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ORIGINAL ARTICLE

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Virulence, biofilm formation ability and antimicrobial resistance of Staphylococcus aureus isolated from cell phones of university students

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Introduction: Contamination of cell phones can contribute to the dissemination of pathogens in the community and/or hospital environment. Objective: To characterize Staphylococcus aureus strains isolated from cell phones of university students. Methods: Samples were collected from 100 cell phones. Detection of genes associated with virulence factors such as biofilm formation (icaA and icaD), enterotoxins production (SEA, SEB, SEC, and SED), and resistance to methicillin (mecA and mecC) was performed in S. aureus isolates by PCR. Typing mecA gene performed by multiplex PCR. Susceptibility to antimicrobials and biofilm formation rate also evaluated by using disk diffusion test and crystal violet staining. Results: S. aureus was present in 40% of the total samples and about 70% of them belonged to Nursing students. Of the isolates, 85% presented resistance to penicillin and 50% were classified as moderate biofilm producers. In addition, 92.5% of isolates contained the gene icaA and 60% of the gene icaD. Approximately 25% of the isolates presented the mecA gene. Typing of the mecA gene showed the presence of staphylococcal chromosome cassette SCCmec I and c III respectively in 20% and 10% of the isolates. 70% of the samples could not be typed by the technique. Regarding the enterotoxins, the most prevalent gene was SEA (30%) followed by the SEC gene (2.5%). The presence of SED and SEB genes not observed in any of the isolates. Conclusion: The cleaning and periodic disinfection of cell phones can contribute to the reduction of the risk of nosocomial infection.

Keywords: Biofilms; cross infection; drug resistance, microbial; enterotoxins; Methicillin-resistant Staphylococcus aureus; virulence.
INTRODUCTION

Cell phones are devices necessary for both personal and professional life. For health professionals, they can improve communication, promoting collaboration and information sharing\textsuperscript{1,2}.

Cell phones have been frequently used in hospitals and healthcare settings. That fact raises major concerns about nosocomial infections as they may be involved in the transmission of bacteria harboring genes of virulence and resistance, especially in areas requiring the highest standards of hygiene\textsuperscript{2}. More than 50\% of health professionals admit to the use of cell phones in the clinical setting, including during physical contact with patients, while bacterial contamination was observed in up to 25\% of the devices\textsuperscript{3}.

Among the possible contaminants, the presence of \textit{S. aureus} is highlighted. \textit{S. aureus} is a common cause of hospital and community-based infections, and methicillin-resistant \textit{S. aureus} (MRSA) is considered an important nosocomial pathogen\textsuperscript{4}. The presence of these microorganisms on cell devices is a crucial point due to the possible dissemination of antimicrobial-resistant bacteria\textsuperscript{5,6}. In addition, these bacteria can produce biofilm, a structure known as a set of bacteria adhered to a surface and which multiply surrounded by a matrix\textsuperscript{7}.

Students in the health field can be potential disseminators of contaminants since practical classes and clinical stages make possible direct contact with fomites and pathogens\textsuperscript{8}. Several studies have already demonstrated that students in the health area are potential transmitters of pathogenic bacteria due to the use of contaminated objects and lack of hygiene. Garcia et al.\textsuperscript{9} observed the presence of bacteria and fungi in ballpoint pens used by university students. Margarido et al.\textsuperscript{10} demonstrated bacterial contamination on the coats of nursing students after their use in health care practices. Zadai et al.\textsuperscript{11}
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reported the presence of pathogenic bacteria on the surfaces of cell phones of medical students.

In this context, cell phones could harbor a diverse range of species of microorganisms including antibiotic-resistant organisms known to cause nosocomial infections.

This study aimed to evaluate the presence of *S. aureus* on the cell phones of university students, as well as to evaluate the antimicrobial resistance, formation of biofilms, and presence of virulence and antimicrobial resistance genes of these microorganisms.

