacteria to third parties. Approximately 20-30% of the general population, being healthy individuals, may present this kind of association, in which *S. aureus* can be found on the skin, skin glands and mucous membranes, especially in the nose [47]. Being that this bacteria is frequently connected with developing human disease and appears to be developing

resistance to a growing number of antibiotics, it is one of the most intensively studied bacterial species [46][48][49].

S. aureus is a Gram-positive opportunistic bacteria which can be ubiquitously found, thus being able to colonize humans, animals and surfaces [50]. This bacteria is very resistant to adverse environment conditions, including drying, and can resist high concentrations of salt (NaCl), being this one of the criteria of their selective media [45]. The fact that humans are a reservoir of these bacteria (asymptomatic carries) can be highly dangerous as it increases the risk of infection, as when the individual is immunocompromised they take the opportunity of developing disease. Most of the patients that develop infection caused by this bacteria, contracted the infection by *S. aureus* bacteria of their commensal flora [49][47].

1.9.1 – *S. aureus* pathology

One of the main causes of hospital acquired and community acquired infections which could lead to serious consequences, is *S. aureus*. When it comes to nosocomial infections caused by this bacteria, it affects the bloodstream, skin, soft tissue and lower respiratory tract. The infections possibly caused by this bacteria are ventilator-assisted pneumonia and central venous catheter-associated bacteremia, also causing deep-seated infections (ex. endocarditis) and toxin mediated diseases, such as toxic shock syndrome (TSS), scalded skin syndrome (SSS) and staphylococcal foodborne diseases (SFD) [51][52][53]. This pathogenic bacteria is particularly important due to its ability to cause life-threating infections and most important, the additional potential to develop resistance to a wide variety of antibiotics, especially the generally used in clinical environments. Additionally this pathogen exhibits various virulence factors (structural and secreted products) which contribute to the ability to develop infection, these being attachment-improving agents, exotoxins and superantigen toxin [49][46].

S. aureus can become more pathogenic by acquiring resistance to antibiotics. One specific antibiotic that is well documented and presents susceptibility in less than

50% of *S. aureus* is Erythromycin (60.4% resistance) [54][55]. The resistance to Erythromycin in staphylococci is conferred by *erm* genes (*ermA*, *B* and *C*), additionally the *msrA* gene also enables this resistance. This antibiotic is widely used for the treatment of human and animal infections [54][56][57].

These genes are more frequently found in *Staphylococcus aureus* rather than in coagulase negative staphylococci (CoNS). Furthermore, among the CoNS the resistance gene that was predominantly found was the *ermC* gene and in *S. aureus*, *ermA* was prevalently detected. However, there are contradictory studies which report that the *ermA* gene is more frequently found in CoNS [54][56]. This demonstrates that resistance to Erythromycin in staphylococci is a known fact and widely detected, thus being extensively studied, as there are studies dated from 1986 [57][58].

Duran and co-workers elaborated a researched with the objective to verify antibiotic resistance genes and susceptibility patterns in staphylococci. Relatively to *S. aureus*, 139 samples were analyzed and it was verified that 92.2% presented resistance to Penicillin and 60.4% were resistant to Erythromycin. Additional resistances were verified as 38.1% to Clindamycin, 23% to Amoxicillin + Clavulanic Acid, 16.5% to Methicillin and 0% to Vancomycin [54]. This study demonstrated that high percentages of resistance are found in staphylococci and this can pose a real public health risk if no other therapeutic agents are effective.

1.9.2 – Methicillin resistant *Staphylococcus aureus* (MRSA)

Methicillin-resistant *Staphylococcus aureus* (MRSA), are *S. aureus* bacteria which have acquired the *mecA* gene, additionally making them resistant to all β-lactam antibiotics. Therefore the chromosomes of the bacteria present a large mobile genetic element (MGE), the SCC*mec*. Just like others Penicillins, the way Methicillin inhibits the dissemination of the bacteria is by blocking the Penicillin binding proteins (PBP), which are responsible for the construction and maintenance of the bacterial cell wall [59][46].

The *mecA* gene, codes for an alternative Penicillin binding protein (PBP2a), which enables a low binding affinity to all β-lactams (native and semi-synthetic), thus the bacteria expresses resistance to them [54]. They are, as the Methicillin-sensitive *S. aureus* (MSSA), highly dangerous in healthcare environments due to their ability to acquire multidrug resistance determinants. Since they are so hazardous, they are easily spread throughout hospitals if special surveillance programs are not carried out, consequently increasing greatly the risk of an outbreak. MRSA, just like MSSA, is able to colonize humans, animals and surfaces [49][50][46].

When analyzing the MRSA distribution throughout the European Union (EU) and the European Economic Area (EEA) for 2012 (figure 6), it can be observed that in Portugal and Romania these bacteria are present in more than 50% of the invasive isolates, which is a very high and preoccupying number. Other countries as Italy, Greece, Malta and Poland present a 25% to <50% range, followed by the majority of countries who represent these areas with 10% to <25% (figure 6) [60].

This representation shows how extensive the dissemination of MRSA is and that it is very present in invasive isolates, in the majority of the countries of the EU/EEA. This dissemination in invasive isolates is especially dangerous as it has a higher mortality rate [61][62]. A report emitted by the WHO indicates that in some settings in Europe 60% of *S. aureus* infections were Methicillin-resistant (MRSA) [9].

There are only 5 countries that presented 1% to <5%, the Netherlands, Denmark, Norway, Iceland and Finland, and only Sweden presented <1% (figure 6). This is verified due to the fact that their healthcare systems are more developed as to attain a better structure with strict hygiene standards [60].

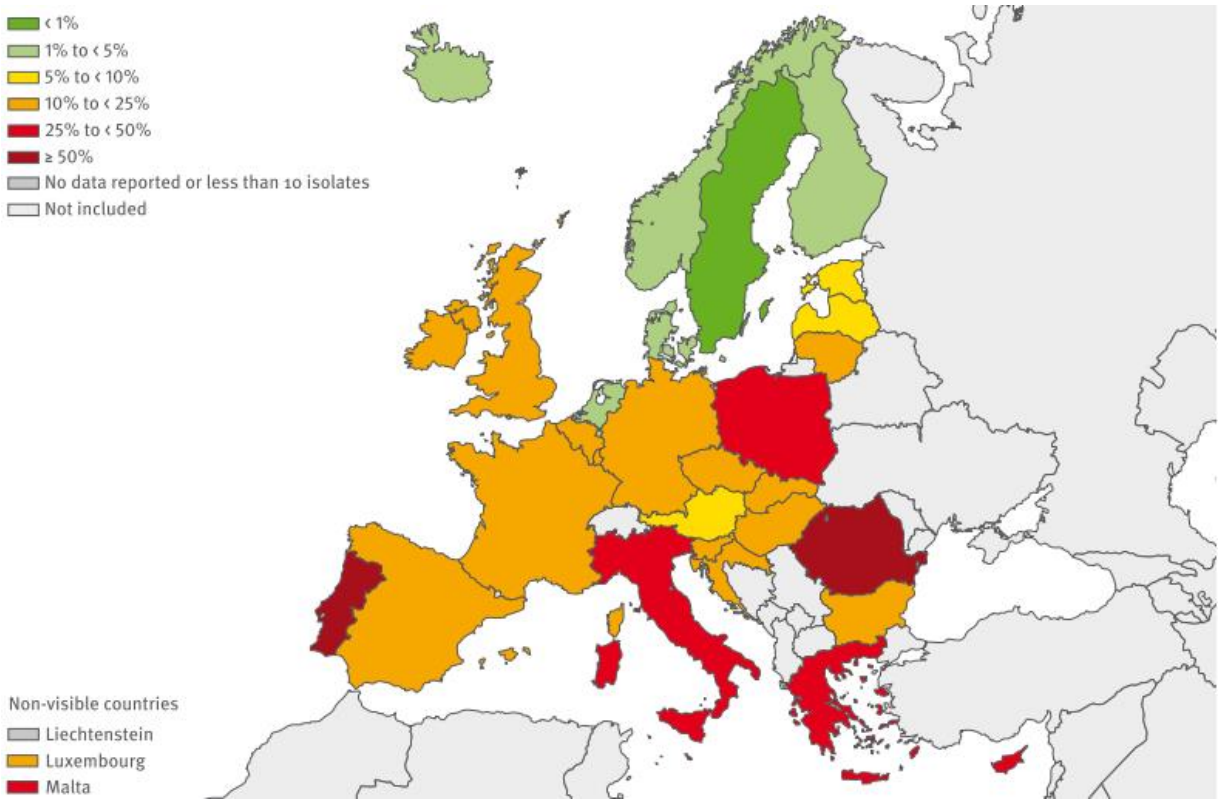


Figure 6: The percentage of Methicillin-resistant *Staphylococcus aureus* in invasive isolates presented by the European Antimicrobial Resistance Surveillance Network's (EARS-Net) annual report of 2012, by country of the European Union (EU)/European Economic Area (EEA), illustration adapted from [60].

A Statistical Bulletin emitted by the Office for National Statistics addressing the deaths involving MRSA in England and in Wales from 2008 to 2012 revealed that these rates have consistently fallen in the latest years [63]. In male individuals, a reduction of 79% was verified and 76% in the female participants, which is highly significant and exhibits an improvement in terms of prevention and treatment. MRSA was only involved in 0.1% and 0.2% of all deaths and in all hospital deaths, correspondingly [63].

As can be verified in figure 7, and being consistent for the United Kingdom, the majority presented lower values for 2012 in comparison to 2011. The overall analysis shows a significant reduction of MRSA in invasive isolates [60]. Although, 7 countries

presented a significant decrease, 4 presented a significant increase, which is preoccupying [60].

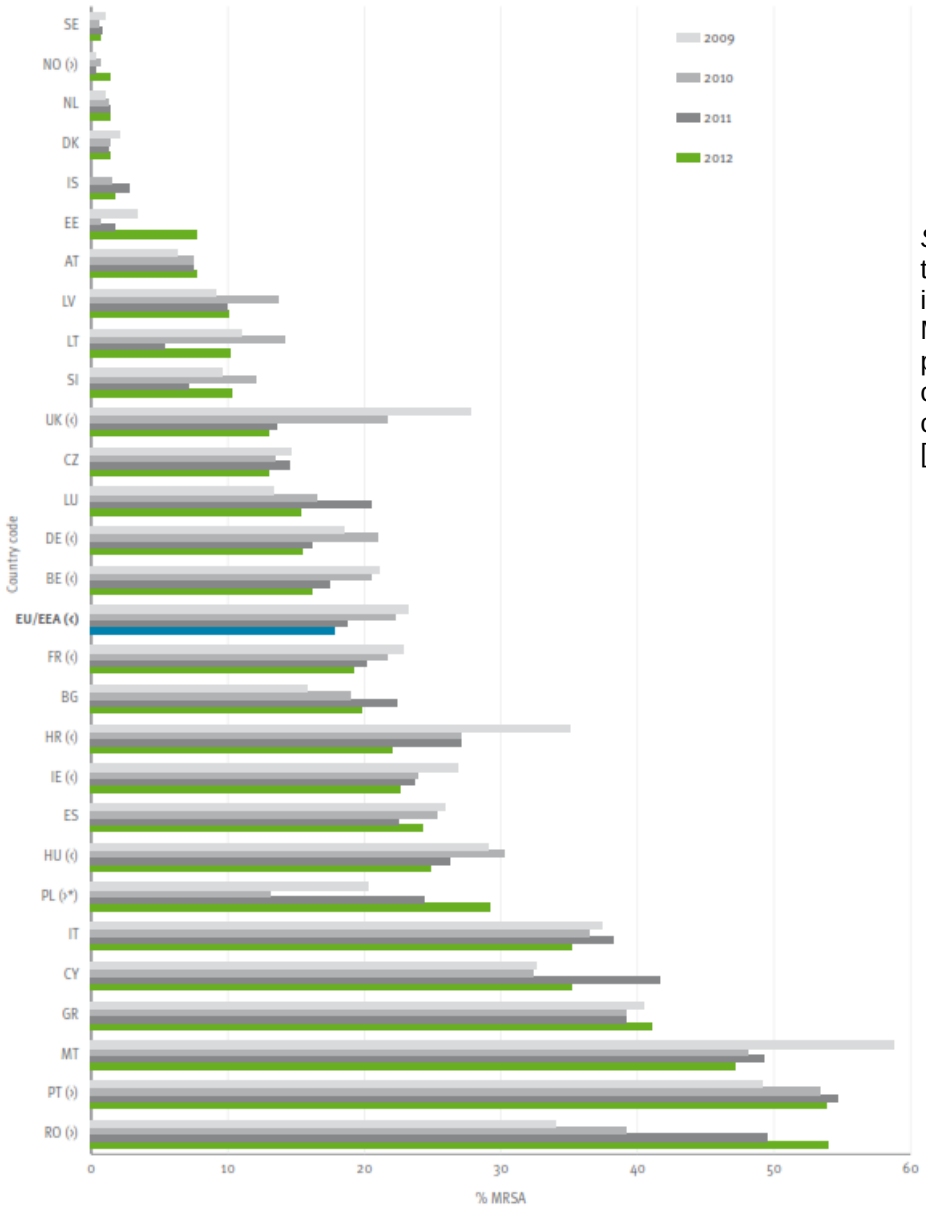


Figure 7: *Staphylococcus aureus* trends of invasive isolates resistant to Methicillin (MRSA) in percentages, by country, EU/EEA countries, 2009 to 2012 [60].

> - significant increase; < - significant decrease; * - significant trends in the overall data were not supported by data from laboratories consistently reporting for all four years.

1.9.3 – Staphylococcal cassette chromosome (SCC)

Staphylococcal cassette chromosomes (SCCs) are relatively large fragments of DNA varying between 21 and 67 kb, which always insert into the same gene on the *S. aureus* chromosome, which is the *orfX* gene (Open Reading Frame). Integration and excision of SCC*mec* by the recombinases occur within a specific attachment site (attBscc) on the *S. aureus* chromosome at the 3' end of *orfX* (figure 8). This SCC transports genes that encode resistance to antibiotics and/or virulence determinants. Many SCCs encode the gene for Methicillin resistance (*mecA*) and thus can be classified into two groups, SCC*mec*, detailed further on, or non-SCC*mec* [49][64][65].

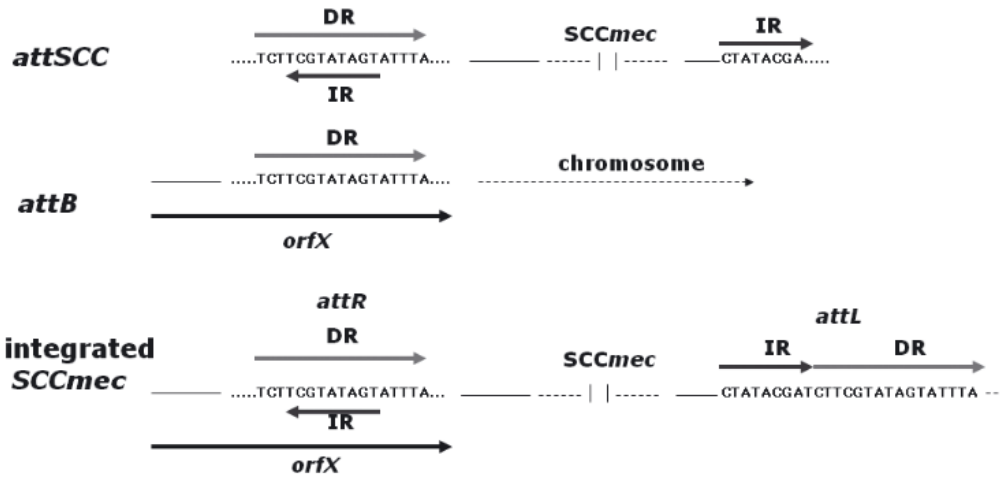


Figure 8: Schematic representation of attachment sites for SCC*mec* integration, illustration adapted from [49].

1.9.4 – SCC*mec*

As previously stated, the SCC*mec* is a mobile genetic element which transports a central genetic determinant, the *mecA* gene which confers resistance to the antibiotic Methicillin and other β-lactam antibiotics, and thus all the MRSA strains contain this

mobile genetic element. MRSA may have acquired the MGE SCC*mec* from *S. sciuri* in a vertical manner (figure 9) [11][46].

The SCC*mec* integrates a *mec* operon (figure 9) where the *mecA* gene is located together with its regulatory genes, *mecI* and *mecR1*, and in addition the MGE encodes chromosomal recombinases (*ccr*A, *ccr*B and *ccr*C) and J regions (joining regions), essential for the horizontal transmission inter and intra-species of SCC*mec* between bacteria (figure 9). The J regions were formerly considered junkyard regions, but it is now known that they may also encode additional antibiotic resistance [64][11][46].

Five different classes of SCC*mec* have been defined (A to E), based on the structural organization of the *mec* operon (figure 10), of which three (A to C) are most commonly found in *S. aureus*. It is relevant to highlight that only the class A SCC*mec* consists of the complete *mecA* regulon (*mecI-mecR1-mecA*), as these regulatory genes are disrupted by insertional sequences in class B and C SCC*mec*, IS1272-Δ*mecR1-mecA* and IS431-Δ*mecR1-mecA* respectively (figure 10). These three classes of the *mec* complex and four different *ccr* allotypes presently define eight *SCCmec* types (I-VIII) (figure 11), although these types can be differentiated further into subtypes depending on J region variations [64][65]. SCC*mec* type IV is the most commonly SCC*mec* type found in MRSA worldwide. This type is also the most variable, presenting eight subtypes, which may be due to its higher mobility compared to the other SCC*mec* types [66][46].

There are most recently described SCC*mec* types, IX, X and XI, which were found to be from animal origin, also known as livestock-associated (LA-MRSA) [11][46]. These three elements carry at least one operon that encodes resistance to heavy metals, this apparently being characteristic for SCC*mec* elements which originate in animals [46]. The SCC*mec* types XI and X exhibit the same *mecA* gene as the previously presented SCC*mec* types (I-VIII), whereas the SCC*mec* type XI harbors a different *mecA* gene homologue ($mecA_{LGA251}$) [65][46]. These SCC*mec* types IX, X and XI present the *ccr* gene complexes crrA1B1, crrA1B6 and crrA1B3, correspondingly [67]. The *mec* gene complexes they exhibit are C2, C1 and E, respectively [68].

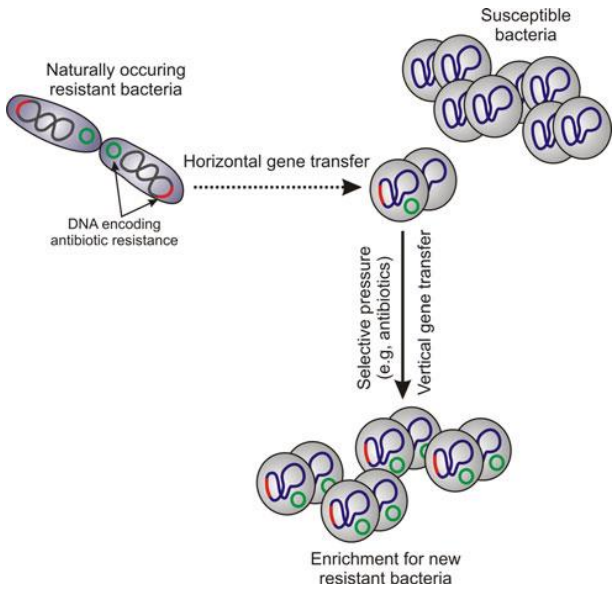


Figure 9: Horizontal and vertical gene/MGE transfer, illustration adapted from [64].

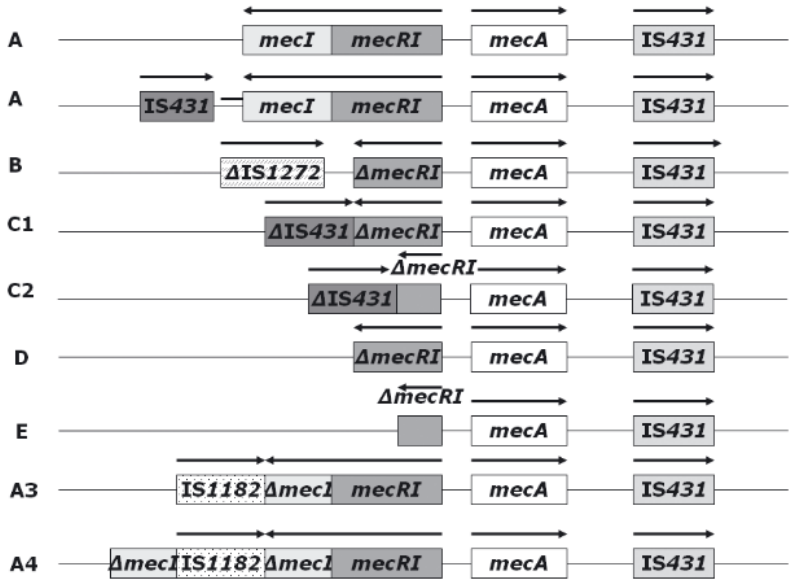


Figure 10: Structural classes of *mec* operon, illustration adapted from [49].

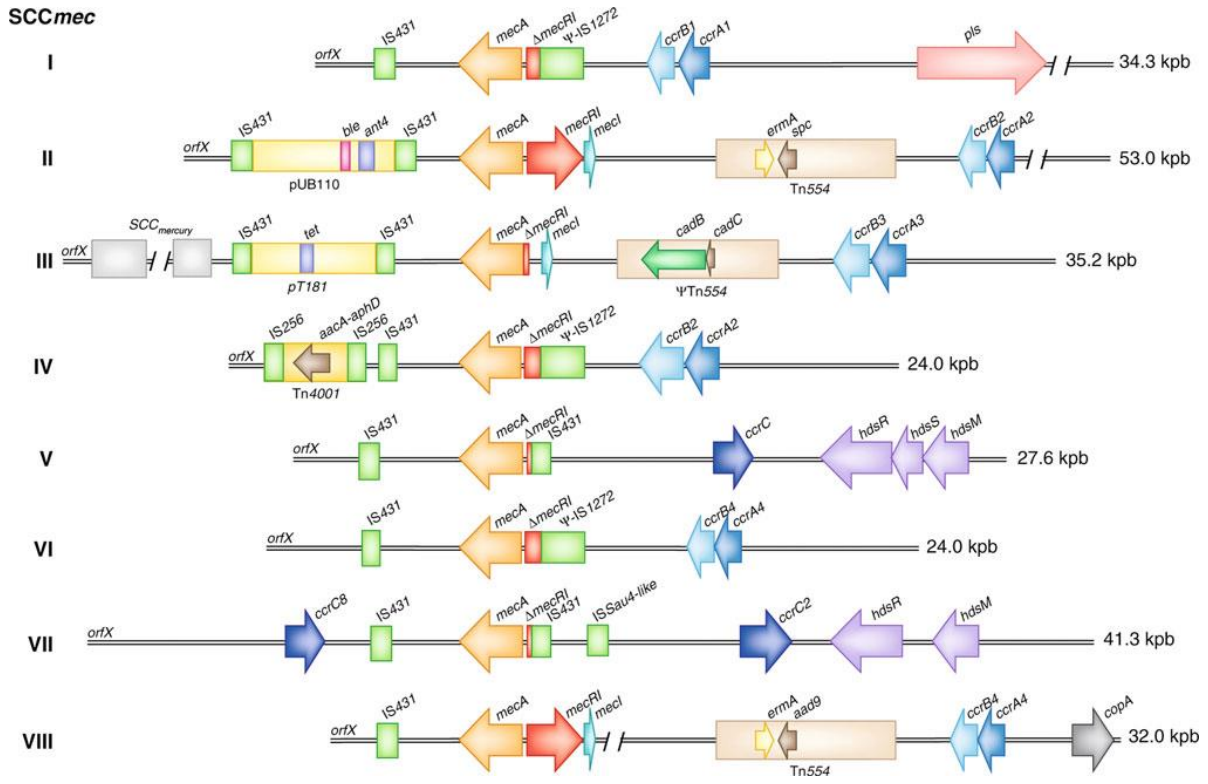


Figure 11: Comparison of SCC*mec* types, illustration adapted from [64].

