

1 Short Research Article  
2 **Staphylococcus and Pseudomonas Isolated**  
3 **from Mobile Phones and Cheek and Ear**  
4 **Locales**

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7  
8 **ABSTRACT**  
9

**Background:** [Write this in a single sentence describing the background of this study]

**Aims:** To isolate and identify *Staphylococcus* and *Pseudomonas* from the cheek, ear, and mobile (cell) phones of college students.

**Study design:** A total of 150 samples were obtained from 50 randomly recruited college students who gave informed consent and answered a brief survey. Three swabs per student were obtained: one each of the swab was from the cheek, the mid-ear, and from the mobile phone.

**Place and Duration of Study:** This study was carried out in the Microbiology Laboratory at the University of Central Missouri, United States from January 2011 until May 2011.

**Methodology:** Swabs were plated in duplicate onto Mannitol Salt Agar (MSA), Oxacillin Resistance Screening Agar Base (ORSAB), Chromagar containing Oxacillin supplement, and Cetrimide Agar. MSA positive colonies were sub-cultured onto ORSAB and Tryptic soy agar plates, which were incubated for 24 hours, and was subjected to a confirmatory PBP2a assay. Statistical analysis was performed using a one-way Analysis of Variance (ANOVA) with significance set at ( $P = 0.05$ ).

**Results:** Twenty-seven (54%) touch screen, 13 (26%) sliders, and 10 (20%) flip phones were swabbed. No *Pseudomonas aeruginosa* was detected. More *Staphylococcus* colonies were recovered from touch screen phones than other phone types ( $P = 0.028$ ). Statistical analysis showed significance between the three locations of bacteria cultured from the phone, cheek, and ear ( $P = 0.034$ ). One male tested positive for methicillin resistant *Staphylococcus aureus* (MRSA) at all three locations swabbed, and one female tested positive from a cheek swab. Two students (4%) were tested positive for MRSA, and 10 (20%) students were harbored methicillin susceptible *Staphylococcus aureus* (MSSA). Most MSSA was on flip (10%) and touch screen (8%) phones. More *Staphylococcus* colonies were recovered from phones belonging to females than males ( $P = 0.0001$ ).

**Conclusion:** [Rewrite the conclusion mentioning the main findings of this study stating the bacterial names with different sites] Bacterial monitoring of mobile phones is important in identifying antibacterial resistance. Furthermore, reinforcing phone disinfection methods to individuals to keep from spreading bacteria with antibiotic resistance will be of global importance.

10  
11 **Keywords:** ~~bacteria~~; *Staphylococcus*; MRSA; methicillin; mobile; resistant; ~~phone~~ *Pseudomonas*

12  
13 **1. INTRODUCTION**  
14

15 Mobile phones, also known as cell(ular) phones, have become utilized globally and are now accepted  
16 ~~socially~~ socially to keep in almost instant contact with others. The mobile phone has been investigated in  
17 clinical settings for the potential role of spreading bacteria to others such as *Staphylococcus*,  
18 *Streptococcus*, *Acinetobacter*, *Klebsiella*, *Escherichia*, and *Pseudomonas* [1-7]. Coagulase negative  
19 ~~Staphylococcus~~ *Staphylococcus epidermidis* can cause skin and urinary tract infections.  
20 *Staphylococcus aureus* causes a wide range of illnesses from minor skin infections like impetigo and  
21 abscesses, to food poisoning, and even life-threatening diseases such as pneumonia, bacteremia, and

22 sepsis [8]. ~~With reported m~~Methicillin resistant *Staphylococcus aureus* (MRSA) cases are increasing;  
23 ~~therefore~~, identifying the bacterium for proper treatment is now of global importance [8]. The *mecA* gene  
24 binds the altered Penicillin Binding Protein (PBP) [9], which is how many of the confirmatory assays are  
25 designed to confirm the gene presence<sup>[U1]</sup>. *Pseudomonas aeruginosa* causes wound infections and  
26 pneumonia, ~~as well as nosocomial infections in hospitalized patients~~ [10]. ~~While Furthermore~~ studies  
27 have shown bacterial recovery from medical personnel such as surgeons, ICU nurses, pharmacists,  
28 medical laboratory scientists and their mobile phones [11]; ~~thus far a case report showing a direct link~~  
29 ~~from a health care worker's mobile phone to a specific patient has been lackin\_g.~~

30 Previous studies have investigated for the isolation of bacteria ~~on from~~ mobile phones of people working  
31 in intensive care units and other areas of the hospital [11], types of cell phones [6,12], patient and visitor  
32 of cell phones [13], veterinary students [14], and college students and community members [5]. Some of  
33 these studies have also included antibiograms of recovered bacteria [15], and have isolated microbes  
34 from public computer keyboards [16]. Thus ~~far~~ studies investigating current epidermal skin flora of an  
35 individual and their cell phone bacteria have also been lacking.