**METHODS**

**Collection of samples and identification of microorganisms**

Samples were collected from 100 cell phones of students from the Biomedicine (20), Pharmacy (20), Dentistry (20), Nutrition (20), and Nursing (20) courses of a private university from São Paulo State, Brazil, by friction with swabs moistened with sterile physiological saline. The swabs were placed in tubes containing 5 mL of Brain Heart Infusion (Oxoid, Basingstoke, Hampshire and England) broth, and incubated at 37°C for 24 h. Then, *S. aureus* identification tests were conducted through biochemical tests according to techniques already established for Gram-positive such as the catalase test and identification of *Staphylococcus* was tested for coagulase. Also, sa442 DNA fragment amplification was used for genotypic identification of *S. aureus* species. The project was approved by the Research Ethics Committee of Universidade do Oeste Paulista (CAAE: 49967115.8.0000.5515).

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Antimicrobial disc-diffusion technique in Agar

The antimicrobial susceptibility test was performed using the agar diffusion technique, as recommended by the Clinical Laboratory Standards Institute-CLSI. The disks used for the evaluation of antimicrobial resistance were oxacillin, cefoxitin, penicillin, clindamycin, erythromycin, and levofloxacin. For the assessment of multidrug resistance, the MAR index (multiple antibiotic resistance) was determined according to Magiorakos et al.

Evaluation of biofilm formation

The isolates were cultured in BHI broth at 37°C for 24 hours. The cultures were adjusted by spectrophotometry at 600 nm to the value of 0.1. Aliquots of 20 μL of the cell suspension from each isolate added to 200 μL of BHI broth present in the wells of 96 well microplates (COSTAR, Corning Inc., Lowell, MA, United States) and then incubated at 37°C for 24 hours. The plates were washed three times with 0.9% (w/v) saline to remove the unbound cells. The adhered cells were stained with 200 μL of 0.1% (w/v) crystal violet (Sigma-Aldrich, St. Louis, MO, USA), for 5 minutes. The dye was removed, the microplate washed again three times and, after drying for 30 minutes in an oven, the dye solubilization was performed with alcohol/acetone solution (80:20) (Sigma-Aldrich, St. Louis, MO, USA). The optical densities of the solution were read at a wavelength of 590 nm. The value found is representative of bacterial cell adhesion. The mean optical density (OD) of the negative control (ODc) was used as the cutoff point. The isolates classified as: Non-adherent (OD ≤ ODc); Weak adherence (ODc < OD ≤ 2xODc); Moderate adherence (2xODc < OD ≤ 4xODc); Strong adherence (OD > 4xODc).
Detection of virulence and resistance genes by PCR

The phenol-chloroform technique was used to extract DNA from microorganisms. In brief, 1000 µl of bacterial pellet sediment were mixed to 500 µl of lysis buffer [100 mM Tris–HCl (pH=8.0), 50 mM EDTA (pH=8.0), SDS 10%] (Sigma-Aldrich, St. Louis, MO, USA) for one hour 60 °C. When incubation was completed, the supernatant was recovered, washed with 1 ml FCI solution (phenol-chloroform-isoamyl alcohol 25:24:1) (Sigma-Aldrich, St. Louis, MO, USA), and centrifuged at 9,000 rpm for 10 min at 4 °C. This procedure was repeated with the CI solution (chloroform-isoamyl alcohol 24:1) and the supernatant incubated with RNAse (Takara Biotechnology Co. Ltd, Dalian, Liaoning China) for 30 min at 37 °C. It was then precipitated with cold isopropanol and 0.1 volumes of 3 M sodium acetate, and incubated overnight at -20 °C. It was then centrifuged at 9,000 rpm for 10 min at 4 °C, the supernatant decanted, and the pellet washed with cold 70% ethanol. Finally, it centrifuged, the supernatant decanted, the remaining contents allowed to dry, and these resuspended in 50 µl TE buffer.