1.9.5 – Community-associated MRSA and Healthcare-associated MRSA

As formerly mentioned, *S. aureus* is an opportunistic bacteria, meaning that they are able to develop infection in individuals when their immune system is compromised. The latest generation of MRSA strains have the propensity to initiate disease development in otherwise healthy individuals living in the community, such as in children and young adults [45][69][46]. MRSA can be divided in two types of associations, community-associated MRSA (CA-MRSA) and Healthcare-associated MRSA (HA-MRSA), because of the fact that this bacteria can be contracted and develops disease in both environments, thus being very important from an epidemiological standpoint. Although they are not a set of features restricted to each type o MRSA association, CA-MRSA and HA-MRSA can be differentiated by epidemiological, clinical and microbiological features (table 1) [46].

CA-MRSA strains are inclined to develop less severe consequence when infection occurs, such as skin and soft tissue infections, although these can be recurring and there are registered outbreaks, and certain cases can progress to invasive tissue infections, bacteremia and even death. The skin soft tissue infections have mainly been described in children, young adults, athletes, prisoners and army recruits [45][69]. However, HA-MRSA has the acquired ability to cause severe infections and therefore has a high mortality rate, such as sepsis and necrotizing pneumonia, which occurs in young patients and is normally preceded by the influenza virus or a similar illness (mortality can exceed 50%) [69].

The main features that permit the differentiation of the two MRSA strains, are clinical, epidemiological, resistance to antibiotics and on a molecular level. It was found that CA-MRSA is resistant to β-lactams and in a molecular stand point, this strain contains a SCC*mec* element of type IV, type V or the newly established type VII. On the other hand, HA-MRSA is typically multidrug resistant and contains the larger type I, type II, type III, type VI or type VIII SCC*mec*, and these elements may encode resistance determinants in addition to *mecA* (table 1). These additional resistance determinants are incorporated into the J regions of SCC*mec*, which are normally encoded by plasmids, transposons or insertion sequences [45][64][69].

Table 1: The main characteristics of HA-MRSA and CA-MRSA strains, adapted from [45].

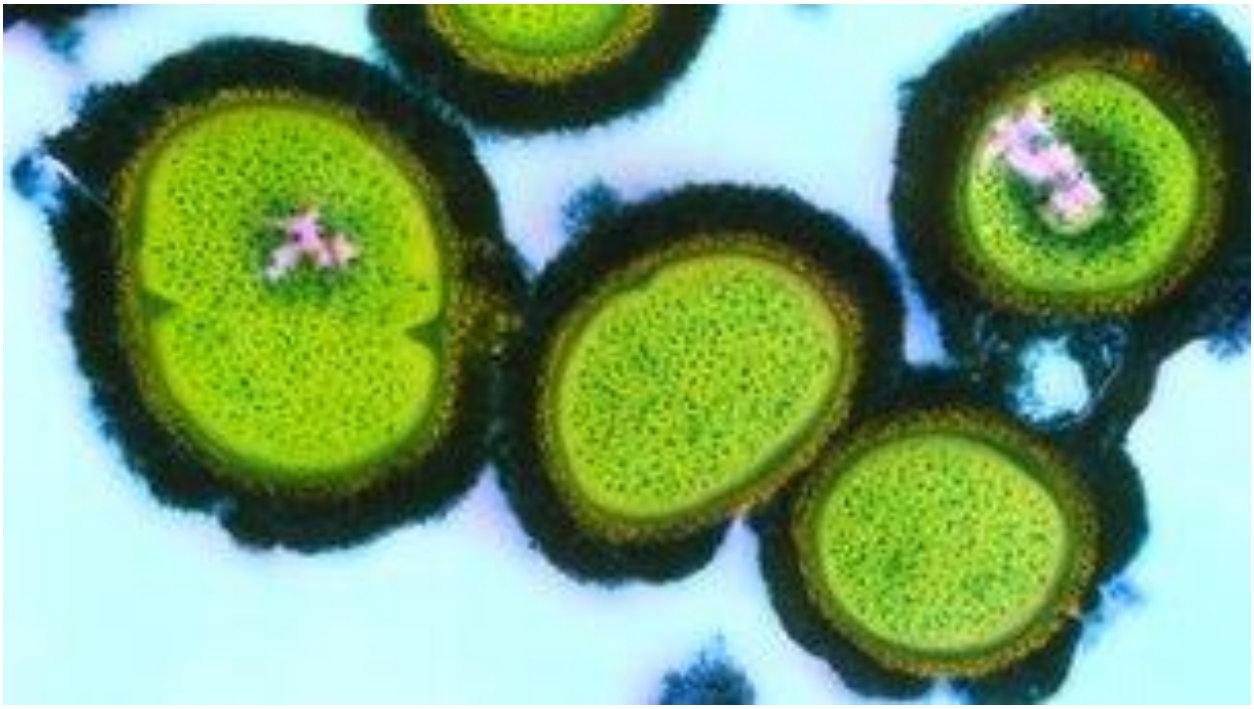
Characteristic	*HA-MRSA*	*CA-MRSA*
Clinical	Surgical site infections and invasive	Skin infections, "bug bites", rarely invasive, multiple and recurrent
Epidemiology	Elderly and healthcare	Young, athletes, drug users, correctional facilities and military
Antibiotic resistance	Multiresistant	B-lactam resistant
Molecular markers	PVL-negative SCC*mec* type I-III, VI and VIII	PVL-positive SCC*mec* type IV,V and VII

HA-MRSA – Healthcare-associated Methicillin-resistant *Staphylococcus aureus*; CA-MRSA – Community-associated Methicillin-resistant *Staphylococcus aureus*; PVL - Panton-Valentine Leukocidin.

Currently most CA-MRSA carry the phage-encoded Panton-Valentine leukocidin (PVL), which is a toxin with the capability of causing lysis in the human leukocytes and necrosis of the epithelial cells. This toxin is primarily related to skin infections and to necrotizing pneumonia, thus most CA-MRSA isolates that cause severe infections produce PVL [70][46][71].

It is noteworthy that currently in Europe the infections due to CA-MRSA are increasing and they belong to a variety of different clones and lineages and the majority carry the PVL genes. The native CA-MRSA strain which was susceptible to most non-β-lactam antibiotics is now evolving into a strain presenting multiresistance to antibiotics, which is highly preoccupying. The most common European CA-MRSA clone is ST80 and is characteristically resistant to fluoroquinolones, tetracyclines and fusidic acid, additionally to the β-lactam resistance [46][65]. The other circulating clone in Europe ST30, has proven itself resistant to different antibiotics including aminoglycosides, and thus both clones demonstrate the veracity of the previous statement relatively to the acquisition and development of resistance to other antibiotics [64][69].

II – OBJECTIVES



(http:// www.bbc.com/news/uk-scotland-edinburgh-east-fife-17541498)

2 - Objectives

This study aimed to analyze the bacteria present on mobile phones and on the hands of their users.

The objective was to isolate the bacteria present and identify them through phenotypic and genotypic characterization and additionally, analyze them quantitatively.

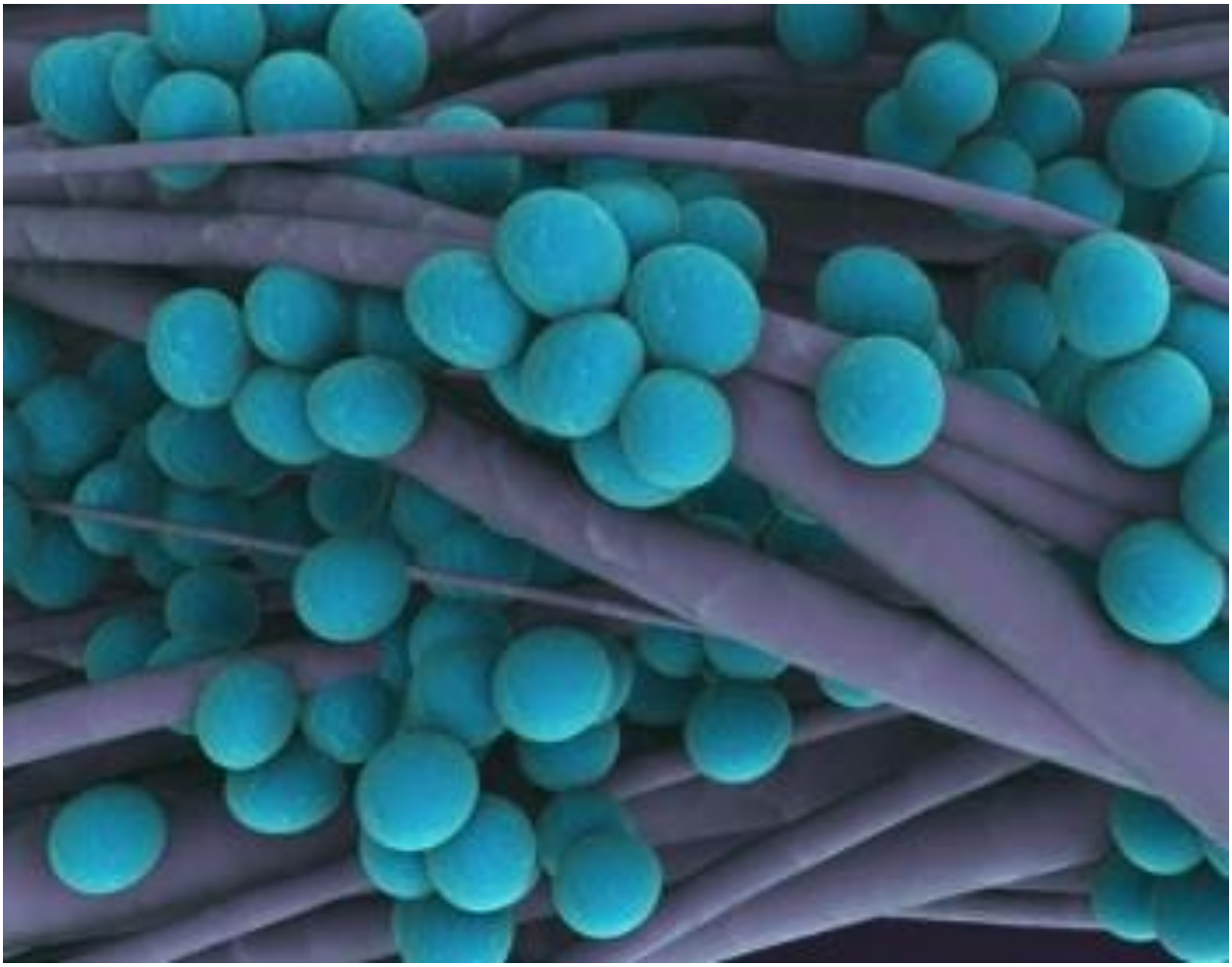
Subsequently their resistance to specific antibiotics and their multiresistance were verified, aiming to obtain the resistance patterns.

These results were analyzed in general, as well as by gender and when possible and found relevant by groups. As such, differences or similarities between genders were aimed to be obtained.

The results were Cross-reference with the individual characteristics of the participants, aiming to verify if there were any factors that contributed significantly to the bacterial counts obtained.

The study also intended to gentotipically characterize the MRSA found, in terms of presenting the *mecA* gene and consequently their SCC*mec* type, being classified in CA-MRSA or HA-MRSA.

III – MATERIALS AND METHODS



(http://www.nutrasilver.com/mrsa)

3 - Materials and Methods

3.1 – Sample collection

Sampling was performed on 30 different male and female students, who frequent 3 different courses at Instituto Superior de Ciências da Saúde Egas Moniz (ISCSEM). A duplicate was obtained for each participant, with the difference of a month as to attain a microflora as close to normal for each individual.

Information about each participant was collected through the submission of these participants to a questionnaire, inquiring about their personal hygiene habits and personal features that were found relevant for the research (appendix 1). The samples of the mobile phones were collected using the Count-Tact agar (CT) medium from bioMérieux®, which enables the detection and enumeration of all microorganisms present, since it is a nonselective nutritional medium [72]. This medium was then incubated at 30°C during 48 hours, as to permit the growth of all bacteria present in the environment. All the procedures that require a sterile environment to be carried out, were performed in a Horizontal Laminar Airflow Cabinet from Biobase®.

In order to obtain the samples from the hand of the subjects, a swab was inserted into a 15 mL sterile tube with 5mL of sterile buffered peptone water 0.1% (SBPW), and thus became soaked with the liquid in question. Subsequently, the hand that is predominantly used to operate the mobile phone was swabbed. The swabbing of the hand consisted in rubbing a sterile swap on the palm of the entire hand (including the digits), in between the fingers and under the fingernails. When the swabbing of the hand was completed, the swab returned to the tube containing SBPW. This set was then agitated by using the Universal Orbital Shaker OS-20 by Boeco®, during 20 minutes at a velocity of 120 RPM.

3.2 – Culture media

The samples obtained were submitted to a quantitative and microbiological analysis, using several culture media. The different culture media were used to account to the numerous diverse bacteria that can possibly be found on the hands of the participants. The culture media employed were the Trypto-casein soy agar (TSA) medium, Columbia agar + 5% horse blood (COH) medium, Mannitol salt agar (CHAP) medium and Drigalski agar (DRIG) medium.

3.2.1 – Trypto-casein soy agar

The Trypto-casein soy agar medium, by Biokar Diagnostics®, is a nonspecific medium it permits the isolation of almost all the microflora of a sample [73].

3.2.2 – Columbia blood agar

The Columbia blood agar medium provided by bioMérieux ® contains 5% of horse blood, in this case, with the purpose of identifying hemolytic bacteria, which cause the lysis of the erythrocytes present in blood. This medium is red in color and permits the growth of various fastidious microorganisms, whether able of hemolysis or not. The bacteria that were relevant to identify were *Staphylococcus aureus* and *Streptococcus pyogenes*, both exhibiting good growth and hemolysis, the first features cream colored colonies and the second pale straw colored colonies [74].

3.2.3 – Mannitol salt agar

The Mannitol salt agar medium, also known as Chapman medium, is a red colored agar and in this case provided by de manufacturer Oxoid®. This is a selective medium, therefore it is used to identify presumptive pathogenic staphylococci, inhibiting most microorganisms due to its high salt content. When the bacteria are possibly

pathogenic and consume the mannitol that is present in the medium, yellow colonies appear surrounded by a bright yellow halo (*Staphylococcus aureus*). However the pathogenicity can only be confirmed by a positive coagulase test, in which rabbit plasma is coagulated due to the conversion of fibrinogen into fibrin by *S. aureus* [49]. Contrarily, when the bacteria are nonpathogenic, the colonies appear to be pink with an unaltered color medium around them (*Staphylococcus epidermidis*) [75].

3.2.4 – Drigalski agar

The Drigalski agar medium is a medium by bioMérieux®, green in color and it is used for the isolation of *Enterobacteriaceae*, therefore being a selective medium (inhibition of Gram-positive bacteria). It is also a differential medium, since it allows the differentiation between the bacteria that use lactose from those that do not use it. *Escherichia coli*, *Proteus mirabilis*, *Salmonella typhimurium* and *Shigella flexneri*, appear as yellow colonies surrounded by yellow medium, blue-grey colonies with greenish center, again blue-grey colonies with greenish center and blue grey-colonies, respectively [76].

3.3 – Sample inoculation

One hundred microliters (100 µL) of the sample contained in the tube was spread in each medium until completely dried. The TSA medium was incubated at 30°C during 48 hours. The Columbia blood agar medium was equally incubated ate 30°C during 48 hours, however it was checked and the bacterial colonies were counted at 24 hours. The Mannitol salt agar medium was incubated at 37°C, during 48 hours, although it was also checked and a colony count was executed at 24 hours. The Drigaslki agar medium was equally incubated at 37°C, though only during 24 hours since it was sufficient to acquire colonies of sensible growth (figure 14 and 15). This temperature is used as to obtain all the bacteria present that are able to colonize the human body.

3.3.1 – chromID MRSA and chromID VRE sample inoculation

ChromID™ MRSA (MRSA) medium and chromID™ VRE (VRE) medium were used, both provided by the manufacturer bioMérieux®. The chromID™ MRSA medium is a reliable MRSA screening method, being selective for methicillin-resistant *S. aureus*, and is a pale, off-white colored medium. The MRSA appear as very distinctive, blue colonies, when resistant to this particular antibiotic [77].

The chromID™ VRE medium detects Vancomycin-resistant enterococci, and is a straw colored clear medium. It allows the presumptive identification of *Enterococcus feacium* and *Enterococcus feacalis*, indigo/purple colonies and light blue colonies, correspondingly [78].

A swab was emerged in the tube containing the sample and then rubbed throughout the media in order to spread out the bacteria in the complete area, until it was completely dry. These media were not submitted to a quantitative analysis as there was no specific volume of sample used, and it is most important to study them microbiologically in order to characterize them genetically, as they are of importance clinically and community wise. Both media were incubated at 37°C during 48 hours and only checked at 48 hours due to the slow development of the bacterial colonies.

3.4 – Microbiological and quantitative analysis

3.4.1 – Drigalski agar

Post 24 hours, the Digaslki agar medium was verified for colony presence and a complete count was executed. If colonies were encountered, they were submitted to an antibiotic susceptibility test resorting to the Mueller-Hinton 2 agar (MH2) medium. The antibiotics applied for the screening of the *Enterobacteriaceae* colonies found were Cefoxitin (FOX), Amoxicillin + Clavulanic Acid (AMC), Ceftazidime (CAZ), Ciprofloxacin

(CIP), Gentamicin (CN), Imipenem (IPM) and Cefotaxime (CTX), provided by Oxoid® and Bio-Rad® (appendix 2).

The bacteria found were also submitted to an indole test, which enables the acknowledgement if the bacteria encountered have the ability to convert tryptophan into indole. The bacteria is considered indole-positive when the broth presents a change in its upper layer to the color red/violet, after incubation, presumably facing an *Escherichia coli* bacteria (figure 12) [79].

Figure 12: Kovac's indole reaction (from left to right – blank, negative and positive), illustration adapted from [80].

3.4.2 – Columbia blood agar and mannitol salt agar

The Columbia Blood agar was examined for the total of bacteria and specifically for hemolytic bacteria, which were separately counted and registered. From these COH media, hemolytic colonies were selected and occasionally other relevant seeming bacteria were chosen for further susceptibility testing.

The bacterial colonies encountered in the Mannitol salt agar medium were counted and the yellow colonies with yellow medium surrounding them, were

specifically counted as they were presumably the pathogenic bacteria *S. aureus*. Seemingly *S. aureus* colonies and some *S. epidermidis* were selected.

All the colonies isolated in the MRSA and VRE media were selected to be studied for other antibiotic resistance. The antibiotics that were used for the susceptibility screening of these bacteria were Cefoxitin (FOX), Amoxicillin + Clavulanic Acid (AMC), Ceftazidime (CAZ), Clindamycin (DA), Erythromycin (E) and Oxacillin (OX), provided by Oxoid® and Bio-Rad® (appendix 2).

3.5 – Antibiotic screening

The susceptibility tests were performed in Mueller-Hinton 2 agar (MH2). This medium promotes the growth of non-fastidious bacteria, such as *Enterobacteriaceae*, non-fermenting Gram-negative bacilli, staphylococci and enterococci [81]. The Mueller-Hinton + 5% sheep blood (MHS), enhances the results by being specific for pneumococci and other streptococci, as they require blood for their growth. Both media were provided by Oxoid® and contain a low concentration of Thymine, which restricts the growth areas around the susceptibility disks and as such provide a more accurate measurement of the zones of inhibition [81].

After 24 hours of incubation, all the inhibition zones for the different antibiotics were measured. Bacteria were considered resistant or susceptible accordingly with the diameter of the inhibition area (appendix 2).

3.6 – Resistant bacteria conservation

Bacteria that presented resistance to one or more antibiotics were selected to be stored for possible future genetic analysis.

3.7 – *mecA* gene detection in presumptive Methicillin-resistant *S. aureus*

3.7.1 – DNA extraction

The DNA extraction was performed using the Invitek® RTP Bacteria DNA Mini Kit, accordingly to the manufacturer instructions (appendix 3).

3.7.2 – Polymerase chain reaction (PCR)

In order to detect the resistance to Methicillin on a genetic level, the gene *mecA* has to be detected in the bacteria's genome, which is characteristic of MRSA [50].

PCR (polymerase chain reaction) was performed [59]. PCR enables the generation of a large amount (over a billions of time the original fragment) of a particular chosen DNA sequence starting from a very small volume of DNA sample.

This DNA amplification method requires a set of essential components, such as primers (forward and reverse), a DNA polymerase, nucleotides (dNTPs) and a DNA sample to be amplified. The primers were a set of specific small DNA sequences that permit the restriction of the amplification to the targeted DNA sequence [82].

3.7.3 – DNA amplification

To proceed with the amplification of the targeted DNA, Ge Healthcare Life Sciences® PuRe-Taq ready-to-go PCR beads were used, which are 0.2 mL Eppendorfs containing a bead that already covers the polymerase enzyme (Taq DNA Polymerase), PCR buffer and the dNTPs [83]. To this mixture 5 µL of sample DNA extracted, 2.5 µL of each primer (table 2) and 40 µL of sterile distilled water (SDW) was added, to a final volume of 50 µL. To certify that there was no contamination when the procedure took place, a blank solution was necessary, which was made up of 45 µL of SDW and 2.5 µL of each primer, containing no DNA. Reactions took place in a MJ Mini Thermal Cycler (Bio-Rad®), and the program used is described in table 3.

Table 2: Primers used for the amplification of the *mecA* gene, adapted from [66].

Primer	*Sequence*
mecA F (forward)	5' – TCC AGA CAA CTT CAC CAG G – 3'
mecA R (reverse)	5' – CCA CTT CAT ATC TTG TAA CG – 3'

Table 3: Amplification program applied, adapted from [66].

Description	*Temperature*	*Time*	*Number of cycles*
Heating	94°C	4 minutes	1
Denaturing	94°C	30 seconds	30
Annealing	40°C	30 seconds	
Extending	72°C	1 minute	
Final extension	72°C	4 minutes	1

3.7.4 – Gel electrophoresis

PCR products were visualized in an agarose gel (2%) electrophoresis. The characteristic *mecA* band presents 162 bp [50].

The DNA marker used was the 25 bp DNA Step Ladder by Promega®, which permits the comparison of its bands to the fragment sizes obtained along the run.

The electrophoresis ran at a voltage of 80V during 60 to 90 minutes (PowerPac Basic Power Supply form Bio-Rad® and Labnet® Enduro Horizontal Gel Box were used) as to obtain a good band separation. Gel results were observed resorting to a UV gel documentation system (UV Transilluminator) which reveals the bands attained thus enabling the localization of the targeted band (162 bp).

3.8 – SCC*mec* typification

3.8.1 – Multiplex PCR

The previously extracted DNA was used to execute the multiplex PCR assay which was performed to attain the type of SCC*mec* element present in the MRSA that were formerly recognized as being *mecA* carriers. When this DNA was no longer viable, the DNA extraction procedure was performed by following the Invitek® protocol of RTP Bacteria DNA Mini Kit (appendix 3). The multiplex PCR assay enables the characterization of multiple DNA fragments that may be present in only one sample, in this case it permitted the characterization of the various components that may constitute the different types of the SCC*mec* element [66]. The components necessary and the manner of operation were the same as the conventional PCR, which was formerly described.

3.8.2 – DNA amplification

The amplification procedures were identical, thus for the sample preparation, Ge Healthcare Life Sciences® PuRe-Taq ready-to-go PCR beads were used [83]. To each Eppendorf, 7 µL of the DNA of the respective sample was added, followed by 2 µL of each primer (forward and reverse) described in table 4, minus the *mecA* primers, of which only 1 µL each was added. The *mecA* primers were added as to function as an internal control, to certify the presence of the *mecA* gene in the amplified samples. To make up the 50 µL solution, 5 µL of SDW was added.