36 The purpose of this present study was to determine ~~the Staphylococcal and Pseudomonad~~ presence of  
37 *Staphylococcus* and *Pseudomonas* on different cell phone types and compare the bacteria to the  
38 person's cheek and ear in direct contact with the cell phone, i.e. facial flora. ~~This study focused on~~  
39 ~~*Staphylococcus aureus* (both methicillin susceptible (MSSA) and methicillin resistant), *Staphylococcus*~~  
40 ~~*epidermidis*, and *Pseudomonas aeruginosa* as the facial flora most likely to be recovered [1, 4, 5, 6, 7].~~  
41 ~~This study utilized three selective agars to initially isolate the desired bacteria – Mannitol Salt Agar (MSA),~~  
42 ~~Oxacillin Resistance Screening Agar Base Chromagar (ORSAB) with Oxacillin supplement, and Cetrimide~~  
43 ~~Agar. Suspected MSSA and MRSA colonies were then subjected to additional isolation, biochemical, and~~  
44 ~~confirmatory tests.~~

## 45 2. MATERIALS AND METHODS

46  
47 This ~~project study~~ was approved by the Institutional Review Board Human Subjects Committee. Fifty  
48 college students who were at least eighteen years of age and possessed a cell phone were randomly  
49 recruited. Each person was asked to complete a short survey ~~and to be swabbed~~. Survey questions  
50 asked cell phone type (~~like~~ flip, slider, or touch screen), participant gender, and participation in collegiate  
51 or intramural athletics. Surveys were assigned a random number, which was also placed on all  
52 corresponding plates and tests.

### 53 2.1 Sample Collection

54 A sterile swab was used to swab the subject's cell phone, the subject's cheek, and the outer mid-to-lower  
55 ear, both locations where phone contact occurred. Standardized 2 cm (length) × 2 cm (width) × 2.54 cm  
56 (height) squares made of cardstock served as templates to ensure equal areas were swabbed for each  
57 person. Swabs were rubbed on the dominant side of the face where the cell phone was usually held  
58 within the template temporal area ~~fifteen times left to right, then fifteen times up and down~~.

### 59 2.2 Culture Media and Growth Conditions

60 Swabs were then plated in duplicate onto Mannitol Salt Agar (MSA) (Oxoid, Basingstoke, UK) and  
61 Oxacillin Resistance Screening Agar Base (ORSAB) Chromagar containing Oxacillin supplement (Oxoid,  
62 Basingstoke, UK) for *Staphylococcus* recovery [17], and Cetrimide Agar (Difco, Detroit, MI) (Difco) for  
63 *Pseudomonas* recovery. Plates were incubated at 37°C for 24 to 48 hours and then results were  
64 recorded. Dark blue ORSAB positive colonies were sub-cultured onto Tryptic Soy Agar (TSA)  
65 (~~Thermofisher~~Thermo fisher Remel, Overland Park, KS) slants, incubated for 24-48 hours at 37°C, ~~and~~  
66 ~~stored at 4°C~~. Colonies that were MSA positive were tested for catalase and coagulase positivity, and  
67 then were sub-cultured onto ORSAB plates and were incubated for 24 hours at 37°C. Colonies testing  
68 positive on both MSA and ORSAB were then sub-cultured onto TSA slants ~~and stored at 4°C~~. Colonies  
69 grown on Cetrimide plates were sub-cultured onto TSA slants ~~and~~. All the isolates were stored at 4°C.  
70 Quality control strains (Hardy Diagnostics, Santa Maria, CA) of *Staphylococcus aureus*, *Staphylococcus*

71 *epidermidis*, *Escherichia coli*, and *Pseudomonas aeruginosa*, were inoculated onto each media type to  
72 ensure proper reactions.