DNA concentration was evaluated with a nano-spectrophotometer (ND-1000 Nanodrop® Thermo Fisher Scientific, Waltham, MA, USA), using the 260/230 and 260/280 wavelength ranges. Quality was determined by electrophoresis in 1% gel to measure sample contamination and degradation. Subsequently, the DNA was quantified, evaluated for purity and quality and maintained at a temperature of -20°C. The genotypic analysis of the strains was based on the genetic amplification from the PCR technique (polymerase chain reaction) by using the amplification protocol and the oligonucleotides (Table 1) used for the detection of genes sa442, IcaA, IcaD, mecA, mecC, SEA, SEB, SEC, and SED as described according to Martineau et al.13.

As described by Milheiriço et al.18 strains positive for the mecA gene typed by the multiplex PCR technique. All assays were performed in a thermocycler. The optimal
cycling conditions were the following: 94°C for 4 min; 30 cycles of 94°C for 30 s, 53°C for 30 s, and 72°C for 1 min; and a final extension at 72°C for 4 min. Each PCR mixture, in a final volume of 50 μl, obtained 5 ng of chromosomal template; 1× PCR buffer with 1.5 mM MgCl₂ (Applied Biosystems, Foster City, California, EUA), 40 μM (each) deoxynucleoside triphosphate; 0.2 μM primers (IDT, Coralville, Iowa, USA) kdp F1 and kdp R1; 0.4 μM primers CIF2 F2, CIF2 R2, RIF5 F10, RIF5 R13, SCCmec III J1F, SCCmec III J1R, SCCmec V J1 F, and SCCmec V J1 R; 0.8 μM primers mecI P2, mecI P3, dcs F2, dcs R1, mecA P4, mecA P7, ccrB2 F2, ccrB2 R2, ccrC F2, and ccrC R2; and 1.25 U of Amplitaq DNA polymerase (Applied Biosystems, Foster City, California, EUA). Primers sequences shown in Table 2.

The evaluation of the obtained results was performed through visualization of electrophoresis in 3% agarose gel (Applied Biosystems, Foster City, California, EUA), stained with ethidium bromide. The controls used in the reaction were the S. aureus strains COL I, PER IA, HV25 III, and MW2 IV described by Milheiriço et al.18 while the strain S. aureus ATCC 25923 used as a negative control for mecA and virulence factors genes. Staphylococcus epidermidis 12228 used as a negative control for the detection of gene sa442.

**Data analysis**

The experiments were performed in triplicate and the final results were submitted to statistical analysis. For statistical analysis, the chi-square test used to compare the results. The data analyzed with Prisma software, considering a level of significance of 0.05.
RESULTS

*S. aureus* identified in 40% of 100 samples. Among the courses evaluated, the samples obtained from the cell phones of students of the nursing course presented the highest percentage of contamination (65%), followed by Dentistry (50%), Biomedicine (40%). The courses Pharmacy (25%) and Nutrition (20%) presented a lower value compared to cell phones of students of the nursing course (p<0.05).

In the present work, the antimicrobial susceptibility of *S. aureus* isolated from the cell devices was also evaluated. A high percentage of resistance to Penicillin (85%) was observed. However, low levels of resistance (p<0.05) were found for Levofloxacin (2.5%) and Clindamycin (7.5%) Figure 1. The presence of multiresistant isolates was also evaluated. Approximately 57.5% of the isolates were considered multiresistant since they demonstrated resistance to two or more of the antimicrobials evaluated. It highlighted a particular sample, isolated from a student's cell phone in the nursing course, which presented resistance to 5 antimicrobials (Table 3), presenting a MAR index of 0.83.

The ability of *S. aureus* isolates to form biofilms was evaluated. Among the isolates, the following were observed: 10% non-adherent, 32.5% weak adherence, 50% moderate adherence, and 7.5% strong adherence (Figure 2). Despite the presence of microorganisms considered to be strong biofilm formers in the cell phones of students of the pharmacy and nursing students, the presence of microorganisms with weak adherence or non-adherence was also observed, demonstrating the great diversity of the samples obtained (p<0.05).