To certify that there were no contaminations during the sample preparation, a blank solution was used which incorporated all the components previously stated, minus the DNA and instead of 5 µL of SDW, 12 µL were added to amount to the 50 µL volume. Consequently these samples, including the blank solution, were amplified resorting to a MJ Mini Thermal Cycler provided by Bio-Rad®, and the program used

was the same as the conventional PCR executed previously and is described in table 3.

Table 4: Primers used in the multiplex PCR assay applied for typifying different SCC*mec* types, adapted from [84].

Primer	Sequence (5' to 3')	Primer specificity	Fragment size (bp)
SCC*mec* I J1 F	TTCGAGTTGCTGATGAAGAAGG	SCC*mec* I J1 region	495
SCC*mec* I J1 R	ATTTACCACAAGGACTACCAGC		
SCC*mec* V *ccr*C F	GTACTCGTTACAATGTTTGG	SCC*mec* V *ccr* complex	449
SCC*mec* V *ccr*C R	ATAATGGCTTCATGCTTACC		
SCC*mec* III J3 F	TTCTTAAGTACACGCTGAATCG	SCC*mec* III J3 region	414
SCC*mec* III J3 R	ATGGAGATGAATTACAAGGG		
SCC*mec* V J1 F	TTCTCCATTCTTGTTCATCC	SCC*mec* V J1 region	377
SCC*mec* V J1 R	AGAGACTACTGACTTAAGTGG		
dcs F	CATCCTATGATAGCTTGGTC	SCC*mec* I, II, IV,VI J3 region	342
dsc R	CTAAATCATAGCCATGACCG		
***ccr*B2 F**	AGTTTCTCAGAATTCGAACG	SCC*mec* II, IV *ccr* complex	311
***ccr*B2 R**	CCGATATAGAAWGGGTTAGC		
SCC*mec* II J1 F	AATCATCTGCCATTGGTGATGC	SCC*mec* II J1 region	284
SCC*mec* II J1 R	CGAATGAAGTGAAAGAAAGTGG		
SCC*mec* III J1 F	CATTTGTGAAACACAGTACG	SCC*mec* III J1 region	243
SCC*mec* III J1 R	GTTATTGAGACTCCTAAACG		
***mecI* F**	ATCAAGACTTGCATTCAGGC	SCC*mec* II, III *mec* complex	209
***mecI* R**	GCGGTTTCAATTCACTTGTC		
***mecA* F**	TCCAGATTACAACTTCACCAGG	Internal control	162
***mecA* R**	CCACTTCATATCTTGTAACG		

F – Forward primer; R – Reverse primer.

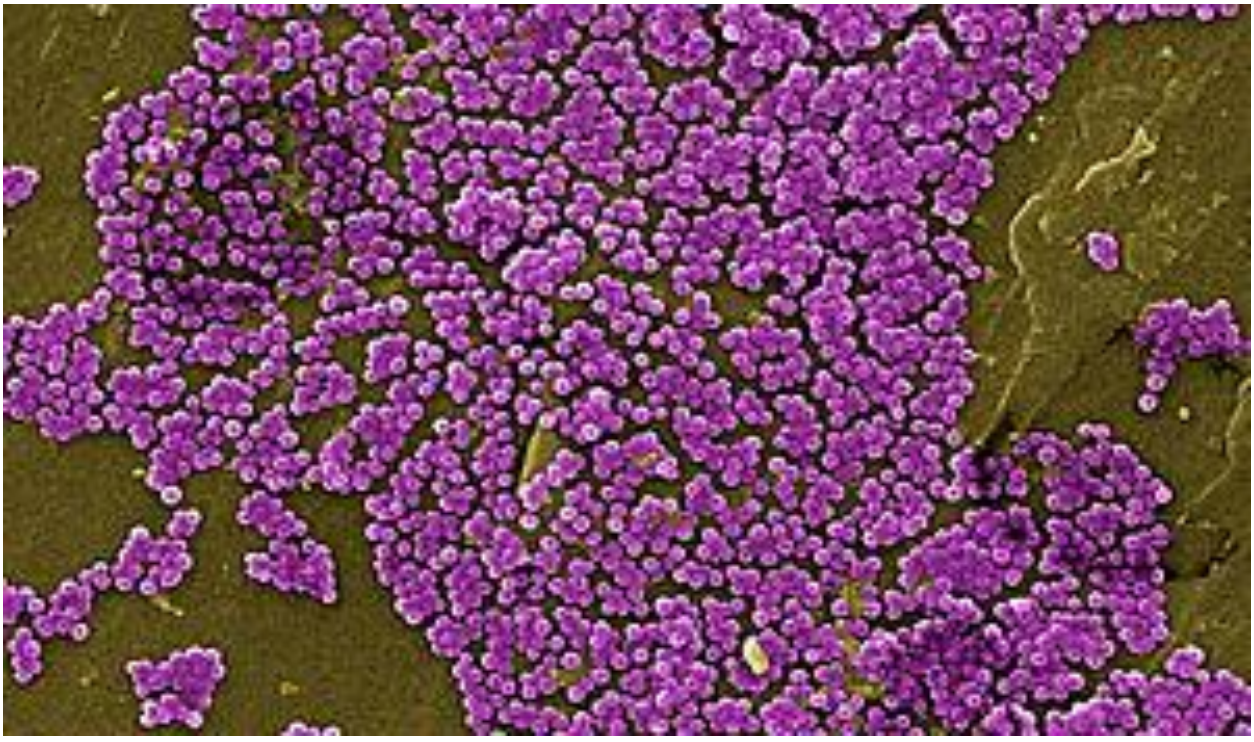
3.8.3 – Gel electrophoresis

The characterization and visualization of the bands of each type of the SCC*mec* elements were done in an agarose gel (2%) after electrophoresis.

The marker used was the GeneRuler Low Range DNA Ladder, Ready-to-Use 25 to 700 bp by Thermo Scientific®, which permits the comparison of its bands to the fragment sizes obtained along the run.

The electrophoresis ran at a voltage of 80V during 90 minutes as to obtain an optimal band separation. The results were observed through an UV gel documentation system (UV Transilluminator) which reveals the bands attained thus enables the determination of the localization of the SCC*mec* elements found and typify them.

IV – RESULTS AND DISCUSSION



(http://science.psu.edu/alert/photos/research-photos/biology/Read-MRSA-CDC.jpg)

4 – Results and discussion

Overall, 30 male and 30 female participants were recruited, 10 of each gender were selected from 3 different courses frequented at Instituto Superior de Ciências da Saúde Egas Moniz (ISCSEM). The participants were selected from the Pharmacy, Forensic Science and Nutrition courses. This reflected on the numbers attributed to each participant, being that the samples numbered 1 to 10 referred to the Pharmacy course, 11 to 20 referred to the Forensic Science course and the samples 21 to 30 referred to the Nutrition course participants. The symbols account to the differentiation of the male samples from the female samples were ♂ and ♀ correspondingly.

The questionnaire which was submitted to the participants and the respective consent form can be found in appendix 1. The questions were based on particular hygiene characteristics that could have the ability to influence the bacterial flora of an individual.

The results were using Microsoft® Excel as in the IBM® SPSS statistics software.

4.1 – Questionnaire analysis

It was found that the majority of the participants had a preference for the utilization of mobile phones with touchscreen (76.67% or 46/60) as opposed to phones with a keypad (23.33% or 14/60) (table 5). This is understandable as nowadays touchscreen mobile phones, more specifically smartphones, are trending and globally promoted, whereas mobile phones with keypad are not and only individuals that really prefer its use own one. It is also the case that the number of smartphones that are manufactured with a keypad is very limited [13].

When the second sampling occurred, it was verified that the number of participants that carried a touchscreen mobile phone increased, this was justified by the reasons previously explained. As such, the participants that carried touchscreen mobile phones increased to 80% (48/60) and the devices with a keypad were only used

by 20% (12/60) of the participants (table 6). These results diverge due to the switch of two participants from keypad mobile phones to the use of touchscreen devices.

It was also important to verify the results based on gender differences. This is mainly important as the general results only indicate an overall perspective and not a visualization of the quantitative analysis in terms of gender, which may present significant variations.

Analyzing the results of the first sample collection, a higher percentage of female participants presented mobile phones with touchscreen use, with a difference of 6.67% from the male candidates (table 5). In the second sampling, both genders increased the percentage of touchscreen mobile phone use, by 3.33% or 1 participant both (table 6). This act of switching to the use of these types of devices is explained by trending behavior in the general community [13]. The difference between touchscreen mobile phones and phones with a keypad in terms of bacterial count will be addressed further on, as it is relevant and interesting to understand which one has the higher affinity to the adherence of bacteria.

Table 5: Percentage of participants, by gender, in the first sampling that used touchscreen devices and mobile phones with a keypad.

Participants	**Type of keyboard (1st sampling)**	
	Touchscreen	Physical
Male	73.33% (22/30)	26.67% (8/30)
Female	80% (24/30)	20% (6/30)
General	76.67% (46/60)	23.33% (14/60)

Table 6: Percentage of participants, by gender, in the second sampling that used touchscreen devices and mobile phones with a keypad.

Participants	Type of keyboard (2nd sampling)	
	Touchscreen	Physical
Male	76.67% (23/30)	23.33% (7/30)
Female	83.33% (25/30)	16.67% (5/30)
General	80% (48/60)	20% (12/60)

The majority of participants had short nails (65% or 39/60) when the sampling occurred, followed by medium size nails (23.33% or 14/60) and a minor percentage has long nails (11.67% or 7/60) (table 7). The size of the nails could influence the amount of the bacteria present on the hands, since nails are a propitious contamination site and are not often cleaned in particular, except when physical and colored contamination can be felt or seen. If resistant bacteria were present, these could remain there throughout long periods of time [42].

The majority of the male candidates presented short nails (76.67%) as opposed to approximately only half of the female participants (53.33%). The medium sized nails had the same percentage of participants and long nails were only verified in 7 of the 30 women submitted to sampling (table 7). Long nails can be problematic, as they are a good reservoir for potential pathogenic bacteria, especially when not thoroughly and frequently cleaned. Even a meticulously hand wash does not imply that the nails are well cleaned [42]. Long nails are not permitted in healthcare environments and even in culinary professions as for safety reasons, such a possible contamination [85][44]. There are cases of healthcare professionals that were tested in terms of bacteria under long nails, and the results presented were preoccupying [44]. As such, the comparison in terms of bacterial count based on nail length will be approached later.

Table 7: Percentage of participants, by gender, that presented short, medium and long nails.

Participants	Nails size		
	Short	Medium	Long
Male	76.67% (23/30)	23.33% (7/30)	0% (0/30)
Female	53.33% (16/30)	23.33% (7/30)	23.33% (7/30)
General	65% (39/60)	23.33% (14/60)	11.67% (7/60)

The majority of the participants had no manicure (73.33% or 44/60), but a small percentage presented manicured nails (26.67% or 16/60) (table 8). The presence of a manicure could lead to an enhanced attachment of bacterial cells. A very low percentage of the participants had rings on their fingers (13.33% or 8/60) and the majority had none (86.67% or 52/60) (table 9). This is an important factor, seeing that rings, just like the nails, are an area where bacteria tend to adhere and as such, accumulate and remain present. It is known in culinary that, when preparing food, it is advised and even a hygienic standard to take of your rings, keep short and no manicured nails as to prevent possible contamination of the food [85].

This is even more important in healthcare setting as the professionals come in close contact with their patients. Healthcare workers are not allowed to carry jewelry when practicing. Their nails are required to be short and without any sort of manicure [44].

The presence of a manicure was only verified in the female candidates, and it was almost equally divided (table 8). Painted nails probably only influence the nail hygiene on a level where the individual is not aware of the contamination that may be present and therefore would not clean them as much. Another aspect were the artificial nails, which are glued on and they can accumulate bacteria. As this kind of nails stay on for a long amount of time, and probably the individual would not clean their nails as much for the reason stated before, these present a threat in terms of hygiene and pathogenic bacteria [85][86].

The presence of rings was only verified in female candidates and in a low percentage (26.67%) (table 9). Rings can be an important source of contamination as mentioned previously, and therefore were also swabbed during the sampling.

Table 8: Percentage of participants, by gender, that presented and did not present manicured nails.

Participants	Presence of manicure	
	Yes	No
Male	0% (0/30)	100% (30/30)
Female	53.33% (16/30)	46.67% (14/30)
General	26.67% (16/60)	73.33% (44/60)

Table 9: Percentage of participants, by gender, that presented and did not present rings on their examined hand.

Participants	Presence of rings	
	Yes	No
Male	0% (0/30)	100% (30/30)
Female	26.67% (8/30)	73.33% (22/30)
General	13.33% (8/60)	86.67% (52/60)

It was verified that the majority of the participants stated that they paid a special attention to the hygiene of their hands (55% or 33/60), whereas 45% stated that they did not (27/60) (table 10). The positive answers included the use of disinfectant and cleaning of the nails. It has also become apparent that the majority of the participants do not have a special attention with the hygiene of their mobile phones (73.33% or 44/60) with 26.67% (16/60) that do pay a special attention such as disinfecting their screen with alcohol or cleaning it with a cloth (table 11).

A study constructed by Bhat and co-workers has revealed that only 6% of healthcare professionals disinfect their mobile phones [7]. Comparing with the results

obtained in this work, it is dangerously low as they pose a higher threat in terms of transmission of pathogenic and multiresistant bacteria.

A special attention to the hygiene of hands and more specifically nails should be paid, for all the reasons explained previously. A lower percentage of male participants (43.33%) pay this kind of attention, although higher than expected. On the contrary, a higher percentage of the female participants employ this special attention (66.67%) (table 10). Aside de difference in numbers, the types of special attention paid in both genders were the same, such as use of disinfectant and cleaning the nails.

A low percentage in both genders admitted that they do pay a kind of special attention to the hygiene of their phones, whilst a higher percentage did not (table 11). By a percentage of 6.67% the female participants pay greater attention to the cleaning of their phones. Although, as was the case formerly stated, the methods used for the maintenance of the clean conditions of the phones were the same in both genders, such as disinfectant and cleaning cloth.

Table 10: Percentage of participants, by gender, that paid and did not pay any special attention to the hygiene of their hands and/or nails.

Participants	**Special hygienic attention to hands/nails**	
	Yes	No
Male	43.33% (13/30)	56.67% (17/30)
Female	66.67% (20/30)	33.33% (10/30)
General	55% (33/60)	45% (27/60)

Table 11: Percentage of participants, by gender, that paid and did not pay any special attention to the hygiene of their mobile phones.

Participants	Special hygienic attention to mobile phone	
	Yes	No
Male	23.33% (7/30)	76.67% (23/30)
Female	30% (9/30)	70% (21/30)
General	26.67% (16/60)	73.33% (44/60)

All the participants indicated that they wash their hands (utilization of soap or any cleansing agent is implied) after using the bathroom (table 12). Although this does not mean that the hands were washed properly, since a minimum time of 1 minute and overall scrubbing is necessary for a thorough cleanse [87].

All the male and female participants admitted to washing their hands after going to the bathroom (table 12).

Table 12: Percentage of participants, by gender, that washed and did not wash their hands after the use of the bathroom.

Participants	Hand washing after bathroom use	
	Yes	No
Male	100% (30/30)	0% (0/30)
Female	100% (30/30)	0% (0/30)
General	100% (60/60)	0% (0/60)

Another important factor to be held in to consideration was the presence of mobile phones when going to the bathroom, and most participants (61.67% or 37/60) indicated that they took it with them, and only 38.33% (23/60) stated that they did not (table 13). This practice can be concerning seeing as the mobile phones can be left in various places of the bathroom, even on top of the toilet flush or its cover which could

be harboring various pathogenic bacteria (ex. *E. coli*), this being more concerning when public bathrooms come into mention [88].

Taking a mobile phone to the bathroom is not advised as it could possibly facilitate the adherence of pathogenic bacteria, as referenced previously. Although the majority of the participants stated that they take their mobile phones to the bathroom, the number were not as high as expected [43]. The differences between the male and female participants were not significant (table 13), but the bacterial count between the participants who took their mobile phones to the bathroom and who did not will be addressed further on.

A survey conducted by 11Mark has shown that approximately 75% of participants in America take their mobile phone to the bathroom, which is significantly higher than the percentage obtained in this study. A higher percentage of men (30%) stated that they would not go to the bathroom without their mobile phone, and 20% of the women [89]. This tendency was not verified in table 13, as both genders presented close values (difference of 1 participant).

Table 13: Percentage of participants, by gender, that brought and did not bring their mobile phones with them when using the bathroom.

Participants	**Bring mobile phone to the bathroom**	
	Yes	No
Male	60% (18/30)	40% (12/30)
Female	63.33% (19/30)	36.67% or (11/30)
General	61.67% (37/60)	38.33% (23/60)

Another factor that is important to the transmission of bacteria is nail biting and biting of the surrounding skin [42]. The numbers were close, since 46.67% (28/60) stated that they do bite their nails/skin and 53.33% (32/60) do not (table 14). This is important due to the bacteria that reside under de nails of an individual and by biting the nails, these bacteria will end up in the oral cavity and if pathogenic, possibly could

cause disease. The behavior of biting nails also exposes various layers of the nail and as such create irregular ridges, which may facilitate the adhesion and permanence of the bacteria in those sites [42].

More than half of the male participants stated that they bite their nails/skin, whereas 20% less of the female participants revealed this habit (table 14). It is a significant difference, and it was expected that males are more prone to this kind of habit. The downsides to this kind of behavior have been formerly presented, and will be analyzed in comparison to the bacterial count.

A study conducted by Ghanizadeh about nail biting, states that only 21.5% of male adults bite their nails [90]. This is a behavior of which its prevalence decreases with age, being predominantly present in children. It was also verified that a higher percentage of males bite their nails than women [90]. The values obtained in this study are discrepancies, as they are drastically higher. Anxiety was thought to be the origin for nail biting, however this research indicates boredom and working on difficult problems [90]. As the individuals who participated in this study were students, this may account for the high percentages obtained.

Table 14: Percentage of participants, by gender, that bit and did not bite their nails and/or surrounding skin.

Participants	**Biting nails/skin**	
	Yes	No
Male	56.67% (17/30)	43.33% (13/30)
Female	36.67% (11/30)	63.33% (19/30)
General	46.67% (28/60)	53.33% (32/60)

The presence of pets in a household also contributes to its hygiene, being that pets can carry bacteria that does not harm them but possibly could harm humans. [91][92] Most candidates do have pets 60% (36/60) and may be more susceptible to bacterial presence than the remaining 40% (table 15). The pets indicated were cats,

dogs, rodents, turtles and birds, some of them could carry bacteria that could be pathogenic for their owners, making this another significant factor to be held into consideration due to the close contact between them [93][94].

Regarding to the presence of pets in the household of the participants, it was noticed that in both genders a higher percentage were pet owners, although with a 13.34% of difference (higher for the male candidates) (table 15). The potential risks associated with the ownership of pets were stated previously, although this will be verified against a bacterial count.

Table 15: Percentage of participants, by gender, that presented and did not present the presence of pets in their household.

Participants	Presence of pets in household	
	Yes	No
Male	66.67% (20/30)	33.33% (10/30)
Female	53.33% (16/30)	46.67% (14/30)
General	60% (36/60)	40% (24/60)

Possibly the most relevant factor was how many times a day do the participants wash their hands, the options were <5x, 5-10x, 10-15x and >15x, with the results being 13.33% (8/60), 60% (36/60), 20% (12/60) and 6.67% (4/60), correspondingly (table 16). Note that the majority washes their hands 5-10x a day, which is not that much as expected, considering meal times and going to the bathroom [95]. Once again, this does not imply that the hand were properly washed, thus a significant reduction of bacterial presence may not have happened.

The majority of the participants washed their hands 5 to 10 times a day. A higher percentage of male candidates washed their hands less than 5 times a day, in comparison to the female participants. This also occurs for the 10 to 15 times of hand washing a day, although the difference was not that meaningful. None of the male

participants answered that they wash their hands more than 15 times a day, whereas 13.33% of the female participants do wash their hands this frequently (table 16).

A study elaborated by Larson and co-workers presented an average of hand washing times a day between 10 and 13, but can differ greatly as standard deviations were found between ±5 and ±10 [95]. However, the results obtained in this work show that the majority tend to the 5 to 10 times a day.

Table 16: Percentage of participants, by gender, that washed their hands less than 5 times, between 5 and 10 times, between 10 and 15 times and more than 15 times a day.

Participants	**Number of hand washes a day**			
	<5x	5-10x	10-15x	>15x
Male	20% (6/30)	56.67% (17/30)	23.33% (7/30)	0% (0/30)
Female	6.67% (2/30)	63.33% (19/30)	16.67 (5/30)	13.33% (4/30)
General	13.33% (8/60)	60% (36/60)	20% (12/60)	6.67% (4/60)

4.2 – Microbiological analysis

It was assumed that the bacteria present on the hands, were the bacteria that also reside on the mobile phones of the participants, since both are in frequent contact throughout the day. The sampling was executed in duplicate and the mean and median were used for interpretation.

The results obtained from the selective culture media employed, thus Columbia blood agar + 5% horse blood, Mannitol salt agar, Digalski, chromID MRSA and chromID VRE, differed greatly. The Count-tact and Trypto-casein soy agar media, demonstrated growth in a 100% of the participants. These both media were used as to attain the total number of bacterial colonies present on the mobile phones and on the hands of the participants, respectively. These bacterial counts and the bacterial range varied considerably as can be perceived in figure 13 and 14. Since both media permit the

growth of all the bacteria possibly present, it was assumed that the bacteria present on the hands of the participants, were the same that were present on the mobile phone due to frequent contact throughout the day. Therefore, only the hands samples were analyzed by using selective media.

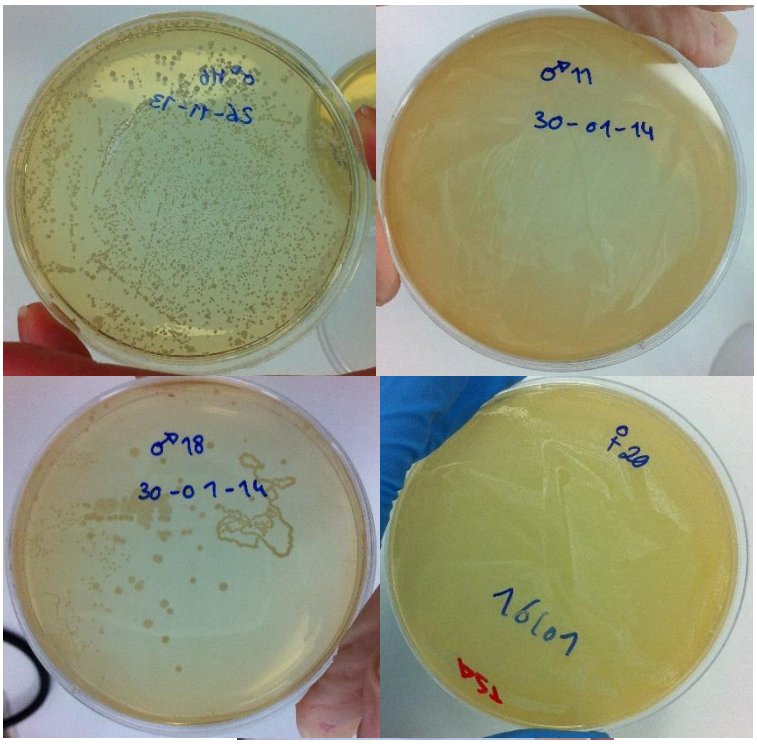


Figure 13: Various TSA media analyzed with different results.

