

Prevalence and Antibiotic Susceptibility Patterns of Methicillin-Resistant *Staphylococcus aureus* Isolated from Pharmacy Students, Tripoli-Libya

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ABSTRACT

A total of 200 nasal swaps were collected from 200 female pharmacy students who participated in this study. 156 of 200 (78%) participants were found to be colonized with multidrug-resistant (MDR) bacterial strains and 44 of 200 (22%) participants were colonized with drug-sensitive bacterial strains. Of 156 positive specimens, 83 (53.2%) specimens contained only one type of bacteria, while 73 (46.8%) specimens yielded more than one bacterial isolate. A total of 229 multidrug-resistant bacterial strains were isolated from the 156 positive carrier participants, and among these, 153 isolates (67%) were gram-positive bacteria and 76 isolates (33%) were gram-negative bacteria. Regarding the gram-positive bacteria, Methicillin Resistant *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus epidermis* represent about 49% (113 of 229) and 17% (40 of 229) of the total isolated strains, respectively. On the other hand, the percentage of gram-negative bacteria identified in this study was distributed in the following manner; *Klebsiella* spp 11% (25 of 229), *Enterobacter* spp 8% (18 of 229), *Citrobacter* 1% (4 of 229), *Serratia* spp 0.9% (2 of 229), and *Pseudomonas* spp 11.7% (27 of 229) of the total isolates.

All *Staphylococcus* spp isolates that represent 67% (153 of 229) of the total isolates, exhibited Methicillin Resistant activity and, thereafter, were classified as Community Associated-Methicillin Resistant *Staphylococcus aureus* (CA-MRSA) and Community Associated-Methicillin Resistant *Staphylococcus epidermis* (CA-MRSE). Besides, almost all confirmed isolates exhibited 100% resistance rates to Augmentin, Clindamycin, and Rifampin. The only effective antibiotics in this study against the isolates were Imipenem and Ciprofloxacin (100% susceptibility for both).

Keywords- Methicillin-Resistant *Staphylococcus aureus*; Female Pharmacy Students.

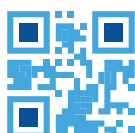
INTRODUCTION

Community-acquired infections (CAIs) are infections acquired everywhere other than in a healthcare facility, in settings such as schools, exercise facilities, prisons, or nursing homes. CAIs are defined as those isolates acquired by persons who have not been recently (within the past year) hospitalized or had an invasive medical procedure. According to what has been published CAI has great potential to occur at the following sites: the respiratory tract, the urinary tract, surgical wounds, the gastrointestinal tract, skin, abscesses, traumatic wounds, bacteremia, and burns.^{1,2}

Unfortunately, CAIs have not been studied as extensively as hospital-acquired infections, and the available information on CAIs mainly on community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA). In general, CAIs involve strains of *Staphylococcus aureus*, *Haemophilus influenza*, *Acinetobacter baumannii*, *Clostridium difficile*, *Streptococcus pneumonia*, *Vancomycin-Resistant Enterococci*, *Pseudomonas aeruginosa*, *E. coli*, *Enterococcus faecium*, and *Enterococcus faecalis*. Such bacteria can cause moderate to severe infectious disease among healthy individuals. For example, community-acquired-*Streptococcus Pneumonia* infection is the biggest cause of potentially life-threatening, community-acquired diseases such as meningitis and pneumonia. It is also the

leading bacterial cause of otitis media and sinusitis where it has evolved to reach unexpected levels of resistance to antibiotics.³

Staphylococcus aureus (*S. aureus*) is among those types of bacteria that have great potential to cause difficult-to-treat infections in humans, although it is an opportunistic pathogen often carried asymptomatically on the human body. But in 1961 a highly resistant strain to methicillin was reported soon after methicillin was introduced into human medicine to treat penicillin-resistant *Staphylococci*. The new strain was defined since then as Methicillin-resistant *Staphylococcus aureus* (MRSA) and involved strains that have acquired a gene giving the resistance to methicillin and essentially all other beta-lactam antibiotics (penicillins and cephalosporins).⁴ This group of organisms has since emerged as a serious concern in human medicine and reported as a nosocomial pathogen in hospitals. Although these organisms cause the same types of infections as other *S. aureus*, hospital-associated strains have become resistant to most common antibiotics, and treatment can be challenging. Since the 1990s MRSA has also become a concern in people who have not been hospitalized or recently had invasive procedures or are in contact with healthcare facilities; the strains that cause such infections are called (community-acquired or community-associated MRSA, CA-MRSA).⁵ CA-MRSA strains emerged as serious infections among prisoners, students at schools,



nursing homes, athletes who share equipment or personal items (such as towels or razors) and children in daycare facilities. Members of the military and those who get tattoos are also at risk. Until recently, these strains were susceptible to many antibiotics other than beta-lactams; however, resistance seems to be increasing and multiple antibiotic-resistant strains have started to emerge.^{6,7}

Researchers suggest that CA-MRSA did not evolve from the hospital-associated MRSA (HA-MRSA) which was further proven by molecular typing of CA-MRSA strains and genome comparison between CA-MRSA and HA-MRSA. By mid-2000, CA-MRSA was introduced into the health care systems, and distinguishing between CA-MRSA from HA-MRSA became a difficult process. Community-acquired MRSA (CA-MRSA) is more easily treated and more virulent, than hospital-acquired MRSA (HA-MRSA). The genetic mechanism for the enhanced virulence in CA-MRSA remains an active area of research.^{4,8,9} However, our focus, hereafter, will be on community-associated-methicillin-resistant bacteria, especially *Staphylococcus aureus*.