The sa442 DNA fragment was used for genotypic identification of *S. aureus* species. In the present study, the amplification of this fragment was evaluated by polymerase chain reaction (PCR) and positivity was observed in 100% of phenotypically identified *S. aureus* samples. It also investigated the presence of IcaA and IcaD genes and
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the isolates presented a frequency of 92.5% and 60%, respectively. Also, it observed that all samples that demonstrated a positive biofilm adhesion profile presented one of the analyzed genes.

Results demonstrated that 25% of the isolates presented the \textit{mecA} gene, however none of the samples were positive for the \textit{mecC} gene. Multiplex PCR assay for typing of Staphylococcal Cassette Chromosome Mec (SSCmec) showed that 20% SCCmec I, 10% SCCmec III, and 70% of the samples could not be typified by the technique, presenting distinct bands of evaluation. The most prevalent enterotoxin gene was \textit{SEA} (30%) followed by the \textit{SEC} gene (2.5%). The presence of \textit{SED} and \textit{SEB} genes was not observed in any of the isolates. The presence of \textit{SEA} and \textit{SEC} genes at the same time was observed in 2% of the samples.

**DISCUSSION**

The constant handling of cell devices favors the transmission of microorganisms, especially those associated with direct contamination through contact with the skin, saliva, and secretions\(^1\). Zakai et al.\(^{19}\) identified \textit{S. aureus} in 16% of samples from the cell phones of medical students. As demonstrated here, the nursing course presented the highest percentage of contamination. Nursing students are likely to become reservoirs of \textit{S. aureus} since hospital practice is part of their education process and usually begins in the first years of the course. This fact could make the adhesion of pathogens easier on the surface of cell devices\(^{20,21}\).

Biofilm plays a key role in the survival of bacterial species in diverse and hostile environments. It’s believed that approximately 65% of human bacterial infections are associated with a biofilm. Bacteria associated with biofilms are generally resistant to antibiotics and present important virulence factors\(^{22,23}\). Marks et al.\(^{24}\) demonstrated that
microorganisms could survive the hostile environment and be spread through a hospital environment contaminated by biofilms. In this way, this work evidenced that mobile phones used in hospital or healthcare settings may be the focus of transmission of pathogenic bacteria due to their ability to form biofilms.

Polysaccharide intercellular adhesion (PIA) is an important aspect of biofilm production and is encoded by the chromosomal intercellular adhesion (ica) locus, consisting of the icaADBC. Among them, the icaA and icaD genes have been reported to play a significant role in biofilm production. All samples that demonstrated a positive biofilm adhesion profile presented one of the analyzed genes. The strains that presented two genes simultaneously (53%) were classified with moderate to strong adherence, in this way, it is possible to correlate that the two genes together play a significant role in the formation of biofilm. A high percentage of non-adherent isolates with single ica locus genes was observed, generating great concern since these genes can be expressed under the effect of a stimulus and thus highlight the need for its expression by quantitative PCR.

Penicillin promotes the blockade of the synthesis of the peptidoglycan layer of the cell wall of the bacteria, thus inhibiting the synthesis of the cell wall. Resistance to this antibiotic had the highest percentage among the isolates of this study. Also, it highlighted the frequent presence of multidrug resistance. Similarly, Silva et al. evaluated the antimicrobial susceptibility of S. aureus isolated from nurses and found that resistance to penicillin was close to 100%.

An important mechanism of S. aureus resistance to antimicrobials is provided by the mecA gene, present in the mobile genetic element designated as the staphylococcal chromosome cassette (SCCmec). Methicillin resistance in S. aureus (MRSA) is due to the presence of the mecA gene. Currently MRSA is an important pathogen that causes
severe morbidity and mortality worldwide. MRSA strains are endemic in many countries and may be present in more than 50% of clinical isolates\textsuperscript{29}. The homolog of the \textit{mecA} gene, called the \textit{mecC} gene, is also located in the SCCmec and produces a similar phenotypic profile of antimicrobial resistance\textsuperscript{29}. In the present study, it was possible to observe that 25\% of the isolates presented the \textit{mecA} gene, however none of the samples were positive for the \textit{mecC} gene. The low number of samples could justify the absence of \textit{mecC} gene in samples, which was a major limitation in our study.