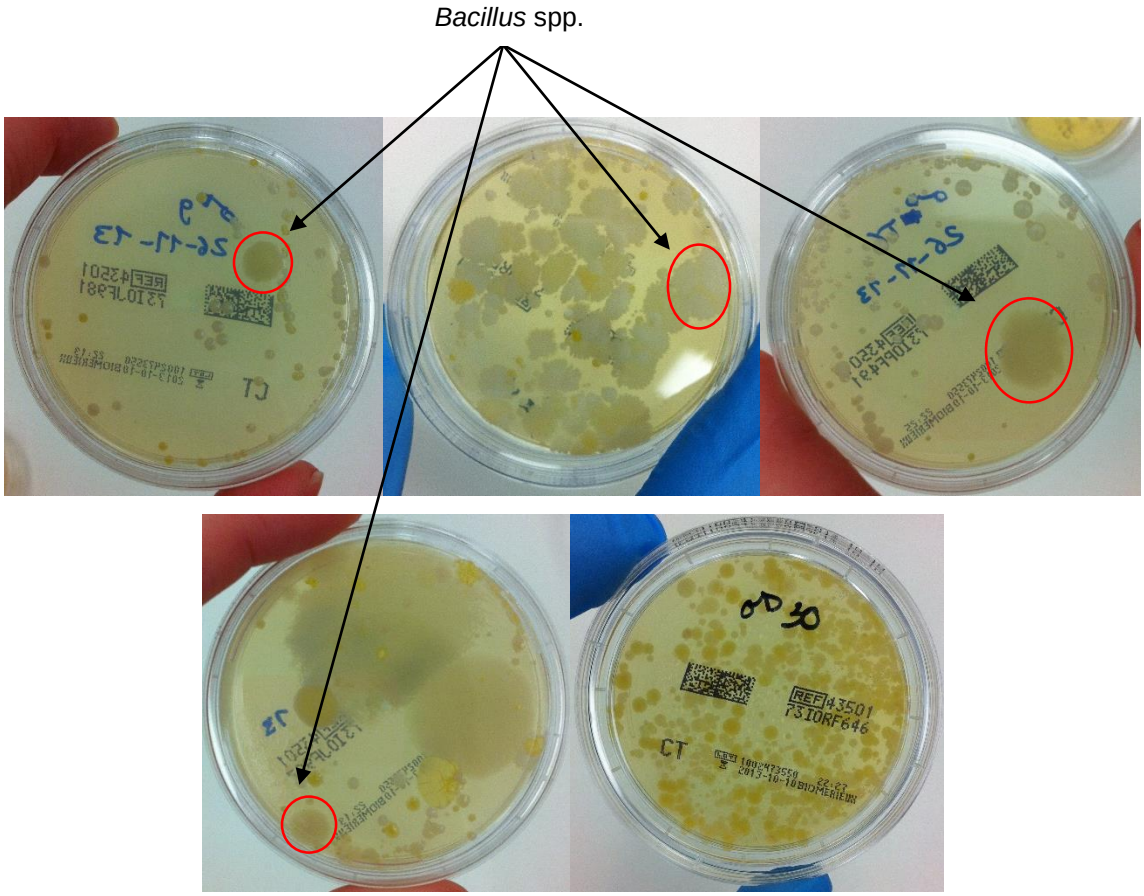


Figure 14: Various CT media analyzed with different results.

4.2.1 – Columbia blood agar and Mannitol salt agar medium

In both genders the presence of *Staphylococcus aureus* and *S. epidermidis*, was observed. These bacteria are Gram-positive, and *S. epidermidis* is the major constituent of the commensal flora of the human skin and mucous membrane, being found all over the body. Although, this bacteria is very capable of developing infection, causing persistent and recurrent disease, especially in hospital settings with patients that present themselves connected to invasive medical equipment [96].

This bacteria is a coagulase-negative staphylococci (CoNS), as opposed to the *S. aureus* bacteria which is coagulase-positive, meaning that when incubated in rabbit plasma, this plasma becomes coagulated due to the conversion of fibrinogen into fibrin by the bacteria [49]. This presumptive test permits the differentiation between these two bacteria [97].

In some participants *Staphylococcus aureus* were detected on the hands as a pure culture (figure 15 and 16).

Staphylococcus aureus bacteria can be divided into two groups, MRSA and MSSA, which means Methicillin-resistant *S. aureus* and Methicillin-sensitive *S. aureus*, being that the first is relevant for this study as it has clinical importance. It is the most important pathogenic staphylococci, especially in terms of hospital acquired infections [96] [98].

Hemolytic bacteria were detected in a great number of participants in both genders.

Figure 15 and 16: Mannitol salt agar medium with *S. epidermidis* colonies (pinkish colonies and red medium) and *S. aureus* colonies (yellow colonies with yellow medium; Mannitol salt agar medium with pure *S. aureus* colonies.

4.2.2 – Drigalski agar medium

In the Drigalski culture media, only one of the samples presented growth, resulting in 2 CFU. This medium permits the growth of *Enterobacteriaceae* (Gram-negative bacteria), being that yellow colonies with yellow medium surrounding were found (figure 17). The presence *Escherichia coli* was confirmed by an indole test, which is a presumptive test for the presence of this bacteria (figure 18).

The *E. coli* bacteria is part of the human and mammal intestinal flora, where its work is beneficial to the organisms themselves. However, there are strains that are largely capable of developing gastrointestinal diseases. *E. coli* is one of the most versatile bacteria and it is greatly used in laboratory research. These bacteria can acquire various different virulence factors which can persist successfully and eventually cause disease in healthy individuals [99].

One of the most well-known and possibly dangerous strains is an enterohemorrhagic serotype of the bacterium *E. coli*, also known as O157:H7. This bacteria is toxin producing and can be transmitted through contaminated food which is undercooked and fecal-oral transmission [100]. The presence of these bacteria on the hands of the participant possibly means the presence of fecal matter, which can be problematic and indicates that the hands were not properly washed, as there are considered indicators of hand hygiene [101].

Figure 17: *Enterobacteriaceae* (Gram-negative bacteria) present in the Drigaslki medium.

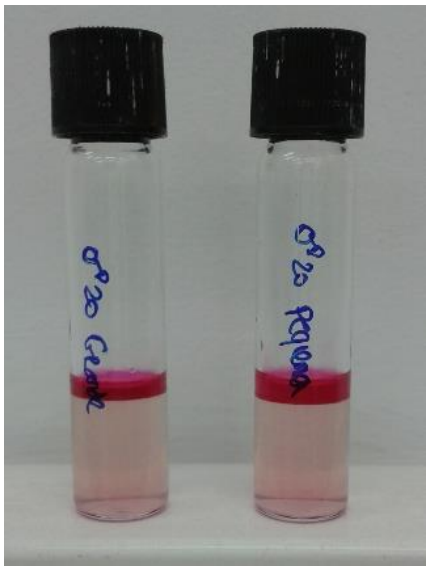


Figure 18: Positive indole test of the *Enterobacteriaceae* found in the Drigalski medium.

4.2.3 – chromID MRSA medium

In the chromID MRSA medium blue/green colonies were found which indicate the growth of Methicillin-resistant *S. aureus* (figure 19). This medium is selective for

MRSA and these bacteria are very common in healthcare environments. This strain of *S. aureus* is resistant to the commonly used antibiotics and the consequences of developing a serious infection because of these bacteria could be problematic.

It is therefore very important to prevent transmission and contamination with these bacteria, especially in immunocompromised individuals. Although healthy individuals may carry the bacteria for several years asymptomatically, not developing disease [47][50]. The bacteria colonizes most frequently the respiratory tract, open wounds, urinary tract and indwelling catheters [102].

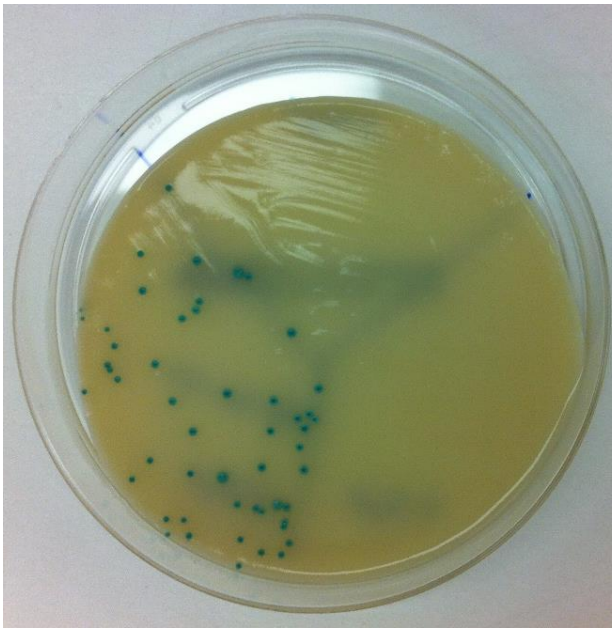


Figure 19: Methicillin-resistant *S. aureus* present in chromID MRSA medium.

4.2.4 – chromID VRE medium

There was no growth in the chromID VRE medium in any of the samples. The Vancomycin-resistant enterococci, as the MRSA, are mostly acquired in healthcare environments and are resistant to a wide range of antibiotics besides Vancomycin. *Enterococcus* are normally a part of the human intestinal tract, acting in a beneficial way, becoming dangerous when VRE are present [103][104].

4.2.5 – Data analysis

The bacteria that were found were, *Bacillus* spp. on both the mobile phone and on the hands, hemolytic bacteria, *Staphylococcus* spp., *Staphylococcus aureus*, *Enterobacteriaceae* and Methicillin-resistant *S. aureus*.

The *Bacillus* spp. were clearly detected in the mobile phone Count-tact media as they are significantly different from the other colonies observed (figure 14). *Bacillus* spp. were more frequently present on the mobile phones than on the hands of their handlers. Being that the percentage of participants that presented *Bacillus* spp. on the devices were 28% in both genders, whilst on the hands, 13% of the male participants presented this bacteria and 2% of the female participants.

The distribution of the presence of *Bacillus* spp. on the mobile phones of both genders analyzed by course were similar (table 17). A higher number of participants of the Nutrition course presented this bacteria on their mobile phones, 35% in both genders.

In terms of *Bacillus* spp. presence on the hands, in all the courses there were male participants that presented this bacteria, whilst only in the Nutrition course it was detected in the female participants. When compared to the other courses, a larger number of male participants of the Nutrition course presented *Bacillus* spp.

The *Bacillus* spp. bacteria were not identified on a species level in this research, although there are two species that are medically significant. *Bacillus cereus* and *Bacillus anthracis* which cause food poisoning and anthrax, respectively. They are both potentially dangerous, being the last referred the worst. They are widely found in soil and water [105][106]. These bacteria can be found when excessive hand washing occurs and when individuals present long and artificial nails [44].

When observing the results for the hemolytic bacteria, it was noticed that both genders had the same percentage of participants who presented growth of this type of bacteria (82%). It was notable that a considerably lower number of both male and female participants presented hemolytic bacteria in the Pharmacy course, 50% and

60% respectively. The other courses exhibited 90% or more of its participants with hemolytic bacteria presence. The Forensics course was the one in which a higher percentage of male and female participants who presented this type of bacteria, 100% and 95% correspondingly.

In both genders and throughout all the courses a very high percentage of participants presented *Staphylococcus* spp., none being lower than 90%. In terms of *Staphylococcus aureus*, a considerably higher percentage of male participants had the presence of these bacteria on their hands (92%), contrasting with 72% of the female participants. The distribution between the courses in both genders, are very similar, not posing any significant information.

The presence of *Enterobacteriaceae* was very scarce, only one male participant between 60 participants in total (1%) presented growth of this bacteria in the Drigaslki medium. This was considered positive as these bacteria can lead to serious illness when not properly treated. This type of bacteria is a normal part of the intestinal flora found in humans and other animals. However, others can be found in water or soil, or can be parasites on a variety of different animals and plants [100][99].

MRSA were mainly found in the male participants, in a very low percentage (10% overall). They were mainly detected in the male Pharmacy participants (25%), none detected in the Forensics participants and in one of the Nutrition participants. Only one of the female participants presented the presence of this bacteria in one of the samplings, frequenting the Forensics course. The females of the other two courses did not present any MRSA growth. *Staphylococcus* spp., are mainly found on the skin or in the nose of individuals, and sometimes healthy individuals. They can also be found in the general environment [70].

The low presence of MRSA was considered positive as these bacteria have the potential to be highly dangerous and in some cases even lead to death [70]. Most probably these MRSA were CA-MRSA, which means they are community acquired and

are not considered multiresistant to antibiotics, thus less persistent and normally do not have this severe consequences [45].

In the study developed by Andrej and co-workers, percentages of bacterial presence were shown for mobile phones. As it was assumed that the bacteria that are on the phones are the same as on the hands, these may serve as an example. A growth of *Staphylococcus* spp. was verified in 57% to 60% of the students, *Bacillus* spp. in 0% to 17% and *Enterobacteriaceae* in 23% to 53% of the participants [2]. However, Andrej stated that almost 50% of users of public toilets so not wash their hands in Slovenia, which was not verified in this study (table 12), which may account for the low percentages obtained for these bacteria. Relatively to the presence of *Staphylococcus* spp., it was considerably higher throughout this work when compared to Andrej's study. When comparing the results for *Bacillus* spp., on the mobile phones they were higher (+11% compared to the higher value of the study) but the hand samples showed the same tendency (table 17).

Table 17: Percentage of participants, by gender and by course, that presented colonies of *Bacillus* spp., hemolytic bacteria, *Staphylococcus* spp., *S. aureus*, *Enterobacteriaceae* and Methicillin-resistant *S. aureus* on their hands.

♂ *Participants*	*Bacillus*[1] *spp.*	*Bacillus*[2] *spp.*	*Hemolytic Bacteria*	*Staphylococcus spp.*	*S. aureus*	*Enterobacteriaceae*	*MRSA*
Pharmacy	(5/20)	(1/20)	(10/20)	(20/20)	(19/20)	(0/20)	(5/20)
(%)	25	5	50	100	95	0	25
Forensics	(5/20)	(2/20)	(20/20)	(19/20)	(18/20)	(1/20)	(0/20)
(%)	25	10	100	95	90	5	0
Nutrition	(7/20)	(5/20)	(19/20)	(20/20)	(18/20)	(0/20)	(1/20)
(%)	35	25	95	100	90	0	5
Total	(17/60)	(8/60)	(49/60)	(59/60)	(55/60)	(1/60)	(6/60)
(%)	28	13	82	98	92	2	10
♀ *Participants*	*Bacillus*[1] *spp.*	*Bacillus*[2] *spp.*	*Hemolytic Bacteria*	*Staphylococcus spp.*	*S. aureus*	*Enterobacteriaceae*	*MRSA*
Pharmacy	(6/20)	(0/20)	(12/20)	(18/20)	(13/20)	(0/20)	(0/20)
(%)	30	0	60	90	65	0	0
Forensics	(4/20)	(0/20)	(19/20)	(20/20)	(15/20)	(0/20)	(1/20)
(%)	20	0	95	100	75	0	5
Nutrition	(7/20)	(1/20)	(18/20)	(19/20)	(15/20)	(0/20)	(0/20)
(%)	35	5	90	95	75	0	0
Total	(17/60)	(1/60)	(49/60)	(57/60)	(43/60)	(0/60)	(1/60)
(%)	28	2	82	95	72	0	2
Overall Total	(34/120)	(9/120)	(98/120)	(116/120)	(98/120)	(1/120)	(7/120)
(%)	28	7.5	82	96.5	82	1	6

[1] – *Bacillus* spp. found in the count-tact medium (mobile phone samples); [2] – *Bacillus* spp. found in the TSA medium (hand samples).

4.2.6 – Quantitative analysis

A quantitative analysis of the results obtained allows an overview of the hygiene of the participants and if the numbers attained are of a preoccupying significance. The participants that presented bacterial counts higher than $1x10^3$ CFU in the culture media, i.e. those who could not be counted, were assumed as $5x10^4$ CFU (1000x50 as 100 µL

of 5 mL was used in each media). The bacterial counts represent the palm surface, the surface between the fingers and under the nails of the hand that is most used in mobile phone handling.

4.2.6.1 – Quantitative analysis by gender and course

As to interpret the results in an adequate manner, the mean for each course was considered for each gender.

It can be observed in table 18 that the number of CFU per cm^2 in the mobile phones of male participants is relatively close, especially between the Pharmacy and Forensics course, being that the Nutrition course only shows a difference of 0.48-0.49 CFU per cm^2.

The bacterial counts of all the mobile phone samples were significantly higher when compared to previous studies. Andrej and co-workers obtained more than 0.05 CFU per cm^2 in 90% of 90 student samples, and the lowest obtained in this study was 0,17 CFU per cm^2. *Bacillus* spp. were mainly found on the phones of the students of the food sciences, this was not verified in this study in terms of mobile phones but in terms of the hands this tendency is verified [2].

Andrej obtained an average of 1,51 CFU and 1,05 CFU per cm^2 for keypad and touchscreen, respectively [2]. These counts are relatively low when compared to the majority of the counts obtained in this study. However, the sampling methods were different as Andrej and co-workers used swabs, and in this study count-tact agar methods were used.

The *Bacillus* spp. detection on the mobile phones was very scarce when perceived in cm^2. Many of the participants did not present any *Bacillus* spp. whereas other exhibited a large quantity (ex. 7.76 CFU per cm^2 in one sample). The lowest detection was in the Nutrition course with 0.012 CFU per cm^2.

The normal bacterial count on healthcare worker's hands ranges between $3.9x10^4$ to $4.6x10^6$ CFU per cm^2 [40]. The size of the hands of participants varied greatly and these were not taken into account in this study, additionally only the palm, between fingers and the skin under the nails were swabbed. As such, the numbers cannot be compared to the normal bacterial count previously presented, although this gives us an idea of the extension of colonization by bacteria that our hands can contain. Another fact to take in to account is that fingertip contamination can reach from 0 to 300 CFU when sampled by agar contact methods [2].

A very high overall bacterial count was verified on the hands of the Pharmacy participants with $\pm 8x10^3$ CFU in difference from the Forensics course. The nutrition course is again the course in which the participants presented a lower CFU number.

Bacillus spp. were highly present in the hand samples of the Nutrition participants, less in the Forensics course and scarcely in the Pharmacy participants. Since they are widely found in water and soil, these could be the potential contamination source.

Hemolytic bacteria were predominantly found in the Forensics participants, and scarcely found in the Pharmacy course.

Staphylococcus spp. were abundantly found in the Pharmacy participants, followed by the Nutrition course and lastly the Forensics course. This could imply that many participants of the pharmacy course carry *Staphylococcus* asymptomatically, or they simply have a large presence of these bacteria on their skin.

S. aureus follows the tendency previously presented for *Staphylococcus* spp., and again this could mean that they are carried asymptomatically, since none presented illness. This could also mean that they could have had any sores or cuts, as many of the male participants indicated that they bit their nails and/or surrounding skin, the last referred to being more propitious to causing wounds.

It could be verified that the Pharmacy course had the participants that carried a larger number of bacteria on their hands and also the ones that are potentially

pathogenic. This was also the course that presented the majority of the MRSA that were found in this study (5/7), where one presented MRSA in both samplings.

Table 18: Number of CFU presented by the male participants, by course and sampling, overall and *Bacillus* spp. on the mobile phones, and overall, *Bacillus* spp., hemolytic bacteria, *Staphylococcus* spp. and *S. aureus* on the sampled hands.

♂ *Pharmacy*	*Overall MP (CFU/cm²)*	*Bacillus[1] spp. (CFU/cm²)*	*Overall H (CFU)*	*Bacillus[2] spp. (CFU)*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
1st Sampling average	2.52	0	$5x10^3$	0	49	2520	$1.42x10^3$
2nd Sampling average	2.74	0.06	$2.98x10^4$	5	140	$6.3x10^3$	$5.29x10^3$
Mean	2.63	0.03	$1.74x10^4$	2.5	94	$4.41x10^3$	$3.36x10^3$
♂ *Forensics*	*Overall MP (CFU/cm²)*	*Bacillus[1] spp. (CFU/cm²)*	*Overall H (CFU)*	*Bacillus[2] spp.*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
1st Sampling average	3.25	0.004	$1.68x10^4$	0	$5.94x10^3$	$1.14x10^3$	320
2nd Sampling average	1.98	0.15	$1.73x10^3$	25	$1.01x10^3$	730	385
Mean	2.62	0.08	$9.27x10^3$	12.5	$3.47x10^3$	933	353
♂ *Nutrition*	*Overall MP (CFU/cm²)*	*Bacillus[1] spp. (CFU/cm²)*	*Overall H (CFU)*	*Bacillus[2] spp. (CFU)*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
1st Sampling average	2.34	0.013	770	110	800	630	260
2nd Sampling average	1.94	0.012	$2.18x10^3$	5	$2.7x10^3$	$3.6x10^3$	$3.36x10^3$
Mean	2.14	0.012	$1.47x10^3$	57.5	$1.75x10^3$	$2.12x10^3$	$1.81x10^3$

[1] – *Bacillus* spp. found in the count-tact medium (mobile phone samples); [2] – *Bacillus* spp. found in the TSA medium (hand samples); MP – Mobile phone sample; H – hand sample; CFU – Colony forming units.

The variation presented by the female participants in terms of bacterial count present on the mobile phones varied 0.49 CFU per cm^2, just as verified in the male participants. However the Forensics participants were the ones that presented the higher value, 1.74 CFU per cm^2 (table 19). The bacterial count obtained were again higher than the value presented by Andrej and co-workers.

Bacillus spp. were scarcely found on the mobile phones throughout the courses, and the highest number was presented by the pharmacy course (0.03 CFU per cm^2), exactly the same number as the male participants of this course. The *Bacillus* spp. value for the Nutrition course in both genders was also similar, although the Forensics course varied greatly from maximum value in the male participants to minimum value in the female participants.

In terms of overall bacterial count of the hands, the Forensics participants presented a considerably larger number, followed by the Pharmacy course and then the Nutrition participants. In the male participants was also verified that the Nutrition course had the lowest CFU values, although it is even lower than the number observed in the female participants ($1.47x10^3$ vs. $2.58x10^3$) (table 19). The largest CFU value presented by the male courses was $1.74x10^4$ CFU (Pharmacy) and the highest value in the female participants was $4.62x10^3$ which is a big contrast. The largest contrast is between courses, within the male participants ($1.74x10^4$ vs. $1.47x10^3$).

The bacterial counts encountered in this study were considerably lower than those stated by the WHO (lowest $3.9x10^3$ per cm^2), as the highest value that was found was $2.98x10^4$ and this for the entirely swabbed area.

Bacillus spp. found on the hands were very uncommon in the female participants, and only found in the Nutrition course. Although compared to the male participants, the CFU value is considerably lower in the female participants if not scarce in total.

The Forensics course and Nutrition course present both a relatively large CFU counts in terms of hemolytic bacteria, as opposed to the Pharmacy course. The

tendency is similar to that formerly found in the male participants. The contrast verified is high.

The number of CFU of *Staphylococcus* spp. lay close together, which was not verified in the male participants. Although the Nutrition course participants present an increased value.

In terms of *S. aureus*, the Pharmacy course follows the hemolytic bacteria CFU tendency, and as *S. aureus* is a hemolytic bacteria this was expected. The nutrition course was the one that presented the highest bacterial count, which was not verified in the male participants, where the Pharmacy course was the one that presented the major CFU count.

In the female participants it was verified that the Forensics course was the one that presented the highest bacterial counts. However, the Nutrition course was the one that presented the highest values for the bacteria that are potentially pathogenic and is therefore more relevant.

This was not consistent with the results obtained for the male participants, meaning that the bacterial count and bacterial flora present on the participants does not have a significant relation to the courses that individuals may frequent.

Only one of the male participants presented 2 CFU of *Enterobacteriaceae*. This participant frequented the Forensics course. As formerly stated, the majority of the MRSA carriers were male participants from the pharmacy course. Other MRSA carriers were a male participant from the Nutrition course and a female participant from the Forensics course (both in one of the samplings). VRE were not detected in any of the participants.

Table 19: Number of CFU presented by the female participants, by course and sampling, overall and *Bacillus* spp. on the mobile phones, and overall, *Bacillus* spp., hemolytic bacteria, *Staphylococcus* spp. and *S. aureus* on the sampled hands.