73 *Staphylococcus aureus* colonies testing positive on ORSAB were then checked for methicillin resistance  
74 using a PBP2a (Alere Wampole, Waltham, MA) latex agglutination assay for confirmation of the *mecA*  
75 gene.

## 76 2.3 Statistical Analysis

77 Statistical analysis was performed using a one-way Analysis of Variance (ANOVA) with the significance  
78 level set at ( $P = 0.05$ ).

## 79 3. RESULTS AND DISCUSSION

80

### 81 3.1 Culture Results

82 Twenty-seven (54%) phones swabbed were touch screen, 13 (26%) were ~~sliders,sliders~~ and 10 (20%)  
83 were flip phones. Fifteen (30%) subjects were male and 35 (70%) were female. None of the human  
84 subjects were student athletes, but eight people participated in intramural sports. None of the participants  
85 were routinely disinfected their cell phone. The number of persons testing positive for MRSA, MSSA, and  
86 *Staphylococcus epidermidis* by gender, phone type, and involvement in intramurals is analyzed shown in  
87 (Table 1).

88 **Table 1: Individuals Testing Positive for Each Staphylococcal Species (Make it simple table)**

Phone Type	#	Gender	Intramurals	MRSA	MSSA	MSSA per Phone Type	<i>S. epidermidis</i>
Flip	7	Female	0	0	5	10%	5
	3	Male	0	0	0		1
Slider	9	Female	1	0	1	2%	5
	4	Male	0	0	0		0
Touch	19	Female	6	1	3	8%	7
	8	Male	1	1	1		5
Totals	50		8 (16%)	2 (4%)	10 (20%)		23 (46%)

89

90 Non-participants in intramurals were averaged 6.4 colony-forming units (CFUs) on cell phones, with  
91 growth on 19, or 45%. Non-participants were averaged 34.9 CFUs per cheek sample, and 54.1 CFUs per  
92 ear sample, ~~both with growth on 38 samples for 90.5%~~. Intramural participants were averaged 9.5 CFUs  
93 on four (50%) cell phones and 28.5 CFUs per cheek sample on six (75%) cheek samples. Ear samples  
94 were averaged 52.8 CFUs on seven (87.5%) ear samples.

95 Colony growth from cell phones on Mannitol Salt plates ranged from zero to 65 CFUs, with a mean of 6.9  
96 CFUs for all cell phones combined. Growth was observed on 23 of 50 (46%) cell phones and is shown in  
97 (Table 2). The raw data of the MSA total and MSA positive CFUs ~~differsdiffer~~ substantially between  
98 phone type. Growth was obtained on 12 (44.4%) touch screen phones, five (38.5%) slider phones, and  
99 six (60%) flip phones, and not all plates had growth. ~~A comparison of the location means per cell phone~~  
100 ~~type can be seen in~~ (Figure 1).

101

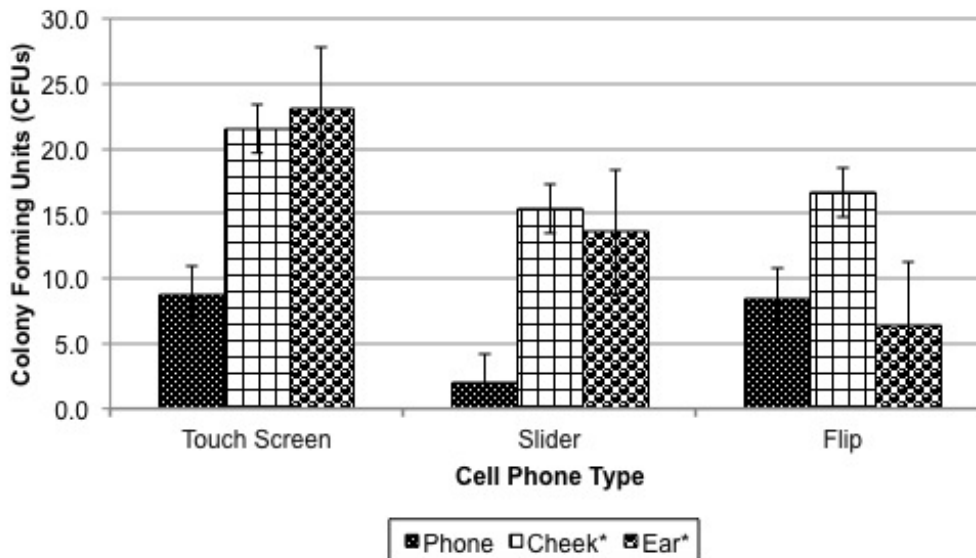
102 **Table 2. Mannitol Salt Agar (MSA) Growth by Location and Phone Type**