According to the combination of ccr allotypes with the mec gene complex, 11 types (I-XI) of SCCmec have already been reported\textsuperscript{29}. The results demonstrated the presence of SCCmecI, SCCmecIII. SCCmec I is the chromosomal cassette that carries no transposons or plasmid that confer resistance to drugs other than methicillin and heavy metals. SCCmec III carries genes like \textit{mecA} and \textit{mecRI}, together with transposons and plasmids that form resistance to metals such as cadmium and mercury and also to antimicrobials such as tetracycline and oxacillin, being considered the oldest truly pandemic MRSA strain\textsuperscript{30}.

In the present work, staphylococcal enterotoxins (SEs) also investigated, since enterotoxigenic \textit{S. aureus} is considered the second most prevalent pathogen in foodborne diseases in Brazil\textsuperscript{31}. The most prevalent gene was the \textit{SEA} toxin, commonly related to cases of intoxication, corresponding to 75\% of outbreaks, and also correlated with human food contamination\textsuperscript{32}.

The data of this work emphasize that cell phones used in the healthcare environment allow the transmission of bacteria that harbor genes of virulence and resistance. The cleaning and periodic disinfection of cellular devices can contribute to reducing the risk of both nosocomial infection rates and those in the community as well as lowering the morbidity/mortality from these infections. In this way, it is necessary to
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raise awareness about the disinfection of mobile phones among health professionals since this is an extremely useful tool in the medical field.

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**Figure 1:** Antimicrobial resistance in *S. aureus* isolated from cell phones. OXA: oxacillin; CFL: cephalothin; PEN: penicillin; CLI: clindamycin; ERY: erythromycin; LEV: levofloxacin. *p<0.05
Figure 2: Evaluation of biofilm formation by *S. aureus* through the microplate adhesion crystal violet staining technique. * Statistically different from *S. aureus* ATCC 25923.
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Table 1: Primers used in PCR amplifications.

| Target gene | Sequence (5’ – 3’) | Amplicon (pb) |
|-------------|---------------------|---------------|
| **Sa442**   | Sa442f: AAT CTT TGT CGG TAC AGC ATA TTC ACG  
             | Sa442r: CGT AAT GAG ATT TCA GTA AAT ACA ACA | 108 |
| **icaD**    | icaDf: ATG TGC AAG CCC AGA CAG AG  
             | icaDr: CGT GCT TTA AAC ATT TAA TG | 198 |
| **icaA**    | icaAf: ACA GTC GCT ACG AAA AGA AA  
             | icaAr: GGA AAT GCC ATA ATG AGA AC | 699 |
| **SEA**     | SEAf: TTG GAA ACG GTT AAA ACG AA  
             | SEAr: GAA CCT TCC CAT CAA AAA CA | 120 |
| **SEB**     | SEBf: TCG CAT CAA ACT GAC AAA CG  
             | SEBr: GCA GGT ACT CTA TAA GTG CC | 478 |
| **SEC**     | SECf: GAC ATA AAA GCT AGG AAT TT  
             | SECr: AAA TCG GAT TAA CAT TAT CC | 257 |
| **SED**     | SEDf: CTA TGG TAA TAT CTC CT  
             | SEDr: TAA TCG TAT TTA TAG GG | 317 |

Table 2: Primers used in multiplex PCR to classify MRSA strains.