♀ *Pharmacy*	*Overall MP (CFU/cm²)*	*Bacillus[1] spp. (CFU/cm²)*	*Overall H (CFU)*	*Bacillus[2] spp. (CFU)*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
1st Sampling average	1.42	0.03	$1.16x10^3$	0	100	$1.15x10^3$	160
2nd Sampling average	1.75	0.02	$6.79x10^3$	0	140	$4.97x10^3$	55
Mean	1.59	0.03	$3.98x10^3$	0	120	$3.06x10^3$	108
♀ *Forensics*	*Overall MP (CFU/cm²)*	*Bacillus[1] spp. (CFU/cm²)*	*Overall H (CFU)*	*Bacillus[2] spp. (CFU)*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
1st Sampling average	2.07	0.01	$6.4x10^3$	0	$6.59x10^3$	$3.05x10^3$	$2.71x10^3$
2nd Sampling average	1.40	0.002	$2.84x10^3$	0	$3.15x10^3$	$2.71x10^3$	$2.31x10^3$
Mean	1.74	0.005	$4.62x10^3$	0	$4.87x10^3$	$2.88x10^3$	$2.51x10^3$
♀ *Nutrition*	*Overall MP (CFU/cm²)*	*Bacillus[1] spp. (CFU/cm²)*	*Overall H (CFU)*	*Bacillus[2] spp. (CFU)*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
1st Sampling average	1.09	0.01	$2.2x10^3$	0	$3.16x10^3$	$2.46x10^3$	$1.76x10^3$
2nd Sampling average	1.42	0.02	$2.96x10^3$	20	$4.21x10^3$	$4.09x10^3$	$3.77x10^3$
Mean	1.25	0.013	$2.58x10^3$	10	$3.68x10^3$	$3.28x10^3$	$2.76x10^3$

[1] – *Bacillus* spp. found in the count-tact medium (mobile phone samples); [2] – *Bacillus* spp. found in the TSA medium (hand samples); MP – Mobile phone sample; H – hand sample; CFU – Colony forming units.

4.2.6.2 – Overall quantitative analysis

It was verified that the male participants, generally present a higher bacterial count than the female participants. Although female participants presented a higher CFU count for hemolytic bacteria and *Staphylococcus* spp., which may be pathogenic (table 20).

Therefore, it was concluded that male participants present a higher bacterial count both on the mobile phones as on their hands, additionally in terms of *Bacillus* spp. They also presented a higher CFU count of *S. aureus,* they account for the majority of MRSA carriers (6/7) and the only participant that carried *E. coli*. They have the more contaminated hands in terms of possible pathogenic and multiresistant bacteria.

A study developed by Pal and co-workers showed that touchscreen mobile phones presented a median CFU count of 0.09 CFU per cm^2, whilst keypad mobile phones exhibited 0.77 CFU per cm^2 [14]. This literature focused on mobile phones carried by healthcare professionals, which should be more contaminated as demonstrated by Bhat and co-workers [7]. As no discrimination was made between keyboard types in this particular analysis, no direct comparison can be made. However, it is clear that the bacterial counts obtained in this work are far superior to those presented by Pal and co-workers.

A study elaborated by Hewitt and co-workers studied the office spaces of male and female individuals and it was found that the men had more contaminated work spaces than female participants [107]. It is known that they are more careless towards hygiene, but it was found that, as they generally have a larger body-size than women and thus present larger skin surface, nasal and oral cavities, they harbor a larger quantity of bacteria [107]. Their body surface permits a proportionally greater surface area for bacterial colonization, which additionally to the inferior hygiene practices increases the bacterial counts significantly.

Table 20: Number of CFU presented by the participants, by gender, overall and *Bacillus* spp. on the mobile phones, and overall, *Bacillus* spp., hemolytic bacteria, *Staphylococcus* spp. and *S. aureus* on the sampled hands.

♂ *vs.* ♀	*Overall MP (CFU/cm²)*	*Bacillus*[1] *spp. (CFU/cm²)*	*Overall H (CFU)*	*Bacillus*[2] *spp. (CFU)*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
♂ *Overall average*	2.46	0.041	$9.36x10^3$	24	$1.77x10^3$	$2.49x10^3$	$1.84x10^3$
♀ *Overall average*	1.53	0.016	$3.72x10^3$	3	$2.89x10^3$	$3.07x10^3$	$1.79x10^3$
Mean	2	0.03	$6.54x10^3$	14	$2.33x10^3$	$2.78x10^3$	$1.82x10^3$

[1] – *Bacillus* spp. found in the count-tact medium (mobile phone samples); [2] – *Bacillus* spp. found in the TSA medium (hand samples); MP – Mobile phone sample; H – hand sample; CFU – Colony forming units.

4.3 – Microbiological results versus individual characteristics

The microbiological analysis in correspondence to the questionnaire permits the understanding of the influence that each factor may carry to the bacterial diversity and CFU number.

4.3.1 – Keyboard type influence

In the first sampling, the large majority presented the utilization of touchscreen mobile phones, both in the male and female participants, 73.33% and 80% respectively (table 5). As can be observed in table 21, keypad present a higher overall bacterial count per cm², in both genders.

The research conducted by Pal and co-workers had the objective to understand if mobile phones with a keypad pose an increased risk of microbial contamination in comparison to touchscreen mobile phones. Although being associated to healthcare workers, the results were considerably lower than those obtained in both sampling in

this work (table 21 and 22), as touchscreen phones presented a median CFU count of 0.09 CFU per cm^2 and keypad mobile phones exhibited 0.77 CFU per cm^2 [14].

On the other hand, Andrej and co-workers obtained an average of 1,51 CFU and 1,05 CFU per cm^2 for keypad and touchscreen, respectively [2]. All the results obtained in this study presented higher CFU counts, both for keypad and touchscreen mobile phones, but the female participants in the second sampling who carried keypad devices (0.96 CFU per cm^2 vs. 1.51 CFU per cm^2) (table 22).

These differences can be justified by the presence of ridges and rugged plastic materials which compose the keyboards, potentially facilitating the adherence and permanence of the bacteria on the devices. However, the touchscreen devices presented a higher CFU count of *Bacillus* spp., whilst the male participants owning a mobile phone with a keypad did not present any growth of these bacteria. This may be due to the materials the mobile phone surfaces are composed of which may permit a higher or lower affinity to the adherence of this type of bacteria.

Table 21: Keyboard influence on the bacterial count of the mobile phones of the 1st sampling, overall count and *Bacillus* spp.

Keyboard type (1st sampling)	♂ **Participants**		♀ **Participants**		**Overall**	
	Overall BC (CFU/cm²)	*Bacillus* spp. (CFU/cm²)	Overall BC (CFU/cm²)	*Bacillus* spp. (CFU/cm²)	Overall BC (CFU/cm²)	*Bacillus* spp. (CFU/cm²)
Touchscreen	2.53	0.008	1.39	0.02	1.96	0.014
keypad	3.16	0	2.07	0.01	2.62	0.005

BC – Bacterial count; CFU – Colony forming units.

In the second sampling it was noticed that the percentage of touchscreen mobile phones usage increased in both genders 3.33% (table 6). In the male participants it was observed that for both the overall bacterial count and the *Bacillus* spp. count, the keypad presented higher values, thus refuting the previously stated that the variation

in *Bacillus* spp. adherence is due to the device's surface composition. Therefore this may be due to the higher presence of these bacteria on the hands of their handlers.

The female participants prove the previously stated, being that both type of surfaces present the same *Bacillus* spp. count (table 22). On the contrary, their touchscreen mobile phones presented a higher overall bacterial count, which may also be explained through the higher bacterial presence on the hands of the users.

Table 22: Keyboard influence on the bacterial count of the mobile phones of the 2nd sampling, overall count and *Bacillus* spp.

Keyboard type (2nd sampling)	♂ **Participants**		♀ **Participants**		**Overall**	
	Overall BC (CFU/cm²)	*Bacillus* spp. (CFU/cm²)	Overall BC (CFU/cm²)	*Bacillus* spp. (CFU/cm²)	Overall BC (CFU/cm²)	*Bacillus* spp. (CFU/cm²)
Touchscreen	1.96	0.02	1.64	0.02	1.8	0.02
keypad	3.07	0.07	0.96	0.02	2	0.05

BC – Bacterial count; CFU – Colony forming units.

4.3.2 – Nail length influence

As previously addressed, nail size may influence the number of bacteria and their permanence on the hands of individuals [86]. The proper cleaning of the nails may reduce these numbers, and this will be verified later on.

The male participants only presented short and medium size nails, and for all but the overall bacterial count of the hands, the previously stated is verified, that longer nails harbor a higher number of bacteria. Thus it is noticed that the possibly pathogenic bacteria were found in an increased number, as opposed to the short nails (table 23) [42].

The female participants presented all nail sizes and it can be observed that the long nails present the highest bacterial count in all parameters, even higher than male

participants in 3 of them. The medium size nails presented lower values for the most important parameters, such as possibly pathogenic bacteria, which may be justified due to the cleaning of the nails and hygiene precautions executed by the participants [42][40].

These findings are consistent with was presented in a report elaborated by the Public Health Agency of Canada, which stated that longer nails, natural and artificial harbor a higher count of microbes and viruses [44]. The area beneath the fingernail (subungual area), is propene to adhering high concentrations of microorganisms, such as coagulase-negative staphylococci, Gram-negative rods (including *Pseudomonas* spp.), *Corynebacteria* and yeasts. These potential pathogens can remain under the fingernails for long periods of time, even after thorough hand washing [44].

Table 23: Bacterial count of the sampled hands of the participants of both genders in correspondence to nail size.

♂ *Participants*	*Overall BC (CFU)*	*Bacillus spp. (CFU)*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
Short	$1.68x10^3$	9	400	723	200
Medium	$1.3x10^3$	75	950	$1.15x10^3$	375
Long	N/a	N/a	N/a	N/a	N/a
♀ *Participants*	*Overall BC (CFU)*	*Bacillus spp. (CFU)*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
Short	513	0	225	513	138
Medium	$1.86x10^3$	0	150	300	75
Long	$2.2x10^3$	14	$2.4x10^3$	$1.33x10^3$	225

BC – Bacterial count; CFU – Colony forming units.

4.3.3 – Manicure and ring influence

Only the female participants presented manicured nails when sampling occurred, which varied from artificial nails to only painted nails. It was clear that besides this fact, the male participants were the ones that presented an overall higher number of CFU on their hands (table 24). This statement was also previously verified in the overall microbiological analysis.

When it comes to the female participants, the ones that present a higher number of bacterial presence on their hands, were the ones that presented manicures when samples were taken, which was expected [86]. However, a higher number of hemolytic bacteria were found on the remaining female participants (table 24).

Nails that were only painted, did not increase significantly the number of bacteria present on the hands, although artificial nails and gel lacquer does have a considerable influence(table 25) [86].

Artificial fingernails may harbor pathogenic microorganisms more frequently than natural nails, and as such they may contribute to transmission of microorganisms to third parties. Literature presented by the Public Health Agency of Canada exhibited the same tendency showed in this study (table 25). Contamination with potentially pathogenic bacteria was observed in individuals that presented artificial and special type of nail art (gel lacquer), whereas individuals that presented unchipped nails polish did not present an increased bacterial contamination [44]. These tendencies do follow what was previously presented in terms of nail length.

Table 24: Bacterial count of the sampled hands of the participants of both genders in correspondence to manicure presented.

♂ *Participants*	*Overall BC (CFU)*	*Bacillus spp. (CFU)*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
Yes	N/a	N/a	N/a	N/a	N/a
No	$1.6x10^3$	24	413	838	213
♀ *Participants*	*Overall BC (CFU)*	*Bacillus spp. (CFU)*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
Yes	$1.23x10^3$	6	175	925	138
No	888	0	338	738	113

BC – Bacterial count; CFU – Colony forming units.

Table 25: Bacterial count of the sampled hands of the female participants in correspondence to the type of manicure they were wearing.

Manicure type	*Overall BC (CFU)*	*Bacillus spp. (CFU)*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
Normal lacquer	475	8	150	425	125
Gel lacquer	$3.43 x10^3$	0	$2.9x10^3$	$1.33x10^3$	125
Artificial nails	$3.15x10^3$	0	$2.36x10^3$	$2.23x10^3$	$1.1 x10^3$

BC – Bacterial count; CFU – Colony forming units.

In terms of ring presence, it stood out that the participants that wore rings when sampling occurred, presented a considerably higher number of CFU in all but two parameters [85]. Only the female participants wore rings, and when compared with the participants that did not wore rings in both genders, it was observed that the male participants revealed higher CFU values, just as many times validated before.

It was interesting that none of the participants who wore rings presented *Bacillus* spp., whilst it would be thought to be a particular prone location for bacteria to reside and perpetuate [85].

The Public Health Agency of Canada and the WHO reported that hand hygiene may be compromised due to wearing rings, as skin underneath that area are present higher contamination than comparable skin areas without rings. Skin can present a higher risk of contamination with *Staphylococcus aureus*, Gram-negative bacilli or *Candida* spp. when the number of rings worn increased [44][40]. It cannot be said that rings result in greater cross- transmission of pathogens, but contaminated jewelry that is not properly maintained might harbor microorganisms that could contribute to the transmission of potential pathogens [44][40]. The tendencies presented in this study were consistent with what was presented in these reports (table 26).

Table 26: Bacterial count of the sampled hands of the participants of both genders in correspondence to ring presence.

♂ *Participants*	*Overall BC (CFU)*	*Bacillus spp. (CFU)*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
Yes	N/a	N/a	N/a	N/a	N/a
No	$1.6x10^3$	24	413	838	213
♀ *Participants*	*Overall BC (CFU)*	*Bacillus spp. (CFU)*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
Yes	$2.81x10^3$	0	$2.01x10^3$	$1.2x10^3$	125
No	600	5	238	513	113

BC – Bacterial count; CFU – Colony forming units.

4.3.4. – Special hygiene with mobile phone influence

It was clearly seen that the participants that did not rendered any special hygiene care to their mobile phones, showed a higher number of CFU on their hands and mobile phones, although exceptions were verified (table 27).

The general tendency of male participants exhibiting higher bacterial count is maintained. An increased number of hemolytic bacteria was verified in both genders for the participants that did present a special attention to their devices, the same being verified for the *S. aureus* CFU count (table 27). This may be explained through the fact that these bacteria were more persistent in terms of elimination with disinfectants and more so when the devices were cleaned with a cloth [2].

It was clear that in both genders the overall bacterial count was higher in the participants that did not pay any special attention to the hygiene of their mobile phones. A higher discrepancy in values is verified in the male participants, as in the *Bacillus* spp. CFU values (table 27). Relatively to the *Bacillus* spp. present on the mobile phones of the female participants, the difference between bacterial count values is very low and close to the lowest value presented by the male participants. This might mean, also by analyzing the previous results attained, that the female participants have a lower overall CFU count of *Bacillus* spp.

The study elaborated by Bhat and co-workers showed that only 6% of healthcare professionals disinfect their mobile phones, whilst 40% use them between examination of patients [7]. It was previously observed that the participants in this study presented higher percentages and this could be verified in the overall bacterial count, although with less impact in the female participants. The bacterial counts are however still high when compared to the CFU counts obtained by Pal and co-workers [14].

The bacteria that were found on the mobile phones in the study conducted by Bhat and co-workers included Methicillin-resistant *S. aureus* (MRSA), Methicillin-sensitive *S. aureus* (MSSA), *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter*, *Enterococcus faecalis* and *Pseudomonas aeruginosa* [7]. *Staphylococcus* spp., also

included in the hemolytic bacteria, were mainly found on the hands of the participants, as can be seen in table 27.

Table 27: Bacterial count of the mobile phones and sampled hands of the participants of both genders in correspondence to special mobile phone hygiene.

♂ *Participants*	*Overall MP (CFU/cm²)*	*Bacillus[1] spp. (CFU/cm²)*	*Overall H (CFU)*	*Bacillus[2] spp. (CFU)*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
Yes	1.04	0.006	925	4	950	825	325
No	2.26	0.05	$1.68x10^3$	30	350	875	200
♀ *Participants*	*Overall MP (CFU/cm²)*	*Bacillus[1] spp. (CFU/cm²)*	*Overall H (CFU)*	*Bacillus[2] spp. (CFU)*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
Yes	1.26	0.012	800	11	375	600	150
No	1.43	0.017	$1.1x10^3$	0	225	875	125

[1] – *Bacillus* spp. found in the count-tact medium (mobile phone samples); [2] – *Bacillus* spp. found in the TSA medium (hand samples); MP – Mobile phone sample; H – hand sample; CFU – Colony forming units.

4.3.5 – Special hygiene with hands/nails influence

The analysis of these results was interesting, as they varied greatly. In the male participants it was observed that the individuals that indicated they pay some kind of special attention to the hygiene of their hands, were the ones that in all parameters present higher CFU counts, whilst this should be on the contrary (table 28).

However, in the female participants, this tendency was met with significant differences. The only bacteria that did not fulfilled the tendency was *Bacillus* spp. and this may be due to the previously stated for the mobile phone hygiene, that the manner of cleaning is not sufficient for the elimination of this type of bacteria [40].

Relatively to the male participants that took special hygiene actions, these results may indicate that the measures they took in order to better maintain the hygiene of their hands is not sufficient or is not appropriate for this purpose, and may even be not properly executed [87]. It can also be due to the excessive cleaning being counterproductive due to skin irritation and dryness of the hands, as was verified by a report elaborated by the WHO and by two studies conducted by Larson and Larson and co-workers [40][95][108].

The nail influence has been previously addressed as an increase in bacterial contamination when they are long, also contributing to this factor. The methods of cleaning should be as thorough as possible to significantly reduce the bacterial presence, although it is not linear [40][44]. The cleaning methods presented in the questionnaire could not achieve this kind of thoroughness, which may justify the CFU count obtained in the male participants that stated they rendered this kind of attention. Bacteria such as coagulase-negative staphylococci, Gram-negative rods (including *Pseudomonas* spp.), *Corynebacteria* and yeasts can be found on the skin underneath the nails [44]. *Staphylococcus* spp. were also significantly present in this work (table 28).

Table 28: Bacterial count of the sampled hands of the participants of both genders in correspondence to special hand/nail hygiene.

♂ *Participants*	*Overall BC (CFU)*	*Bacillus spp. (CFU)*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
Yes	$1.68x10^3$	38	825	850	325
No	$1.53x10^3$	13	400	723	200
♀ *Participants*	*Overall BC (CFU)*	*Bacillus spp. (CFU)*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
Yes	888	5	238	688	125
No	$1.16x10^3$	0	$1.78x10^3$	$1.08x10^3$	300

BC – Bacterial count; CFU – Colony forming units.

4.3.6 – Taking mobile phone to bathroom influence

It was expected in this analysis that the participants that did not take their mobile phone with them to the bathroom would present a significantly lower or only a somewhat lower bacterial count on their hands and mobile phones or at least a lower number of potentially pathogenic bacteria (table 29). However, it was highlighted that only in one of the participants fecal bacteria were detected and in a very low quantity (2 CFU).

Mobile phones that are brought into the bathroom have the potential to adhere various bacteria, especially of fecal origin [88]. In both genders, *Bacillus* spp. was present in larger numbers in the participants that did not take their mobile phones to the bathroom, verifying the same with *S. aureus*.

Relatively to the overall mobile phone CFU count, in both genders, it was verified that a higher bacterial count was attained, with a greater difference in the male participants, additionally verified in the *Bacillus* spp. CFU count (table 29). These results respect the tendency that was expected [88]. The *Bacillus* spp. bacterial count that was obtained for the female participants did not follow this expectation, presenting an increased CFU value in the participants that did not take their mobile phone to the bathroom. This may be justified by the fact that these female participants had longer nails than the others, which carry a larger number of *Bacillus* spp. as found in table 23 [42][40].

The study conducted by Andrej and co-workers presented that mainly staphylococci and *Enterobacteriaceae*, which includes bacteria as *E. coli*, were found [2]. In the same research, it was verified that the bacterial counts of the mobile phones were an average of 1,51 CFU and 1,05 CFU per cm^2 for keypad and touchscreen, respectively, which is considerably lower for the male participants than what was obtained in this work (table 29).

Table 29: Bacterial count of the mobile phones and sampled hands of the participants of both genders in correspondence to taking mobile phone to the bathroom.

♂ *Participants*	*Overall MP (CFU/cm²)*	*Bacillus[1] spp. (CFU/cm²)*	*Overall H (CFU)*	*Bacillus[2] spp. (CFU)*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
Yes	2.29	0.064	$1.49x10^3$	14	188	888	200
No	1.82	0.004	$1.8x10^3$	40	813	738	300
♀ *Participants*	*Overall MP (CFU/cm²)*	*Bacillus[1] spp. (CFU/cm²)*	*Overall H (CFU)*	*Bacillus[2] spp. (CFU)*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
Yes	1.43	0.008	975	0	300	775	125
No	1.26	0.029	$1.35x10^3$	9	200	875	225

[1] – *Bacillus* spp. found in the count-tact medium (mobile phone samples); [2] – *Bacillus* spp. found in the TSA medium (hand samples); MP – Mobile phone sample; H – hand sample; CFU - Colony forming units.

4.3.7 – Biting nails and/or surrounding skin influence

Contradictory results were obtained when analyzing these CFU counts. In the male participants it was found that the individuals who bit their nails and/or surrounding skin presented considerably higher overall bacterial counts. Additionally, the CFU values for hemolytic bacteria and *Staphylococcus* spp. were also significantly higher. The bacterial count for *S. aureus* was likewise higher but not in those proportions (table 30).

These elevated numbers may present dangerous repercussions if the bacteria present are pathogenic and/or resistant to antibiotics, since the nails are in direct proximity of the oral cavity allowing their transmission from and to the fingers [42][86].

The female participants presented the contrary, the individuals that did bit their nails and/or surrounding skin presented significantly lower bacterial counts than the remaining individuals (table 30). This may be due to better hygiene conduct and maintenance. This behavior facilitates the transmission of the bacteria present on the

hands which may be potentially pathogenic and/or resistant to antibiotics, and therefore increasing the chance of developing disease [42][86].

The study conducted by Ghanizadeh stated that only 21.5% of male adults bite their nails, and that it is more prevalent in children, especially boys [90]. A high percentage of participants stated that they bit their nails and/or surrounding skin, this may damage the tissue around the nail and lead to infection, increasing the bacterial contamination of the hands and consequent transmission. This is consistent for the values obtained for the male participants, although the female participants do not follow this tendency (table 30). The quantity and nature of the bacteria that were present may be concerning as they are potentially pathogenic and may be resistant to antibiotics. They can possibly lead to disease development as the nails and surrounding skin are in close contact with the oral cavity of the individual.

Table 30: Bacterial count of the sampled hands of the participants of both genders in correspondence to biting nails and/or surrounding skin.

♂ *Participants*	*Overall BC (CFU)*	*Bacillus spp. (CFU)*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
Yes	$2.08x10^3$	12	$1x10^3$	$1.1x10^3$	275
No	555	40	200	700	200
♀ *Participants*	*Overall BC (CFU)*	*Bacillus spp. (CFU)*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
Yes	800	0	200	375	75
No	$1.35x10^3$	5	375	925	225

BC – Bacterial count; CFU – Colony forming units.

4.3.8 – Having pets influence

As previously stated, owning pets may influence both the bacterial count and types of bacteria present in a household. The transmission of bacteria from animals to pets is possible, even antibiotic resistant bacteria, which may not cause illness in the animal but may develop disease in the owner [93][92].

The overall bacterial count in both genders was lower in the pet owners, which may be due to the more frequent hand washing habits developed by this ownership. The number of *Bacillus* spp. is higher in both genders that keep pets (table 31).

The CFU count for *Staphylococcus* spp. is higher in both gender that own pets, this is commonly documented, also in MRSA that pets contract these types of bacteria from their owners and vice versa [69][109]. In the female participants, the bacterial count of *S. aureus* is considerably higher to those who do not keep pets, however this difference is not verified in the male participants (table 31).