Phone Type	Location	MSA Plates with Growth	MSA Total CFUs	MSA Mean CFUs	MSA Positive CFUs
Touch Screen	Phone	44%	237	8.8	8
	Cheek	85%	582	21.6	220
	Ear	85%	621	23	457
Slider	Phone	38%	24	1.9	2
	Cheek	85%	200	15.4	56
	Ear	92%	177	13.6	14
Flip	Phone	60%	85	8.5	17
	Cheek	100%	166	16.6	8
	Ear	100%	64	6.4	9

103

104 MSA Total CFUs = Both *S. epidermidis* and *S. aureus* are included.  
 105 MSA Positive CFUs = *S. aureus*.

106  
 107  
 108



109

110 | **Figure 1:** Mean Colony Forming Units (CFUs) recovered from swabs taken from each phone type,  
 111 cheek, and ear

112 Cheek and Ear CFUs: \*  $P = .03$

113 Mean  $\pm$  S.E.M = Mean values  $\pm$  Standard error of means of colony counts of experiment performed in duplicate.

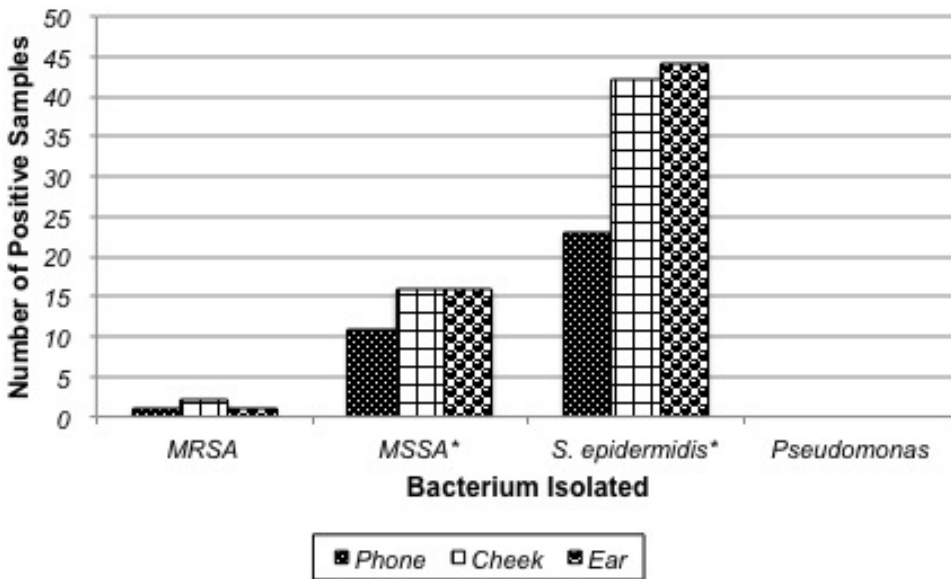
114

115 | Based on location, a total of 23-(46%) cell phone samples, 44 (88%) cheek samples, and 45 (90%) ear  
 116 samples were yielded growth. The mean cell phone sample grew 6.9 CFUs, with cheek swabs growing  
 117 33.9 CFUs, and ear swabs growing 53.9 CFUs. [U2]By gender, males had a mean of 9.7 CFUs and  
 118 females had a mean of 5.7 CFUs. For cheek samples, males averaged 47.3 CFUs and females averaged  
 119 28.1 CFUs. For ear samples, male averaged 50.1 CFUs and female averaged 55.5 CFUs.[U3]

120 For cell phones, a total of 319 colonies were *S. epidermidis*, and 27 colonies were MSA positive. For  
 121 cheek samples, 1410 colonies were *S. epidermidis* and 284 colonies were MSA positive. For ear  
 122 samples, a total of 2212 colonies were *S. epidermidis* and 483 colonies were MSA positive. Colonies  
 123 testing MSA positive were scrutinized for proper colony color, size, shape and were underwent catalase  
 124 and coagulase screening before being sub-cultured to ORSAB media. A total of 44 samples were sub-  
 125 cultured onto ORSAB Chromagar, and of those, 34 (77.3%) were tested negative and 10 (22.7%) were  
 126 tested positive. No growth was obtained on the Cetrimide agar for *Pseudomonas aeruginosa*.