| Oligonucleotide | Sequence (5’ - 3’) | Amplicon (bp) |
|-----------------|---------------------|---------------|
| CIF2 F2         | TTC GAG TTG CTG ATG AAG AAG G | 495 |
| CIF2 R2         | ATT TAC CAC AAG GAC TAC CAG C | 449 |
| ccrC F2         | GTC TCT GTT ACA ATG TTT GG | 414 |
| ccrC R2         | ATA ATG GCT TCA TGA TTA CC | 377 |
| RIF5 F10        | TTC TTA AGT ACA CGC TGA ATC G | 342 |
| RIF5 R13        | ATG GAG ATG ATT TAC AAG GG | 311 |
| SCCmec V J1 F   | AGA GAC TAC TGA CTT AAG TGG | 284 |
| SCCmec V J1 R   | TTC TCC ATT CTT GTT CAT CC | 243 |
| dcs F2          | CAT CCT ATG ATA GCT TGG TC | 209 |
| dcs R1          | CTA AAT CAT AGC CAT GAC CG | 162 |
| ccrB2 F2        | AGT TTC TCA GAA TTC GAA CG | 124 |
| ccrB2 R2        | CCG ATA TAG AAW GGG TTA GC | 124 |
| kdp F1          | AAT CAT CTG CCA TGG GTG ATG C | 124 |
| kdp R1          | CGA ATG AAG TGA AAG AAA GTG G | 124 |
| SCCmec III J1 F | CAT TTG TGA AAC ACA GTA CG | 124 |
| SCCmec III J1 R | GTC ATT GAG ACT CCT AAA GC | 124 |
| mecl P2         | ATC AAG ACT TGC ATT CAG GC | 124 |
| mecl P3         | CGG GTT TCA ATT CAC TTT GC | 124 |
| mecl P4         | TCC AGA TTA CAA CTT CAC CAG G | 124 |
| mecl P7         | CCA CTG CAT ATC TTT TGA CG | 124 |

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Table 3. Results obtained by antimicrobial susceptibility test, biofilm formation and PCR amplification from *S. aureus* isolates from cell phones of university students