A study developed by Costa and co-workers has revealed that transmission of the bacteria carried by pets to human co-habitants can occur directly (skin to skin contact) and contact with bacteria in the saliva or feces, or indirectly, through the household environment [110]. Due to different behavior inside and outside the household, contamination of their hair, skin and mouth with fecal and other potentially pathogenic bacteria can occur. A study is presented in which the same multiresitant *E. coli* was found in various sites on the pet as on the owner and involving environment [110]. The presence of multiresistant *S. aureus*, *E. coli* and *Enterococcus* spp. have been detected, although the transmission antibiotic resistant bacteria between household animals and humans needs to be studies further [110]. Elevated *Staphylococcus* spp. counts were verified in both genders that stated that they own pets (table 31), which is consistent with was previously presented.

Table 31: Bacterial count of the sampled hands of the participants of both genders in correspondence to owning pets.

♂ *Participants*	*Overall BC (CFU)*	*Bacillus spp. (CFU)*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
Yes	$1.6x10^3$	31	525	863	213
No	$3.92x10^3$	10	412	738	225
♀ *Participants*	*Overall BC (CFU)*	*Bacillus spp. (CFU)*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
Yes	563	6	213	975	225
No	$1.16x10^3$	0	338	488	100

BC – Bacterial count; CFU – Colony forming units.

4.3.9 – Hand washing influence

None of the male participants stated that they washed their hands more than 15 times a day. It should be seen that the participants that wash their hands less presented higher bacterial counts as opposed to the participants that wash their hands the most exhibited lower CFU values.

This was not verified in the results, as the participants that wash their hands the most presented higher bacterial counts than should be expected. However there are studies that revealed that excessively washing hands can work in a counterproductive way, meaning that more bacteria are presented when hands are washed too frequently. This is due to the disruption of the normal skin microflora, increasing the chance of developing infection and creation of wounds, thus exhibiting higher bacterial counts [108][40].

Additionally, studies revealed by the Public Health Agency of Canada and the WHO stated that hand washing with plain soap can also result in increased bacterial counts as soaps can lead to skin irritation and dryness. Soap can also become

contaminated and as such lead to colonization of the user's hands with Gram-negative bacilli, although the hazard associated is minor [40][44]. An increase in bacterial count was found in this study when hand washing was more frequent (table 32), being consistent with was previously stated. The *Bacillus* spp. bacterial count does increase but no significant information can be drawn.

The male participants to not follow the tendency firstly referred to, and as can be seen, the CFU values increase drastically in some parameters. However, *Bacillus* spp. does not follow this pattern. Considerably high hemolytic bacteria counts were found as it was for *Staphylococcus* spp. The female participants follow both tendencies, being confirmed that excessive hand washing does not always mean that hands were less colonized by bacteria (table 32) [108][95].

Table 32: Bacterial count of the sampled hands of the participants of both genders in correspondence to hand washing times a day.

♂ *Participants*	*Overall BC (CFU)*	*Bacillus spp. (CFU)*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
<5x	$1.05x10^3$	25	738	688	200
5-10x	$1.8x10^3$	34	400	825	200
10-15x	$6.05x10^3$	0	$1.13x10^3$	$1.1x10^3$	325
>15x	N/a	N/a	N/a	N/a	N/a
♀ *Participants*	*Overall BC (CFU)*	*Bacillus spp. (CFU)*	*Hemolytic Bacteria (CFU)*	*Staphylococcus spp. (CFU)*	*S. aureus (CFU)*
<5x	$1.98x10^3$	0	$4.54x10^3$	$2.03x10^3$	$1.65x10^3$
5-10x	$1.1x10^3$	5	225	775	150
10-15x	475	0	250	$1.23x10^3$	125
>15x	975	0	950	$1.76x10^3$	138

BC – Bacterial count; CFU – Colony forming units.

4.3.10 – Overall influence results

It was overall found that in terms of mobile phones, the factor that influences the bacterial count the most was when the devices presented keypads, 3.16 CFU per cm^2 in the male participants and 2.07 CFU per cm^2 in the female participants, when highest. The influence on the CFU count by taking the mobile phones to the bathroom and not rendering a special attention to the devices was close in the male participants (2.29 CFU per cm^2 vs. 2.26 CFU per cm^2). This influence of both factors was the same in the female participants (1.43 CFU per cm^2).

In terms of influence on the bacterial counts of the hands, results differed between male and female participants. In the male participants it was noticed that excessive hand washing (10 to 15 times a day) was the major reason for a significantly increased bacterial count ($6.05x10^3$ CFU), which is confirmed by the report emitted by the Public Health Agency of Canada [44]. The second highest bacterial count found was for the male participants that did not own pets ($3.92x10^3$ CFU). This may be contrary to what is thought but individuals who own pets may be more conscious hygienically speaking, and therefore present lower bacterial counts. The third factor that influenced the male bacterial count the most was nail biting ($2.08x10^3$ CFU), which is explained by Ghanizadeh due to the fact that this behavior results in the possibility of skin damaging and the increased adherence of bacteria, adding to the contact with bacteria present in the oral cavity [90].

The most influential factor on the bacterial count of the female participants was the presence of nail art, namely gel lacquer and artificial nails ($3.43x10^3$ CFU and $3.15x10^3$ CFU, respectively). The second factor that influences the CFU count the most is wearing rings ($2.81x10^3$ CFU). The third most contributing factor is the nail size, particularly long nails ($2.2x10^3$ CFU). All these three factors are widely described in various literatures, additionally reports published by the Public Health Agency of Canada and the WHO as gravely influencing bacterial counts due to facilitating the adherence and permanence of possibly pathogenic and multiresistant bacteria [41][45]. It is a health standard both in the healthcare sector as in the food industry that nails

should be kept short, not manicured or have any type of nail art and jewelry should not be worn [44][40][86]. This shows that great variations are found between male and female individuals.

4.3.11 – *Enterobacteriaceae*

Only in one of the participants in one of the samplings, 2 *Enterobacteriaceae* colonies were found. These were both tested positive for indole production. This may indicate that the bacteria were *E. coli*.

Due to the very low percentage (1%) of this bacteria in the overall presence in the samples (table 17), it was not formerly presented in the microbiological analysis versus the questionnaire questions. Instead the participant's characteristics will be exposed next.

The individual was a male participant, who carried a mobile phone with a touchscreen keyboard, which harbor lower bacterial counts as stated by Pal and co-workers [14].

His nails were short, not presenting any manicure or rings, which by the Public Health Agency of Canada and the WHO are low contamination factors [44][40].

He did not render any special hygiene precautions both to his mobile phone and hands and/or nails, as is common as was stated by Bhat and co-workers [7]. However, the study conducted by Andrej and co-workers revealed the presence of *Enterobacteriaceae* in 39% of the students samples, which include various bacteria associated with normal gut flora [2][101].

He did indicate that hand washing was always executed after using the bathroom and that the mobile phone was not taken with him in that situation, which should reduce the exposure to fecal related bacteria.

The participant does have pets and does bite his nails and/or surrounding skin. Being documented that household pets can transmit *E. coli*, as stated by Costa and co-

workers, this is a contributing factor [110]. The frequency of hand washing throughout the day was 10 to 15 times, which does not mean that they were washed properly as stated by the WHO, possibly contributing to the contamination [40].

The most pertinent factors that could contribute to this kind of bacterial presence, were the ones that indicated a lack of proper hygiene methods implementation, like not properly washing of the hands after bathroom use and taking the mobile phone to the bathroom, which can increase the contamination with fecal associated bacteria [88][111]. Although the participant does wash his hands frequently, this does not always mean that it is beneficial. Frequent hand washing can lead to damage of the skin, which can enhance the harboring of pathogenic bacteria [108]. And the act of hand washing may not always be performed correctly, being so that a high number of bacteria may still be present [43].

He had short nails which normally harbor a smaller number of bacteria, however the participant does bite his nails and/or surrounding skin which can be preoccupying when these bacteria are present, as damaging and infection of the skin can occur [90]. In the case of *E. coli*, this bacteria has the potential to cause gastroenteritis when ingested, and this kind of behavior can lead to that without the individual knowing where it has originated from [99].

This participant did not present any factor that could contribute significantly or conclusively justify the presence of *Enterobacteriaceae*. Probably the bacteria was transiently present on the hands as he could have touched something that harbors it. Most likely it could have been harbored on the mobile phone for the reasons previously stated, related to the lack of hygiene methods rendered to the device, along with the possibility of not properly washing his hands. Mobile phones are a reservoir for various bacteria as stated by Walia and co-workers and enterococci are assumed to be indicators of fecal contamination of the hands, i.e. hand hygiene, as showed by Boehm and co-worker [6][101].

4.4 – Antibiotic resistance screening

4.4.1 – Overall antibiotic screening

Certain bacteria, mainly hemolytic and *S. aureus*, from each participant were selected from the Columbia blood agar media and from the Mannitol salt agar, as to be submitted to antibiotic susceptibility testing (figure 20). The antibiotics tested for each sample were, Cefoxitin (FOX), Amoxicillin + Clavulanic Acid (AMC), Ceftazidime (CAZ), Clindamycin (DA), Erythromycin (E) and Oxacillin (OX) (appendix 2). From the male participants, 138 samples were analyzed and from the female participants 126, this is due to the fact that for some participants more than two bacteria were analyzed per sampling whilst other participants did not present bacterial growth to be analyzed.

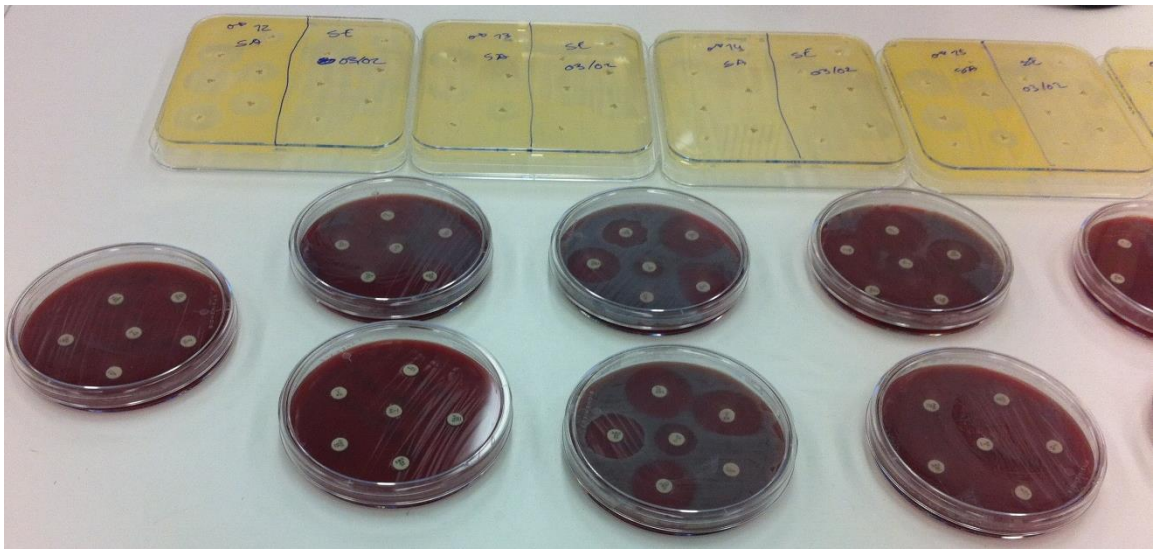


Figure 20: Various samples submitted to antibiotic susceptibility testing.

Resistance to Cefoxitin, Amoxicillin + Clavulanic Acid and Ceftazidime were rarely verified, especially for Amoxicillin + Clavulanic Acid and only in the female participants (table 33). A study developed by Duran and co-workers presented

resistance to Amoxicillin + Clavulanic Acid in 28.9% of staphylococci screened and in 23% of *S. aureus* samples [54]. In comparison to the percentage obtained in this study (0.4% overall), the values of Duran and co-workers are significantly higher. Contrarily to Duran and co-workers, not only *S. aureus* were screened in this work, but this cannot solely account for the difference in values, as the strains screened could not have acquired this resistance do Amoxicillin + Clavulanic Acid.

Ceftazidime has been connected to antibiotic resistance by bacteria found on mobile phones and hands, although in higher percentages than presented in this study (1.9%) (table 33) [112]. Arora and co-workers presented resistance to Ceftazidime ranging 39.5%, from strains isolated from the hands [112]. It can be clearly seen that it is significantly higher than those presented in this study.

However, for Clindamycin and Oxacillin, the percentages verified were higher, ranging from 5.7% to 2.9% and 5.7% to 24.2% when resistant, respectively. The study conducted by Duran and co-workers which researched the susceptibility patterns in staphylococci, presented resistance to Clindamycin in 47% of the screened staphylococci and in 38.1% of the *S. aureus* sampled, which is considerably higher than what was found in this work [54].

Additionally, a study conducted by Srikanth and co-worker presented sensibility to Clindamycin in 83.6% of MSSA isolates screened, being close to what was found in this work [113]. Nevertheless, not only staphylococci were screened in this work, which may partially justify the fluctuation in percentages.

The study developed by Srikanth and co-worker also revealed that Oxacillin susceptibility among MSSA isolated was 10.6%, which is very high as the overall resistance obtained in this study was 12.5% [113]. Again, not only *S. aureus* were screened, which may account for this difference, as the difference in samples.

As will be addressed further on, Oxacillin and Cefoxitin may indicate the detection of Methicilln-resistant *S. aureus* [59]. Although the percentages of resistance for Cefoxitin were low (0.8%), for Oxacillin the percentages ranged to 24.2% in the

Pharmacy course by male participants (table 33). This is consistent with the facts previously states as this group presented the majority of the MRSA detected (5/7), however they did not present any resistance to Cefoxitin, which is contradictory.

Clindamycin resistance was verified in higher numbers in the male participants (8.1% to 20.9%). The overall percentages obtained for each gender varied significantly from 13.8% in the male participants to 5.5% in the female participants. This may be due to the fact that a lower percentage of female participants presented *S. aureus* and MRSA growth (table 17). This antibiotic has been documented to be ineffective towards *S. aureus* and MRSA, which may justify the percentages obtained as many participants presented high bacterial counts for *S. aureus* [54][50]. As previously showed, Srikanth and co-worker obtained a susceptibility to Clindamycin in 83.6% of MSSA and in 29.3% of MRSA [113]. Rahimi and co-workers obtained a resistance of 82.9% in MRSA isolates [114], proving what was previously stated and what will be observed further on.

Relatively to the antibiotic Erythromycin, it presented the highest resistance rates in both genders and throughout all the courses. In the male participants, a very high percentage of resistance to this drug was found in the pharmacy course (69.7%). On the contrary, the female participants frequenting the same course exhibited the lowest percentage (25.7%). However, analyzing the overall percentages by gender, it was verified that the female participants showed a slightly higher value (+1%) than the male participants. This might be explained due to the fact that more male samples were analyzed and that their participants from the Nutrient course scored particularly low (30.2%).

The resistance to Erythromycin in staphylococci is not very uncommon, and the percentages obtained in this study, 44.7% overall, were lower than those verified in other researches, for example by Duran and co-workers and Srikanth and co-worker which presented 60.4% of resistance and 82.8% of susceptibility in *S. aureus* respectively [54][113][115]. This specific resistance will be addressed and discussed later on.

Table 33: Percentage of participants, by gender, that presented bacteria resistant to the used antibiotics.

♂ *Participants*	*FOX*	*AMC*	*CAZ*	*DA*	*E*	*OX*
Pharmacy	(0/33)	(0/33)	(0/33)	(5/33)	(23/33)	(8/33)
(%)	0	0	0	15.2	69.7	24.2
Forensics	(1/62)	(0/62)	(1/62)	(5/62)	(25/62)	(13/62)
(%)	1.6	0	1.6	8.1	40.3	21.0
Nutrition	(0/43)	(0/43)	(2/43)	(9/43)	(13/43)	(0/43)
(%)	0	0	4.7	20.9	30.2	0
Total	(1/138)	(0/138)	(3/138)	(19/138)	(61/138)	(21/138)
(%)	0.7	0	2.2	13.8	44.2	5.2
♀ *Participants*	*FOX*	*AMC*	*CAZ*	*DA*	*E*	*OX*
Pharmacy	(0/35)	(1/35)	(1/35)	(0/35)	(9/35)	(3/35)
(%)	0	2.9	2.9	0	25.7	8.6
Forensics	(1/56)	(0/56)	(1/56)	(5/56)	(29/56)	(7/56)
(%)	1.8	0	1.8	8.9	51.8	12.5
Nutrition	(0/35)	(0/35)	(0/35)	(2/35)	(19/35)	(2/35)
(%)	0	0	0	5.7	54.3	5.7
Total	(1/126)	(1/126)	(2/126)	(7/126)	(57/126)	(12/126)
(%)	0.8	0.8	1.6	5.5	45.2	9.5
Overall Total	(2/264)	(1/264)	(5/264)	(26/264)	(118/264)	(33/264)
(%)	0.8	0.4	1.9	9.8	44.7	12.5

FOX – Cefoxitin; AMC – Amoxicillin + Clavulanic Acid; CAZ – Ceftazidime; DA – Clindamycin; E – Erythromycin; OX – Oxacillin.

The study conducted by Duran and co-workers researched the susceptibility patterns in staphylococci and antibiotic resistance genes [54]. This revealed that *S. aureus* exhibits high resistance to Erythromycin (60.4%), Clindamycin (38.1%) and Amoxicillin + Clavulanic Acid (23%) [54]. The high resistance to Erythromycin was verified in this study (table 33), however to Clindamycin (9.8%) and Amoxicillin + Clavulanic Acid (0.4%) the resistance was low. However, not exclusively *S. aureus*

were screened and those strains who were screened may not have acquired resistance to these antibiotics.

The sample that presented resistance to Amoxicillin + Clavulanic Acid was presumptive *S. aureus*. The samples that exhibited resistance to Clindamycin were 1 *S. epidermidis*, 7 presumptive *S. aureus* and 18 hemolytic bacteria, which also include *S. aureus*. Although the percentages obtained were low, these results were consistent with the Duran and Srikanth study [54][113].

The 2 *Enterobacteriaceae* that were detected and presumptively found as being *E. coli*, were submitted to an antibiotic screening of 7 different antibiotics. The antibiotics used were Cefoxitin (FOX), Amoxicillin + Clavulanic Acid (AMC), Ceftazidime (CAZ), Ciprofloxacin (CIP), Gentamicin (CN), Imipenem (IMP) and Cefotaxime (CTX) (appendix 2).

As can be verified in table 34, both the bacteria were susceptible to all the antibiotics employed. This were positive results as bacteria that originated from contact with fecal matter, direct or indirectly, can be potentially dangerous as previously explained [88].

A study developed by Prakash and co-worker found that 34.15% of the screened *E. coli* bacteria presented susceptibility to Ciprofloxacin, 26.08% to Gentamicin, 34.78% to Cefotaxime and 92.68% to Imipenem. However, these were urinary samples and not hand samples but a general idea can be perceived.

Another study developed by Tansarli and co-workers showed that 16% to 86% of *E. coli* samples were susceptible to Amoxicillin + Clavulanic Acid, 89% to 98% to Cefoxitin, 92% to 99% to Cefotaxime, 100% to Imipenem, 68% to 91% to Ciprofloxacin and 64% to 98% to Gentamicin, which all confirm what was found in this study (table 34) [116].

Table 34: Antibiotic susceptibility results of the only two *Enterobacteriaceae* colonies obtained.

Enterobacteriaceae	*FOX*	*AMC*	*CAZ*	*CIP*	*CN*	*IMP*	*CTX*
♂*20DRIG-GR*	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
♂*20DRIG-PQ*	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive

FOX – Cefoxitin; AMC – Amoxicillin + Clavulanic Acid; CAZ – Ceftazidime; CIP – Ciprofloxacin; CN – Gentamicin; IMP – Imipenem; CTX – Cefotaxime.

4.4.2 – Multiresistance to antibiotics

Various selected samples presented resistance to the antibiotics employed, as previously presented. From these samples, excluding MRSA, a high number presented resistance to more than one of the antibiotics applied. These could range from resistance to 2 antibiotics to resistance to 4 of the antibiotics used in the screening, which was verified in two samples one male and one female (table 36). However, the antibiotics used present an overlap in classes which means that only a total of 4 antimicrobial classes were present.

Magiorakos and co-workers stated that the most used definition to classify Gram-positive and Gram-negative bacteria as being multidrug resistant is when they present resistance to 3 or more antimicrobial classes [117]. In this case, β-lactams were used which include both Penicillins (Oxacillin and Amoxicillin + Clavulanic Acid) and Cephalosporins (Cefoxitin and Ceftazidime), additionally Macrolides (Erythomycin) and Lincosamides (Clindamycin) were used.

Analyzing table 35, it was found that in the male and female participants both presented a very scarce percentage of multiresistant bacteria, 0.7% and 0.8% of the overall samples respectively. Both genders did not present any multiresistant bacteria during the first sampling and only one in the second sampling.

When observing table 35 for the results obtained for the percentage of participants that presented multiresistant bacteria, it was found that only one individual of each gender had their hands contaminated with them (3.3%). It was noticed that the

overall results for both genders were the same and the percentages were low which was very positive.

In an overall approach, 0.8% of the screened bacteria, excluding MRSA, presented resistance to 3 different antibiotic classes that were used and 3.3% (2/60) of the participants presented these multiresistant bacteria. These two participants frequented the Forensics course and both samples were hemolytic bacteria.

Table 35: Percentage of screened samples and participants, by gender, that presented bacteria with multiresistance to antibiotics.

Multiresistance	*1st sampling*	*2nd sampling*	*Sampling mean*	*Participants*
♂ *Participants*	(0/72)	(1/66)	(1/138)	(1/30)
(%)	0	1.5	0.7	3.3
♀ *Participants*	(0/66)	(1/60)	(1/126)	(1/30)
(%)	0	1.7	0.8	3.3
Overall Total	(0/138)	(2/126)	(2/264)	(2/60)
(%)	0	1.6	0.8	3.3

4.4.3 – Resistance to Erythromycin

It was noted that a high percentage of the selected bacteria that were screened for antibiotic resistance, presented resistance to Erythromycin (44.7%) (table 33). The resistance to Erythromycin in staphylococci is conferred by *erm* genes (*ermA*, *B* and *C*), additionally the *msrA* gene also enables this resistance. This antibiotic is a macrolide as can be verified in appendix 2, and is widely used for the treatment of human and animal infections.

The study elaborated by Duran and co-workers detected a resistance to Erythromycin in 48.7% of the staphylococci screened and in 60.4% of *S. aureus*, being

consistent with the percentage obtained in this work as not all bacteria screened are exclusively *S. aureus* [54][56][57].

Lower percentages were verified in a study developed by Srikanth and co-worker, as 82.8% of susceptibility by Methicillin-sensitive *S. aureus* (MSSA) were verified [113]. These studies come to show that resistance to Erythromycin can be a common occurrence in staphylococci.

Although genetic methods were not applied to verify the presence of the different resistance genes, previous studies, including Duran's research, demonstrated that these genes were more frequently found in *Staphylococcus aureus* rather than in coagulase negative staphylococci (CoNS) [54].

4.4.4 – Oxacillin and Cefoxitin as presumptive indication of MRSA

The antibiotics Oxacillin and Cefoxitin could be used as presumptive screening of the presence of Methicillin-resistant *Staphylococcus aureus* [118]. This presumptive screening was executed by a disk diffusion test using the Mueller-Hinton agar as described in the chapter "Materials and Methods". They are both β-lactams and Oxacillin is a Penicillin whilst Cefoxitin is a Cephalosporin (appendix 2).

Nowadays Methicillin is not used in susceptibility testing, being replaced by Oxacillin due to the fact that it is a more stable anti-staphylococcal Penicillin [69]. The Clinical and Laboratory Standards Institute (CLSI), recommends the implementation of a Cefoxitin or an Oxacillin disk diffusion test as alternative methods of screening for MRSA presence. The chromID MRSA media permits the identification of MRSA colonies, as previously detailed, and these media contain the antibiotic Cefoxitin in their composition [45][69][118][59].