127 **3.2 MRSA Confirmation**

128 Of the 10 ORSAB positive *Staphylococcus aureus* colonies, four (40%) samples (2.6% of total samples  
 129 taken overall) tested positive on the PBP2a confirmatory assay test for MRSA. [The results of the PBP2a  
 130 confirmatory test showed one male subject, who was not involved in intramurals, tested positive on all  
 131 three locations swabbed – the phone, cheek, and ear. [U4] In addition, one female also tested positive from  
 132 a cheek swab. Thus, two individuals out of 50 (4%) were positive for MRSA and the gender distribution  
 133 for this study was 50% male and 50% female (Figure 2). Figure 2 shows the number of positive samples,  
 134 ~~or plates with growth of each bacterial type, from the cheek, ear, and phone swabs taken.~~



135  
 136 **Figure 2:** Number of plates with growth, out of the 50 swabs taken, that grew the specific  
 137 **bacterium from a phone, cheek, or ear swab**  
 138 *Staphylococcus aureus* (MSSA) and *S. epidermidis*: \*  $P = .01$   
 139

140 **3.3 Statistical Analysis**

141 Statistical analysis showed significance between the three locations of bacteria grown on MSA cultured  
 142 from the phone, cheek, and ear ~~with~~ ( $P = 0.03$ ). Phones belonging to females harbored more recovered  
 143 microbes than males, ~~with~~ ( $P = 0.001$ ). Furthermore, touch screen phones appeared to harbor more  
 144 *Staphylococcus* overall than the other two types of phones ( $P_p = .03$ ). However, when the means for  
 145 each phone type were used in the ANOVA, ( $P_p = 0.4$ ).

146 ~~When t~~The total number of CFUs obtained on MSA were compared between the types of phones, ( $P_p =$   
 147  $0.01$ ). Because *S. epidermidis* is usually not associated with high mortality rates like *S. aureus*, the  
 148 ANOVA was re-ran without *S. epidermidis* figures to see if significance still existed with *S. aureus* levels.



149 | Significance was still seen ~~with~~ ( $P = 0.02$ ). Since no growth was seen for *Pseudomonas*, it was not used  
150 | in any calculations.

### 151 | 3.4 Cell Phone and Facial Flora Discussion

152 | —Based on ~~our~~ ~~these~~ findings, there did not appear to be a correlation between the bacterial growth  
153 | recovered from the cell phone, cheek and ear as correlation coefficients were 0.45, 0.46, and 0.18  
154 | respectively. When only *Staphylococcus aureus* growth was compared, the cheek and ear correlation  
155 | coefficient increased to 0.7, and the other two ~~were~~ decreased to -0.04 and -0.07, respectively. A large  
156 | CFU recovered from a person's cheek did not mean ~~that~~ a large CFU was recovered on the person's cell  
157 | phone and vice versa. ~~The cheek and ear plates had a higher colony count than cell phones on average,~~  
158 | ~~which was expected as *Staphylococcus spp.* is normal skin flora~~<sup>[U5]</sup>. The average colony count for cell  
159 | phone samples on both touch screen and flip phones was very close. ~~We hypothesized that~~ touch  
160 | screen cell phones would harbor more bacteria than the other two phone types. On average, slider  
161 | phones had lower colony counts than the other two cell phone types. This could be due to less direct  
162 | contact while talking on the phone, or less environmental exposure while not in use. ~~Also, the keypad of~~  
163 | ~~slider phones may not get as much use as the one in flip or touch screen phones because many slider~~  
164 | ~~phones can be used without the keypad much of the time.~~ <sup>[U6]</sup> Flip phones should have less exposure to  
165 | the environment when not in use, so less bacteria would come in contact with the screen and keypad, but  
166 | the bacteria deposited onto the keypad are protected for longer periods of time. <sup>[U7]</sup>