| Strain  | Course  | Resistance               | Biofilm Formation | icaA | icaD | meCA | meCC | SCCmec1 | SCCmecIII | SEA | SEB | SEC | SED |
|---------|---------|--------------------------|-------------------|------|------|------|------|---------|-----------|-----|-----|-----|-----|
| 1       | Biomedicine | PEN,                     | Weak              | +    | -    | -    | -    | -       | -         | -   | -   | -   | -   |
| 3       | Biomedicine | PEN, OXA,               | Moderate          | +    | +    | -    | -    | -       | -         | -   | -   | -   | -   |
| 4       | Biomedicine | PEN, OXA,               | Moderate          | +    | +    | -    | -    | -       | -         | -   | -   | -   | -   |
| 6       | Biomedicine | PEN, ERY,               | Moderate          | +    | -    | +    | -    | -       | -         | -   | -   | -   | -   |
| 7       | Biomedicine | PEN, OXA,               | Moderate          | +    | -    | +    | -    | -       | -         | -   | -   | -   | -   |
| 9       | Biomedicine | PEN, ERY, OXA,          | Moderate          | +    | +    | -    | -    | -       | -         | +   | +   | +   | -   |
| 14      | Biomedicine | PEN, OXA, CFL,          | Moderate          | +    | +    | +    | -    | -       | +         | +   | +   | +   | -   |
| 16      | Biomedicine | PEN,                     | Weak              | -    | -    | -    | -    | -       | -         | +   | -   | -   | -   |
| 21      | Dentistry  | PEN, OXA,               | Moderate          | +    | +    | -    | -    | -       | -         | -   | +   | -   | -   |
| 22      | Dentistry  | PEN,                    | Moderate          | +    | +    | -    | -    | -       | -         | -   | +   | -   | -   |
| 23      | Dentistry  | PEN, OXA,               | Weak              | +    | +    | -    | -    | -       | -         | -   | -   | -   | -   |
| 24      | Dentistry  | PEN,                    | Moderate          | +    | -    | -    | -    | -       | -         | -   | -   | +   | -   |
| 25      | Dentistry  | PEN,                    | Moderate          | +    | +    | -    | -    | -       | -         | -   | -   | +   | -   |
| 26      | Dentistry  | PEN, ERY,               | Moderate          | +    | +    | -    | -    | -       | -         | -   | +   | +   | -   |
| 29      | Dentistry  | PEN,                    | Moderate          | +    | -    | -    | -    | -       | -         | -   | -   | +   | -   |
| 30      | Dentistry  | PEN,                    | Moderate          | +    | +    | -    | -    | -       | -         | -   | +   | +   | -   |
| 31      | Dentistry  | PEN,                    | Moderate          | +    | +    | -    | -    | -       | -         | -   | +   | +   | -   |
| 40      | Dentistry  | PEN,                    | Moderate          | +    | +    | -    | -    | -       | -         | -   | +   | +   | -   |
| 53      | Nutrition  | PEN, ERY,               | Moderate          | +    | +    | -    | -    | -       | +         | +   | -   | -   | -   |
| 56      | Nutrition  | PEN,                    | Weak              | +    | -    | -    | -    | -       | -         | -   | -   | -   | -   |
| 57      | Nutrition  | PEN,                    | Not adherent      | +    | -    | -    | -    | -       | -         | -   | -   | -   | -   |
| 58      | Nutrition  | PEN,                    | Weak              | +    | -    | +    | -    | -       | -         | -   | -   | -   | -   |
| 62      | Pharmacy   | PEN, ERY, OXA, CFL,     | Weak              | +    | -    | +    | -    | -       | +         | -   | -   | -   | -   |
| 66      | Pharmacy   | PEN, ERY,               | Weak              | +    | -    | -    | -    | -       | -         | -   | -   | -   | -   |
| 67      | Pharmacy   | PEN, ERY, OXA, CFL,     | Weak              | +    | -    | +    | -    | -       | -         | -   | -   | -   | -   |
| 71      | Pharmacy   | PEN, ERY, OXA, CFL,     | Weak              | +    | -    | +    | -    | -       | -         | -   | -   | -   | -   |
| 80      | Pharmacy   | PEN, ERY,               | Strong            | +    | -    | -    | -    | -       | -         | -   | -   | -   | -   |
| 82      | Nursing    | PEN, ERY, OXA, CLI,     | Moderate          | +    | +    | -    | -    | -       | -         | -   | -   | -   | -   |
| 83      | Nursing    | PEN, ERY,               | Weak              | +    | -    | -    | -    | -       | -         | -   | -   | -   | -   |
| 85      | Nursing    | PEN, CFL,               | Strong            | +    | +    | -    | -    | -       | -         | -   | -   | -   | -   |
| 86      | Nursing    | PEN, ERY, OXA,          | Not adherent      | +    | -    | -    | -    | -       | -         | -   | -   | -   | -   |
| 87      | Nursing    | PEN,                    | Weak              | +    | -    | -    | -    | -       | -         | -   | -   | -   | -   |
| 88      | Nursing    | PEN, ERY,               | Weak              | +    | -    | -    | -    | -       | -         | -   | -   | -   | -   |
| 90      | Nursing    | ERY,                    | Weak              | +    | -    | -    | -    | -       | -         | -   | -   | -   | -   |
| 93      | Nursing    | PEN,                    | Weak              | +    | -    | -    | -    | -       | -         | -   | -   | -   | -   |
| 94      | Nursing    | PEN, OXA, CFL,          | Not adherent      | +    | -    | -    | -    | -       | -         | -   | -   | -   | -   |
| 95      | Nursing    | ERY,                    | Moderate          | +    | +    | -    | -    | -       | -         | -   | -   | -   | -   |
| 96      | Nursing    | PEN, ERY, OXA, CFL, CLI,| Weak              | +    | -    | -    | -    | -       | -         | -   | -   | -   | -   |
| 97      | Nursing    | PEN, ERY, OXA, CFL, CLI,| Weak              | +    | -    | -    | -    | -       | -         | -   | -   | -   | -   |
| 100     | Nursing    | PEN, ERY,               | Not adherent      | +    | -    | -    | -    | -       | -         | -   | -   | -   | -   |

Legend: OXA: Oxacillin; CFL: Cephalothin; PEN: Penicillin; CLI: Clindamycin; ERY: Erythromycin; LEV: Levofloxacin. + positive - negative

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