Cefoxitin is a potent inducer of the *mecA* regulatory system, being therefore widely used as screening tool for the detection of *mecA* gene-mediated Methicillin resistance. In the presence of Cefoxitin, MRSA strains presenting inducible resistance

to Methicillin exhibit a far more rapid growth, than in the presence of Oxacillin. This fact occurs due to the capability of Cefoxitin to enhance the induction of PBP2a [59].

In more recent studies, including Rao's research, it has been revealed that the Cefoxitin disk diffusion test is far more efficient than most phenotypic methods employed, such as the Oxacillin disk diffusion test, formerly mentioned, as Cefoxitin enables only the detection of MRSA that express a *mecA*-mediated resistance mechanism [59].

All the bacteria that presented resistance or intermediate sensibility to the antibiotics Oxacillin and Cefoxitin are presented in table 36. The samples that show "(2nd)" refer to bacteria collected from the second sampling. Only two exhibited resistance to Cefoxitin, whilst all presented resistance to Oxacillin. It is noteworthy that only one of the six samples (♂7COH-C. Preta (2nd)) presented growth in the chromID MRSA medium, however it only presented intermediate sensibility to Cefoxitin.

Table 36: Antibiotic susceptibility patterns of the samples that presented resistance to the antibiotic Oxacillin.

Samples	*FOX*	*AMC*	*CAZ*	*DA*	*E*	*OX*
♂7COH-C. Preta (2nd)	Intermediate (18 mm)	Sensitive	Intermediate (16 mm)	Sensitive	Sensitive	Resistant
♂18COH-Hemo (2nd)	Resistant	Intermediate (20 mm)	Sensitive	Resistant	Resistant	Resistant
♀2CHAP-SA	Intermediate (15 mm)	Resistant	Intermediate (15 mm)	Sensitive	Sensitive	Resistant
♀12COH-C. Preta	Intermediate (21mm)	Sensitive	Sensitive	Sensitive	Intermediate (19 mm)	Resistant
♀15COH-Hemo (2nd)	Resistant	Intermediate (17 mm)	Intermediate (16 mm)	Resistant	Resistant	Resistant
♀28COH-Hemo (2nd)	Intermediate (15 mm)	Sensitive	Intermediate (16 mm)	Sensitive	Sensitive	Resistant

FOX – Cefoxitin; AMC – Amoxicillin + Clavulanic Acid; CAZ – Ceftazidime; DA – Clindamycin; E – Erythromycin; OX – Oxacillin.

When the percentage of growth in the chromID MRSA media by all the samples was analyzed, it was found that of the 120 samples (60 of the 1st sampling and 60 of the 2nd sampling), only 7 in total presented growth. Of these 7 samples, only one was female (♀17). When observing their antibiotic resistance screening, it was found that 5 out of the 7 bacteria presented resistance to Oxacillin, which may indicate MRSA presence. Relatively to Cefoxitin, only one presented resistance to this antibiotic, although 3 presented intermediate susceptibility.

Sensibility to this antibiotic is exhibited when their inhibition zones were ≥ 22 mm and resistance is verified when the inhibition zones are <15 mm. The intermediate susceptibility presented were closer to the resistance inhibition zone determinant than to the sensibility determinant inhibition zone.

Resistance and intermediate susceptibility dominates in table 37, more so in the last 4 included samples (♂6 (2nd), ♂7 (2nd), ♂22 and ♀17), where sensitivity is verified to 0 to 2 antibiotics. Interestingly but consistent, these were the only samples, out of the 7 genetically tested, that had the presence of the *mecA* gene which will be addressed in another section further on. It was found that all the true MRSA exhibited multiresistance to antibiotics, characteristic of HA-MRSA previously detailed [70].

A study developed by Gonsu and co-workers demonstrated that multiresistance to antibiotics is highly present among MRSA. From the 18.4% of samples that presented multiresistant bacteria, 76% of those bacteria were MRSA [119]. In this study 2 MRSA were multiresistant, and it is preoccupying as samples were taken from participants with supposedly no frequent contact with healthcare settings, contrarily to the healthcare professionals as in the Gonsu study.

The research developed by Srikanth and co-workers presented susceptibility patterns found for MRSA in healthcare settings [113]. Relatively to Erythromycin, Oxacillin and Clindamycin, sensitivity was found in 37.2%, 6.8% and 29.3%, correspondingly [113].

In a study conducted by Rahimi and co-workers, it was found that in healthcare settings 89.8% of MRSA were resistant to Erythromycin and 83.9% resistant to Clindamycin, which are values considerably higher than what was presented by Srikanth's research [114].

However, the results obtained in this work do follow the tendency presented by both studies, excluding what was obtained for Clindamycin.

Table 37: Antibiotic susceptibility patterns of the bacteria that presented growth in the chromID medium.

Samples	*FOX*	*AMC*	*CAZ*	*DA*	*E*	*OX*
♂*1*	Sensitive	Intermediate (14 mm)	Intermediate (20 mm)	Sensitive	Resistant	Sensitive
♂*2*	Sensitive	Sensitive	Intermediate (15 mm)	Sensitive	Resistant	Resistant
♂*7*	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Sensitive
♂*6 (2nd)*	Intermediate (17 mm)	Intermediate (16 mm)	Resistant	Sensitive	Sensitive	Resistant
♂*7 (2nd)*	Resistant	Resistant	Resistant	Sensitive	Intermediate (20 mm)	Resistant
♂*22*	Intermediate (16 mm)	Resistant	Resistant	Sensitive	Resistant	Resistant
♀*17*	Intermediate (18 mm)	Intermediate (16 mm)	Intermediate (16 mm)	Resistant	Resistant	Resistant

FOX – Cefoxitin; AMC – Amoxicillin + Clavulanic Acid; CAZ – Ceftazidime; DA – Clindamycin; E – Erythromycin; OX – Oxacillin.

4.4.5 – Individual characteristics of the participants that presented MRSA

The individual characteristics have been addressed previously, although it was found important to analyze the differences and congruencies between the participants whose samples presented potentially MRSA growth (table 38).

It is to be noted that only one of the six samples who presented growth was from a female participant. That indicates that MRSA were mainly present in male participants. Only one of the samples was inoculated from a mobile phone with a keypad, which could designate that touchscreens are more prone to adhere this kind of bacteria, which is contradictory to the research developed by Pal and co-workers [14]. However, the sample pool of participants that own a mobile phone with keypad was reduced and as such it cannot be assumed as a significant evaluation.

In terms of nail length, five of the individuals presented short nails, which supposedly should carry a considerably lower number of bacteria, as stated by the Public Health Agency of Canada and the study developed by Rothrock and co-workers [44][86].

On the other hand, most of these participants (4/5) stated that they did not have special hygienic attention with their hands and nails, which could explain the presence of these bacteria, as can be verified by the study developed by Bhat and a report emitted by the Public Health Agency of Canada and the WHO [7][41][45].

The female participant, exhibited long nails and although stated that a special attention to the hygiene of the hands and nails was rendered, MRSA was found. The reported of the Public Health Agency of Canada stated that even when hand washing and cleaning is thorough, that bacteria can still be found persistently on the skin underneath the nails [44].

Five out of six of the participants stated that that they did not have a special hygienic care of their mobile phones, which could possibly prolong the time that these devices serve as reservoirs of these kinds of resistant bacteria, and potentially cause bacterial transmission to their handlers and third parties, thus being considered potentially dangerous, as shown by Bhat, Shahaby and Famurewa [7][4][120].

The majority does not take their mobile phone with them to the bathroom, which is considered a preventive behavior, as the act of taking the devices to the bathroom

could potentially increase the bacterial count and the risk of contracting pathogenic bacteria, especially fecal bacteria (ex. *E. coli*) [88].

In terms of hand washing behavior all the participants stated that they washed their hands after the use of the bathroom and that they washed their hands 5 to 10 times a day, which is the normal average considering the standard deviation presented by Larson and co-workers [108][95].

Nail and/or skin biting was observed in half of the participants, which is high considering that Ghanizadeh reaffirmed that it is mainly a behavior of children which gradually disappears [90]. This behavior could possibly be dangerous due to the fact that bacteria can lodge themselves underneath the nails and by putting the fingers in direct contact with the oral cavity, the present pathogenic bacteria can be transmitted directly to the individual's oral cavity and potentially cause disease development. It can also contribute to damaging the surrounding skin which can lead to infection, and as such harbor higher bacterial counts as stated by Ghanizadeh [90].

As previously stated, pets can carry bacteria that are potentially dangerous to humans but do not affect them [91] [94]. The majority of the participants own pets and in conjunction with the nail and/or skin biting, not properly washing hands, not cleaning nails, etc. this could be dangerous, as animals also carry MRSA and *E. coli* which could induce disease in individuals due to close contact with the pets, as demonstrated by Costa and co-workers [110][69][99].

Table 38: Individual characteristics, attained by the questionnaire, of the participants that presented MRSA growth.

Questions	♂*1*	♂*2*	♂*6*	♂*7*	♂*22*	♀*17*
Keyboard type	Keypad	Touch screen	Touch screen	Touch screen	Touch screen	Touch screen
Nail length	Short	Short	Short	Short	Short	Long
Special hygiene hands/nails	Yes	No	No	No	No	Yes
Special hygiene Mobile phone	No	No	No	No	No	Yes
Hand washing after bathroom use	Yes	Yes	Yes	Yes	Yes	Yes
Take mobile phone to bathroom	No	Yes	Yes	No	No	No
Nail/skin biting	Yes	Yes	No	Yes	No	No
Pet owner	Yes	Yes	No	Yes	Yes	Yes
Hand washing a day	5-10x	5-10x	5-10x	5-10x	5-10x	5-10x

4.5 – MRSA genetic characterization

The MRSA bacteria presents the characteristic *mecA* gene which is encoded by a mobile genetic element (MGE) Staphylococcal cassette chromosome (SCC) which can be acquired by *S. aureus*, as formerly extensively explained, and thus confer β-lactam resistance to the bacteria [49]. They can also confer resistance to other antibiotics, increasing the health risk they pose.

There exist a variety of *SCCmec* elements, being divided in classes (A to E) and types (I to VIII). The classes were differentiated based on the structural organization of the *mec* operon [64][11]. These classes of the *mec* complex additionally to the four different *ccr* allotypes define the eight SCC*mec* types described to the present day (table 38). Depending on the J region variations, they can be further subtyped [64].

Livestock-associated MRSA (LA-MRSA) SCC*mec* elements were recently discovered and classified, IX, X and XI [46]. Although, the SCC*mec* type XI presents a divergent *mecA* gene homologue (*mecA*$_{LGA251}$) [65].

4.5.1 – *mecA* gene detection

As previously stated, to detect the presence of a MRSA the *mecA* gene requires to be present, which is found at the 162 bp band [66]. The seven samples collected that presented growth in the chromID MRSA medium were submitted do DNA extraction with subsequent DNA amplification. The primers used are specific for the targeted *mecA* encoding area and were formerly described in the chapter "Materials and Methods".

After the corresponding procedures and the certification that they were properly executed in optimal conditions, the results can be observed in figure 22 and 23. There was no contamination of the samples, as can be verified in both gels by the lack of bands in the negative control well run. The extra bands verified in the wells of the samples ♂1, ♂2 and ♂7 in figure 25 were due to unspecific linkage. It is clearly verified that in this run only one of the 4 samples presented the *mecA* gene, sample ♀17, whilst all of them presented growth in the specific MRSA medium.

Still from the first sampling, the sample ♂22 presented the *mecA* gene band as is exhibited figure 25. It may not be a bright band, due to the low concentration in DNA, but is obviously present.

From the second sampling, both samples ♂6 and ♂7 presented the *mecA* gene as is shown in figure 23. The photograph was somewhat overexposed in terms of light, hindering the perception of the genetic marker’s bands but the distinctive 162 bp band can clearly be identified.

From the seven samples that presented bacterial growth in the chromID MRSA medium, only four presented themselves to be MRSA. These were submitted to

multiplex PCR as described in the chapter "Materials and Methods", in order to be able to typify their SCC*mec* element and understand if the strain contracted is originated from a healthcare setting or community setting.

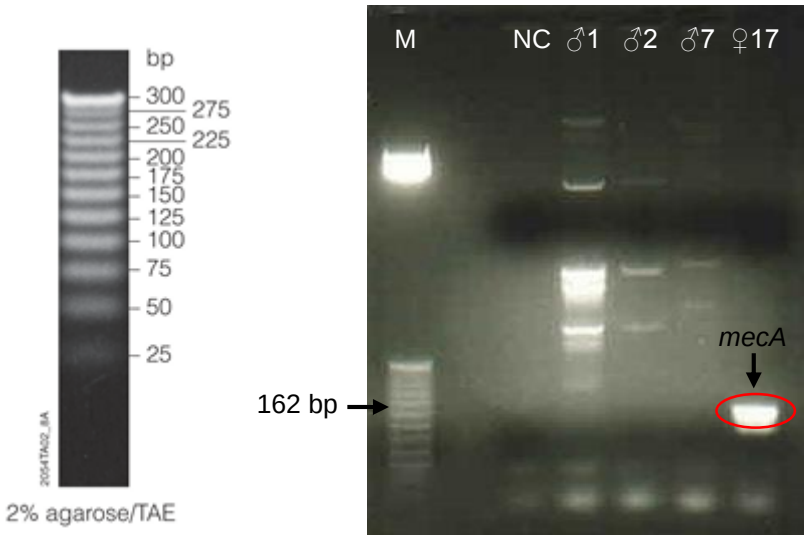


Figure 21 and 22: Genetic marker used (25 bp DNA Step Ladder by Promega®); Electrophoresis gel results from the *mecA* gene amplification of the samples ♂1, ♂2, ♂7 and ♀17. Aditional M – DNA marker and NC – Negative control.

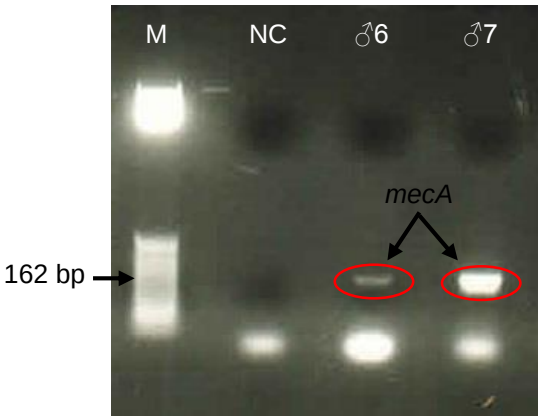


Figure 23: Electrophoresis gel result from the *mecA* gene amplification of the samples ♂6 (2nd sampling) and ♂7 (2nd sampling). Aditional M – DNA marker and NC – Negative control.

4.5.2 – SCC*mec* element detection and typification

The SCC*mec* element is inserted into the *orfX* gene in the *S. aureus* chromosome [64]. There are different types (I to XI) as previously explained, although the primers used, that were described in the "Materials and Methods"- chapter only permit the identification of SCC*mec* element I to VI. By executing the multiplex PCR and thus adding various primers corresponding to a diversity of specific encoding regions, it was possible to typify those six different types of SCC*mec* by only running one amplification program [66].

The gel observed in figure 25 demonstrates that there was not contamination of the samples, as can be verified by the negative control. The marker's bands, although faint, present a good separation enabling a good reading of the bands resulted from the samples. With the multiplex PCR executed, the *mecA* corresponding primers were also amplified as to function as an internal control. All but one of the samples presented the characteristic 162 bp band, whilst could be due to the DNA saturation of the other primers thus being overshadowed and not being visible.

Only two of the four samples presented bands for SCC*mec* typification, samples ♂6 (2^{nd}) and ♂7 (2^{nd}). These presented the bands 284 and 342, and 243, respectively. It was noted that with only these bands, the SCC*mec* elements could not be classified, as the 209 bp band in both and additionally the 414 in the ♂7 (2^{nd}) sample were not present (figure 25). This may be due to the primers not working properly in the annealing phase of the amplification. Therefore, the amplification was repeated many times, with adjusted settings for the PCR and electrophoresis, but this was the optimal result that could be obtained (figure 25). The ♂6 (2^{nd}) sample presented two bands that did not correspond to any bands that characterizes de SCC*mec* element, one above the 700 bp band and another above the 500 bp band, this may be due to the occurrence of nonspecific linkage.

Taking this into account, the samples can be presumptively classified as SCC*mec* type II for sample ♂6 (2^{nd}) and SCC*mec* type III for sample ♂7 (2^{nd}), as can

be verified in table 39. Both samples were presumptively determined as being healthcare-associated MRSA (HA-MRSA) as can be observed in table 41, which is preoccupying as this type of MRSA acquired resistance to various other antibiotics, therefore increasing the health risk. If these can really be found in community settings, such as Universities, this may present a public health risk if not properly diagnosed and controlled [69][45].

The samples ♀17 and ♂22 did not present any bands other than the 162 bp *mecA* gene band, this could indicate that they are a SCC*mec* type other than the reach of the multiplex PCR executed (SCC*mec* I to VI). Thus these samples could not be typified.

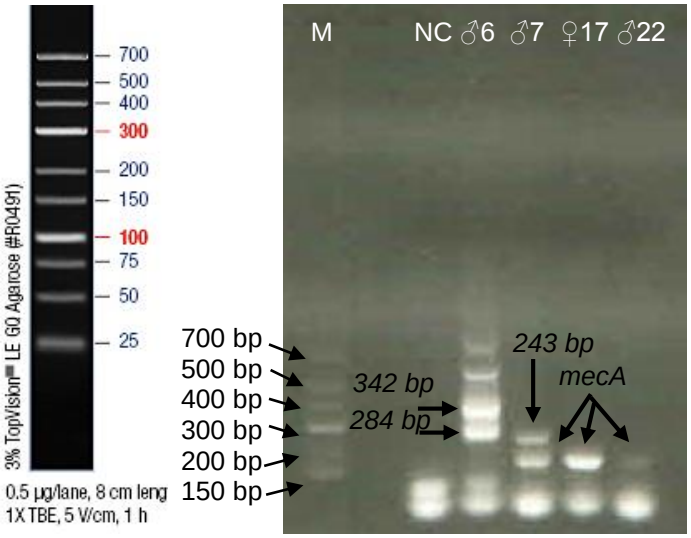


Figure 24 and 25: Genetic marker used (GeneRuler Low Range DNA Ladder, Ready-to-Use 25 to 700 bp by Thermo Scientific®); Electrophoresis gel result from the amplification of the different SCC*mec* types of the samples ♂6 (2nd sampling), ♂7 (2nd sampling), ♀17 and ♂22. Aditional M – DNA marker and NC – Negative control.

Table 39: Characterization of the different SCC*mec* types when resorting to multiplex PCR strategy, adapted from [84].

SCCmec type	*Number of bands*	*Band size*	*Varients*
SCCmec I	2	495 bp 342 bp	-
SCCmec II	3	342 bp 284 bp 209 bp	-
SCCmec III	3	414 bp 243 bp 209 bp	SCC*mec* IIIB – Absence of the bands 414 and 243 bp
SCCmec IV	2	342 pb 311 pb	SCC*mec* IVE and IVF – Absence of the band 342 bp
SCCmec V	2	449 bp 377 bp	-
SCCmec VI	1	342 bp	-

Bp – Base pair.

The SCC*mec* types persumptively obtained for this research were SCC*mec* element II and SCC*mec* element III, samples ♂6 (2nd) and ♂7 (2nd) respectively. As can be observed in table 40, they may present other resistance determinants. In this study, only resistance to Erythromycin was tested out of all the antibiotics presented in this table.

Resorting to table 37, it can be seen that the sample ♂6 (2nd), which was presumptively assumed to be SCC*mec* type II, presented susceptibility to Erythromycin, which should not be exhibited. As previously stated, the classification of the SCC*mec* types was not conclusive as bands were absent. Since there exist more SCC*mec* elements than tested for in this research (tested: I to VI; exist: I to XI) and some characteristics overlap as the *ccr* genes and *mec* complex, this MRSA sample

may be actually another SCC*mec* type, although this could not be confirmed (table 40 and 41) [11].

Relatively to the SCC*mec* type III of the sample ♂7 (2nd), it is also verified that it should present additional resistance to Erythromycin. As can be seen in table 37, this sample only presented intermediate resistance to Erythromycin and a large inhibition zone of 20 mm which is closer to the susceptibility inhibition zone (≥ 22 mm) than to the resistance inhibition zone (<17 mm) (appendix 2). This is also not consistent with what was expected, which can also be explained by the reasons previously stated for the sample ♂6 (2nd).

Table 40: Characteristics of the eight types of the SCC*mec* elements, adapted from [49].

SCCmec type	*mec complex*	*ccr genes*	*size*	*Other resistance determinants*
I	Class B-E	*ccr*A1B1	34 kb	None
II	Class A	*ccr*A2B2	52-58 kb	Erythromycin, spectinomycin, bleomycin, tetracyclin
III	Class A	*ccr*A3B3	67 kb	Erythromycin, spectinomycin, tetracyclin, mercury, cadium
IV	Class B-E	*ccr*A2B2 or *ccr*A4B4	20-25 kb	None
V	Class B-E	*ccr*C	28 kb	None
VI	Class B	*ccr*B4	20-25 kb	None
VII	Class C	*ccr*C2, *ccr*C8	28-30 kb	None
VIII	Class A	*ccr*A4, *ccr*B4	32 kb	Erythromycin, spectinomycin

Kb – Kilo-base.

Table 41: Characteristics of the three recently found types of SCC*mec* elements, adapted from [67][68].

SCCmec type	*mec complex*	*ccr genes*
IX	Class C2	*ccr*A1B1
X	Class C1	*ccr*A1B6
XI	Class E	*ccr*A1B3

As formerly presumptively obtained, the SCC*mec* types obtained (II and III), were both classified as healthcare-associated MRSA (HA-MRSA). In table 42 the difference between HA-MRSA and CA-MRSA can be observed. It is noted that HA-MRSA are far more dangerous strains than CA-MRSA, although the Panton-Valentine Leukocidin (PVL) gene is present in this last type and produces toxins that increase the health risk when these bacteria are contracted [45][11].

It was found strange that HA-MRSA were found in the ISCSEM faculty as these bacteria are associated with healthcare setting, although it is a healthcare faculty and a part of the campus incorporates a dental clinic, the participants of this study have no reason to come in close contact with this clinic, only if they were to be treated there. So the origin of the MRSA that they carry could not be justified this way. If HA-MRSA is becoming mainstream in community settings it could become a general public health problem if proper measures are not taken, as can be verified in table 42 and as has already been formerly stated.

A study developed by Chawla and co-workers researched pathogen presence on mobile phones of healthcare professionals and other arbitrary individuals. It was found that MRSA was found on 20% of the healthcare professionals mobile phones whilst none was found on the remaining individuals [121]. As it was assumed that the bacteria present on the hands are the same as can be found on the mobile phones, this study once again shows that MRSA should not be easily found in the community, especially HA-MRSA.

Table 42: Differences between healthcare-associated MRSA and community-associated MRSA, adapted from [84].

Characteristics	*HA-MRSA*	*CA-MRSA*
SCCmec type	I-III, VI and VIII	IV, V and VII
Antibiotic resistance	Multiresistant	Normally limited to β-lactams and erythromycin
Toxin presence	Few	Many
PVL gene	Rare	Common
Epidemiology	Associated to healthcare settings and the elderly	Young individuals, athletes, military soldiers and substance abusers
Infections	Septicemia, urinary and respiratory infections	Skin and soft tissue infections

HA-MRSA – Healthcare-associated Methicillin-resistant *Staphylococcus aureus*; CA-MRSA – Community-associated Methicillin-resistant *Staphylococcus aureus*; PVL - Panton-Valentine Leukocidin.