### 167 | 3.5 *Staphylococcus* Discussion

168 | Recovery of coagulase negative Staphylococci in the highest concentration also ~~support~~ ~~s~~ <sup>support</sup> the  
169 | findings of Bhoonderowa et al. (2014). <sup>[U8]</sup> MRSA isolated from touch screen phones more than other  
170 | types of phones supports Lee et al. (2013) and Pierson (2013), <sup>[U9]</sup> but differed from those of Pal et al.  
171 | (2013) <sup>[U10]</sup>. ~~Our~~ ~~This~~ 4% MRSA finding is slightly higher than the estimated 2% listed by the Centers for  
172 | Disease Control and Prevention (CDC) in the United States, but ~~our~~ 20% MSSA recovery ~~of this study~~ is  
173 | lower than the CDC's estimated 33% in the nares [9]. This could be attributed to the cheek area not being  
174 | as favorable of an environment as the nares, or attributed to the immune system production of fatty acids  
175 | [18] or other molecules.

176 | —No samples were knowingly taken from collegiate athletes; however, a small number of participants  
177 | listed involvement in intramurals. Previous studies have shown a higher prevalence of *Staphylococcus*  
178 | *spp.* on athletes [19], but ~~our~~ ~~the~~ participants had lower means on their cheeks and ears compared to  
179 | nonparticipants. However, they did have higher CFU means on their phone than nonparticipants. ~~The~~  
180 | ~~higher phone CFUs might be that they more frequently contact transient bacteria.~~ <sup>[U11]</sup> Potential reasons  
181 | for the lower facial flora averages could include frequent showers lowering cheek and ear flora via pH  
182 | changes or cell disruption from soaps and detergents, antibacterial inhibition or killing from soaps or body  
183 | washes [20], and mechanical action dislodging bacteria from washing and drying off with towels. <sup>[U12]</sup>

184 | ~~Our~~ ~~The~~ findings of phones belonging to females harboring higher overall CFUs than males supported the  
185 | findings of Bhoonderowa et al. (2014) <sup>[U13]</sup> but contradicted the findings of Amala and Ejikema (2015) <sup>[U14]</sup>.  
186 | ~~These results could be caused by a variety of factors including personal hygiene, more females involved~~  
187 | ~~in intramurals, or more frequent use of earrings and makeup by females in the population studied.~~  
188 | ~~Because disproportionately more females participated in this study, a study involving more males would~~  
189 | ~~be beneficial to see if this pattern continues.~~ <sup>[U15]</sup>

### 190 | 3.6 *Pseudomonas* Discussion

191 | ~~We were surprised no *Pseudomonas* was recovered on any plates as based on previous studies we~~  
192 | ~~expected approximately 1-6% [1, 3, 15]. Swabs were also plated onto TSA plates, and no characteristic~~  
193 | ~~odor, swarming, or fluorescent colonies were noted. Control organisms grew on the medium, and the age~~  
194 | ~~of the healthy population studied could explain the lack of results.~~ <sup>[U16]</sup>

### 3.8 Conclusion

### 3.7 Summary

In ~~summary conclusion~~, ~~our the~~ findings did not show a correlation between cell phone, cheek and ear recovery of *Staphylococcus aureus*, but did find a difference in growth from touch screen phones ( $P = .03$ ), between locations ( $P = .03$ ), and phones owned by females ( $P = .00$ ). The 4% MRSA rate was slightly higher ~~than the CDC estimates, even though our 20% MSSA rates were below estimates.~~ bacterial monitoring of electronics such as mobile phones and tablets is important in identifying antibacterial resistance. Furthermore, reinforcing phone disinfection methods to individuals to keep from spreading bacteria with antibiotic resistance will be of global importance.

### 3.8 Conclusion

~~In conclusion, bacterial monitoring of electronics such as mobile phones and tablets is important in identifying antibacterial resistance. Furthermore, reinforcing phone disinfection methods to individuals to keep from spreading bacteria with antibiotic resistance will be of global importance.~~

## ETHICAL APPROVAL

This project was approved by the University Institutional Review Board human subjects committee.

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262 **DEFINITIONS, ACRONYMS, ABBREVIATIONS**

263

264 **CFU:** Colony Forming Unit

265 **MRSA:** Methicillin Resistant *Staphylococcus aureus*

266 **MSSA:** Methicillin Susceptible *Staphylococcus aureus*

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