4.5.3 – MRSA classification

The MRSA that were found in this study were classified as being HA-MRSA. In table 43 a detailed list of difference can be found between HA-MRSA and CA-MRSA. It is found that many characteristics differentiate these two types of MRSA and that HA-MRSA poses a higher threat to individuals. This is due to the fact that it develops more dangerous and serious illness in susceptible individuals, and has the ability to acquire resistance to various antibiotics [65].

As can be verified in table 43, supposedly, there is little spread among household contacts and community spread is rare. This contradicts what was verified in this study, as both MRSA typified were considered to be HA-MRSA. The participants were healthy young adults and University students who probably do not come in contact with healthcare settings unless necessary.

The samples collected from the chromID MRSA medium did present resistance to various antibiotics, especially those who presented the *mecA* gene and were classified as true MRSA. The resistance to Erythromycin was widely observed throughout all samples but it was rare to attain samples that presented resistance to antibiotics of more than 3 different antibiotic classes that were screened for, besides those who were genetically characterized for *mecA* gene presence and SCC*mec* element typification.

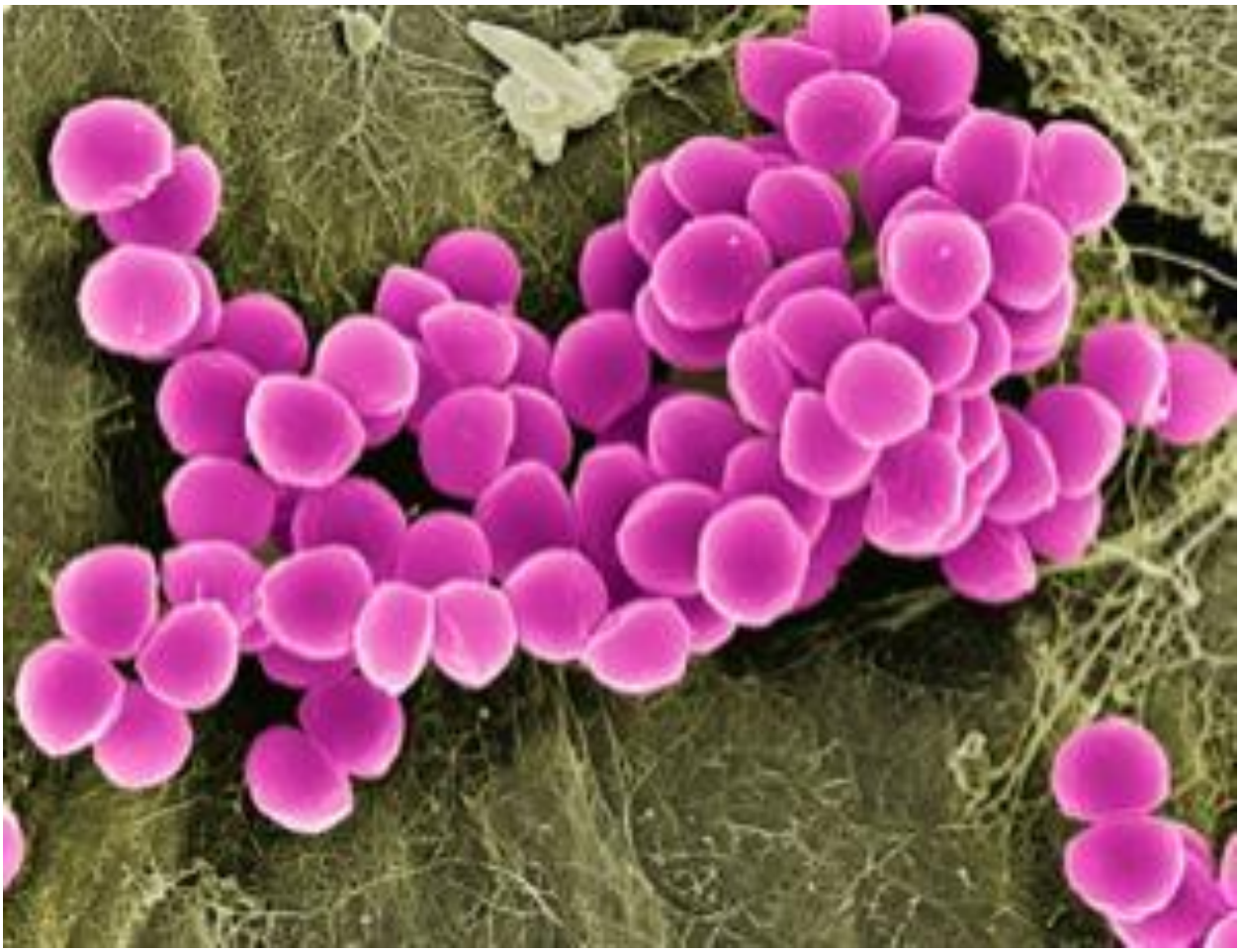
One of the characteristics that might have assisted in the differentiation of HA-MRSA and CA-MRSA in this case, was the detection of the Panton-Valentine Leukocidin gene in the samples. However this was not done due to time limitations.

Table 43: Main differences between healthcare-associated MRSA and community-associated MRSA, adapted from [109].

Characteristics	*HA-MRSA*	*CA-MRSA*
Normally infected individuals	Elderly, immunocompromised, critically or chronically ill.	Young healthy individuals, students, professional athletes and military personnel.
Infection site	Bacteremia with no obvious source of infection, surgical wounds, open ulcers, IV lines, catheters and ventilators	Skin and soft tissue infection, producing cellulitis and abscesses.
Transmission	Within healthcare settings; little spread among household contacts	Community acquired; may spread in families and sports teams
Diagnosis setting	In an in-patient setting (hospitalization)	In an outpatient or community setting
Medical history	History of MRSA colonization, infection or recent surgery; hospitalization; antibiotic use; dialysis; permanent indwelling catheter and other intravenous devices	No significant medical history or healthcare contact
Strain virulence	Community spread is rare and PVL genes are usually absent	Community spread occurs easily and PVL genes often present
Antibiotic susceptibility	Multiresistant with a very limited choice of therapeutic agents	Resistant to β-lactams and normally susceptible to more antibiotics than HA-MRSA

HA-MRSA – Healthcare-associated Methicillin-resistant *Staphylococcus aureus*; CA-MRSA – Community-associated Methicillin-resistant *Staphylococcus aureus*; PVL - Panton-Valentine Leukocidin.

V – CONSLUSIONS AND FUTURE PERSPECTIVES



(http://blog-galvinengineering.com.au/wordpress-3.2.1/wordpress/wp-content/uploads/2011/11/MRSA.jpg)

5 – Conclusions and Future Perspectives

It was found that male participants had more contaminated hands than the female participants, due to the overall higher bacterial count for potentially pathogenic bacteria and MRSA.

Female participants were more conscious in terms of hygienic attention to both their mobile phones and their hands, and therefore presented lower CFU counts.

It was found, in general, that mobile phones with a keypad were more contaminated than touchscreen keyboards, and that both can effectively be reservoirs of potentially pathogenic and multiresistant bacteria.

The individual characteristics significantly influenced the level of contamination of both mobile phones and hands.

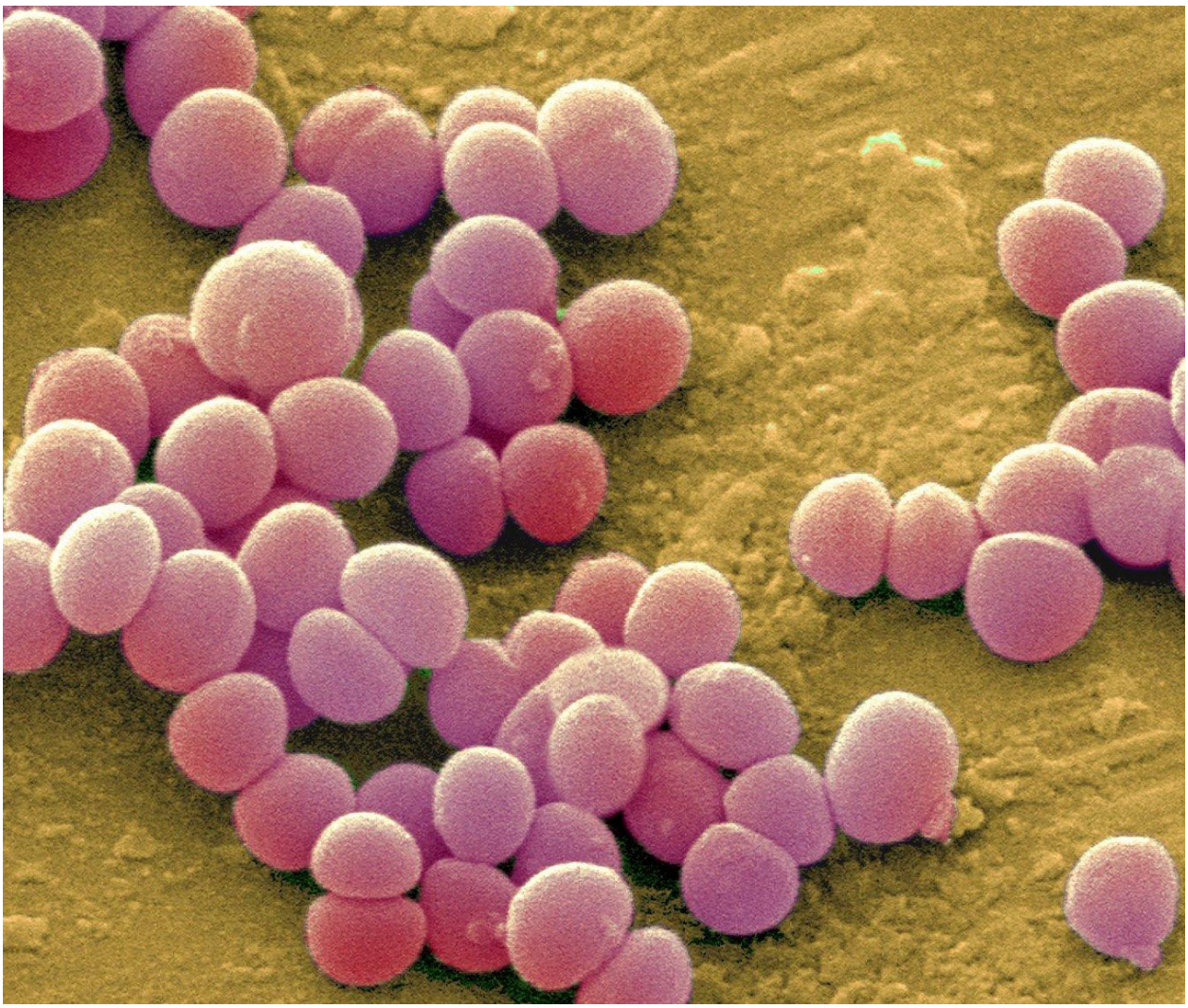
A high resistance to Erythromycin was detected and a low percentage of participants carried bacteria resistant to multiple antibiotics. Presumptive HA-MRSA was found in healthy young adults.

Awareness should be raised to the general public, as to understand that mobile phones can carry potential pathogens. Rigorous hygienic standards should be established in healthcare settings in relation to these devices, and to the touchscreens that are present in their medical devices.

Innovations as developed by the Copper Development Association Inc. and Corning Inc. should be carried on as they are crucial to battle the transmission of pathogenic and antibiotic resistant bacteria in healthcare settings and in the general public, as the threat will continue to increase.

Bacterial presence on these devices should be further investigated in terms of microflora, as specific strains of bacteria may be present. It should also be verified which types of MRSA are mainly present on touchscreens and the threat they pose to public health. HA-MRSA can be disseminated throughout the community by the mobile phones and even hands of healthcare professionals.

VI – Bibliography



(http://galleryhip.com/mrsa-bacteria.html)

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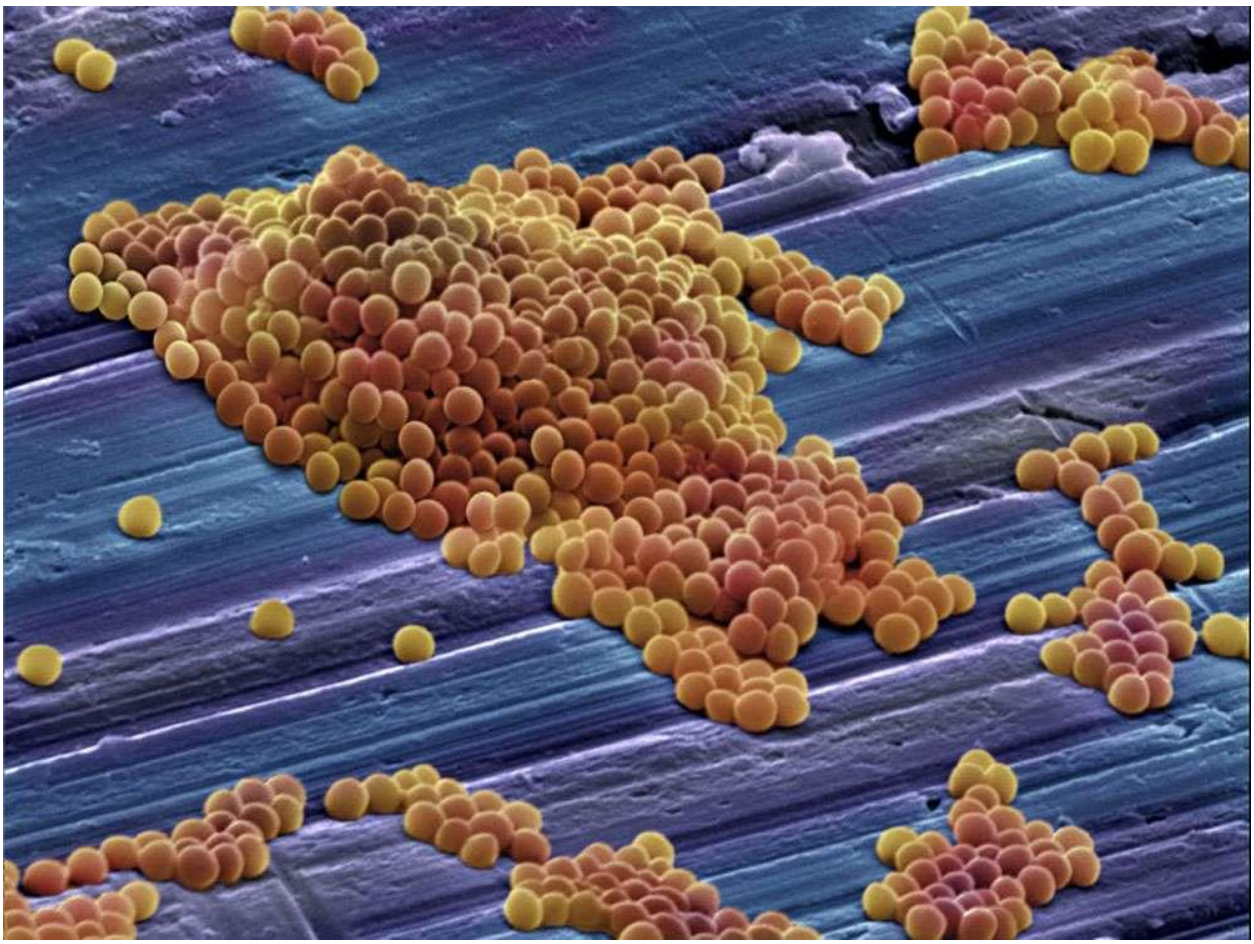
VII – APPENDIX



(http:// www.sanger.ac.uk/about/press/2010/100121.html)

7.1 – Informed consent form and questionnaire

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Informação ao participante

Leia com atenção a informação que se segue:

Eu, Ana Petronella Vasconcelos Danen, aluna de segundo ano de Mestrado em Microbiologia, na Universidade de Aveiro, estou a elaborar uma dissertação que irá centrar-se na hipótese de os ecrãs tácteis dos telemóveis reflectirem a flora comensal do seu utilizador, e gostaria de solicitar a sua participação neste estudo.

Para a elaboração deste estudo necessitarei de recolher amostras das mãos e do telemóvel do participante. As recolhas serão efectuadas com uma zaragatoa, sem causar dano ao telemóvel ou invasão para com o portador. Estas amostras serão analisadas a nível fenotípico e genotípico de modo a verificar se existe correspondências entre o dispositivo e o utilizador, e se existem bactérias que possam ter um risco associado. Adicionalmente, necessitarei de efectuar um questionário confidencial de modo a entender se existe uma correlação entre os cuidados de higiene indicados e os resultados obtidos. Será posteriormente efectuada uma nova recolha de amostras das mãos e do telemóvel, de modo a verificar se ocorreram alterações ao nível da flora comensal.

Todas as informações recolhidas para o desenvolvimento deste estudo não serão utilizadas para quais quer outras actividades, sendo confidenciais, não revelando informações pessoais nem a identidade do participante.

O objectivo do estudo é verificar se a partir do telemóvel será possível identificar o seu portador e se tanto o portador como o telemóvel são possíveis reservatórios de bactérias específicas. É também um objectivo, entender se no telemóvel e nas mãos se podem encontrar bactérias multirresistentes, constituindo um factor de risco para a saúde do indivíduo e terceiros. Irão ser englobados indivíduos com e sem factor de risco (profissionais de saúde).

Condições de participação

Neste estudo não será efectuado qualquer tipo de diagnóstico, os dados recolhidos serão somente utilizados para um estudo estatístico e comparativo.

O estudo abrange qualquer tipo de participante que possua um telemóvel com ecrã táctil ou teclas, do sexo masculino ou feminino, indivíduos aleatórios e profissionais de saúde.

O participante tem de concordar fazer uma segunda recolha de amostras e a participar no questionário. Não poderá efectuar qualquer acto de higiene fora do comum ou específico antes da recolha das amostras, comprometendo assim a análise. Relativamente ao questionário, deve responder com veracidade, de modo a obter resultados consistentes e não comprometer o estudo.

Obrigado.

Consentimento Informado

Tomei conhecimento e foram prestadas todas as informações relacionadas com os objectivos e métodos do estudo, tendo sido esclarecido(a) em todas as minhas dúvidas e questões. Além disso fui informado(a) que sou livre de aceitar ou recusar participar neste estudo. Poderei em qualquer momento pedir informação complementar sobre o mesmo, e, se o desejar, parar a minha participação sem suportar nenhuma responsabilidade.

Aceito participar neste estudo e autorizo a recolha de amostras das mãos e telemóvel, o armazenamento da minha informação e a transferência e publicação dos meus dados.

Nome do(a) Participante:

Assinatura do(a) Participante:

Confirmo que, sobre este estudo, tudo foi explicado ao/à participante acima referido(a).
Assinatura da aluna:

Monte da Caparica,___de_____de 2013/2014

universidade de aveiro
departamento de biologia
debio

Formulário de participação no estudo de dissertação 2013/2014.

Sexo: Tipo de ecrã:

Idade: Tamanho das unhas:

Profissão: Manicure:

Marca e modelo de telemóvel: Anéis:

Perguntas:

1- Tem uma atenção especial com a higiene das unhas/mãos?
   - ○ Sim Qual:
   - ○ Não

2- Tem uma atenção especial com a higiene do telemóvel?
   - ○ Sim Qual:
   - ○ Não

3- Lava as mãos depois de utilizar a casa de banho?*
   - ○ Sim
   - ○ Não

4- Leva o telemóvel consigo para a casa de banho?
   - ○ Sim
   - ○ Não

5- Roí as unhas/peles?
   - ○ Sim
   - ○ Não

6- É dono(a) de animais de estimação, e se sim qual?
   - ○ Sim Qual:
   - ○ Não

7- Quantas vezes por dia lava as mãos?*

- Menos de 5 vezes
- 5 a 10 vezes
- 10 a 15 vezes
- Mais de 15 vezes

*Por lavar das mãos entende-se que seja utilizado qualquer tipo de sabonete.

7.2 – Antibiotic information and inhibition zones

Antimicrobial Agent	*Classification*	*Disk code*	*Potency*	*Sensitive (Diameter in mm)*	*Intermediate (Diameter in mm)*	*Resistant (Diameter in mm)*
Amoxicillin + Clavulanic Acid	β-lactam/ Penicillin	AMC	20/10 µL	≥ 21	14-20	< 14
Cefotaxime	β-lactam/ Cephalosporin	CTX	30 µL	≥ 21	15-20	< 15
Cefoxitin	β-lactam/ Cephalosporin	FOX	30 µL	≥ 22	15-21	< 15
Ceftazidime	β-lactam/ Cephalosporin	CAZ	30 µL	≥ 21	15-20	< 15
Ciprofloxacin	Quinolone/ Fluoroquinolone	CIP	5 µL	≥ 22	19-21	< 19
Clindamycin	MLSK Group/ Lincosamide	DA	2 µL	≥ 15	N/a	< 15
Erythromycin	MLSK Group/ Marcolide	E	15 µL	≥ 22	17-21	< 17
Gentamicin	Aminoglycoside	CN	10 µL	≥ 16	14-15	< 14
Imipenem	β-lactam/ Penem	IMP	10 µL	≥ 22	17-21	< 17
Oxacillin	β-lactam/ Penicillin	OX	5 µL	≥ 20	N/a	< 20

Source: "Interprétation des zones d’inhibition.", provided by bioMérieux and [122].

7.3 – RTP Bacteria Mini Kit DNA extraction protocol

Protocol 2: Isolation of DNA from bacteria pellets (1 x 10^9 bacteria cells)

Please read protocols prior the start of the preparation and complete preparing steps!!

Important note: *Switch on heating blocks (e.g. thermomixer) to 65℃ and 95 ℃*

Take an aliquot of the bacteria culture and spin it down at 9.300 x g (10.000 rpm) for 3 min. Remove the complete supernatant careful.

1a. For gram positive bacteria

Add 400 µl **Resuspension Buffer R** to the pellet and resuspend the pellet by pipetting up and down. Transfer the resuspended sample into the **Extraction Tube L** and vortex shortly.

Incubate the sample in a thermomixer for 10 min at 37℃ and at 65℃ for 10 min (continuous shaking

increases the lysis procedure, after 8 min its possible to switch the heating block to 65℃ and do the heating of the block to 65° with the sample inside, if the blo ck is not too slow > 4℃ per min, you just do then incubation for 12 min, if your block is slower, you have to elongate incubation time). Continue with step two.

1b. For gram negative bacteria

Add 400 µl **Resuspension Buffer R** to the pellet and resuspend the pellet by pipetting up and

down. Transfer the resuspended sample into the **Extraction Tube L** and vortex shortly. Incubate the sample in a thermomixer at 65℃ for 10 min (continuous shakin g increases the lysis procedure).

Continue with step two.

2. Place the **Extraction Tube L** into a thermomixer and incubate at 95℃ for 5 - 10 min (continuous shaking increases the lysis efficiency).

3. Add 400 µl **Binding Buffer B6** to the sample and vortex shortly.

4. Load the sample onto the **RTA Spin Filter Set** and incubate for 1 min. Centrifuge at 13.400 x g

(12.000 rpm) in a standard table centrifuge for 1 min. Discard the filtrate and place the RTA Spin Filter back into the RTA Receiver Tube.

5. Add 500 µl **Wash Buffer I** and centrifuge at 9.300 x g (10.000 rpm) for 1 min.

   Discard the filtrate, and the RTA Receiver Tube.

   Place the RTA Spin Filter into a new RTA Receiver Tube.

6. Add 600 µl **Wash Buffer II** and centrifuge at 9.300 x g (10.000 rpm) for 1 min. Discard the filtrate, place the RTA Spin Filter back into the RTA Spin Filter and finally centrifuge for 3 min at max. speed to remove the ethanol completely.

7. Place the RTA Spin Filter into a new 1.5 ml Receiver Tube and add 200 µl of **Elution Buffer D**.

   Incubate for 1 min at room temperature. Centrifuge for 1 min at 5.900 x g (8.000 rpm).

Note: *The DNA can also be eluted with a lower volume of Elution Buffer D (depends on the expected yield of bacterial DNA).*