MATERIALS AND METHODS

Study Design, Setting, and Population

This investigation was a prospective, community-based, point prevalence study conducted at the Department of Microbiology and Immunology, Faculty of Pharmacy, University of Tripoli. The study proposal was reviewed and approved by the Department Review Board. Participant informed consent was obtained in all cases. 200 healthy female pharmacy students were subjected to the study and individuals who were admitted to hospitals in the last 6 months were excluded from the study. Demographic data and information on the risk factors of all participants were recorded.

Bacterial isolation and Identification

The specimens for culture were obtained from both anterior nares using rotating a pre-moistened cotton swab around the sampling site. The swab was immediately inoculated onto nutrient broth tubes which were then incubated for 24 hours at 35°C. After that, a sample from the broth was streaked directly on nutrient agar and a methicillin (5 µg) disk was placed on the surface and incubated at 35°C for 24 hours (Oxoid, Tripoli, Libya). Any discernible growth within the zone of inhibition, when seen using transmitted light, is treated as methicillin resistance. In addition, the isolates are considered methicillin-resistant when they grow in plates with zone diameters of ≤9 around the methicillin disk (CLSI 2011).¹⁰ The isolates were further confirmed by gram stain and test strips (API Staph, bioMérieux) for identification of presumptive *S. aureus*. The identification and isolation procedures were conducted on methicillin-resistant isolates only using gram stain, capsule stain, mannitol salt agar, MacConkey agar, triple sugar iron (TSI) test, indol

test, oxidase test, and API20E kit. All media and reagents were used according to the manufacturer's instructions.

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was determined using the Kerby-Bauer disk diffusion method against vancomycin, rifampin, trimethoprim/sulfamethoxazole, imipenem, augmentin, clindamycin, ciprofloxacin, and ceftriaxone. All the procedures and interpretive criteria were according to the latest National Committee for Clinical Laboratory Standards (NCCLS) recommendations.¹⁰ After 24-h incubation at 37°C, the zone diameter was measured and compared to NCCLS guidelines *S. aureus* ATCC 29213 (sensitive) and 27R MRSA (resistance) were used as control strains. Double-disk diffusion tests (D-tests) were performed for each isolate to evaluate the presence of inducible clindamycin resistance (MLS_{Bi}). Isolates with positive D-tests were reported as resistant to clindamycin.¹¹

Statistical analysis

The Sigma plot was employed for statistical analysis. *P*-values were calculated using X²-test and *P* < 0.05 was considered statistically significant.

RESULTS

Characteristics of participants

Demographic characteristics and risk factors of our study population are outlined (Table 1). A total of 200 nasal swabs were collected from 200 healthy female pharmacy students who participated in this study. All the students were attending the Faculty of Pharmacy daily and sharing a theatre for an average of three hours in the morning. The specimens were collected on the same day at the Department of Microbiology and Immunology, Faculty of Pharmacy, University of Tripoli. The age of the participants was normally distributed ranging from 20 – 26 years with a mean of 22.5 years and all of them were residents in the area of the study. All the cases had no chronic health problems and without a history of hospitalization in the last 30 days. Data on risk factors has resolved that 36 of 200 (18%) participants were suffering from bacterial infection. Of these, respiratory tract infection was reported in 31 of 200 (18%) participants and otitis media in 9 of 200 (4.5%) participants only. The allergic reaction was reported in 27 of 200 (13.5%) participants with the daily antihistaminic course and no one of the participants was under an antibiotic regimen (Table 1).

Etiological and causative organisms

In the study group, 156 of 200 (78%) participants were found to be colonized with multidrug-resistant bacteria, and 44 of 200 (22%) participants were colonized with antibiotic-sensitive bacteria. Of 156 positive specimens, pure culture media was obtained from 83 specimens, while 73 specimens yielded mixed culture media with more than one bacterial isolate (Table 2). A total of 229



Table 1: Demographic characteristics and risk factors of the study subjects colonized with methicillin-resistant bacteria.

Variable	Colonized group (%) n= 200
Age, (mean year)	22.5
Gender (female)	200
Antibiotic use	0 (0%)
Hospitalization in the last 30 days	0 (0%)
Infection - Respiratory tract infection	31 (18%)
- Otitis media	(4.5%)
Rhinitis / Allergic reaction	27 (13.5%)
Smoking	0 (0%)

resistant bacterial strains were isolated from 156 nasal swaps collected from female pharmacy students.

Among these, 153 isolates (67%) were gram-positive bacteria and 76 isolates (33%) were gram-negative bacteria. Regarding the gram-positive bacteria, *S. epidermis* and *S. aureus* represent about 17.6% (40 of 229) and 49% (113 of 229) of the total isolated strains, respectively. These strains were highly resistant to methicillin disk on culture media and, subsequently considered as community-associated-methicillin-resistant bacteria, and all subsequent discussions were based on these isolates. On the other hand, the percentage of gram-negative bacteria identified in this study was distributed in the following manner; *Klebsiella* spp 11% (25 of 229), *Enterobacter* spp 8% (18 of 229), *Citrobacter* 1% (4 of 229), *Serratia* spp 0.9% (2 of 229), and *Pseudomonas* spp 11.7% (27 of 229) of the total isolates (Table 3).

Risk factors associated with MDR

In the study, we addressed the correlation between some reported medical conditions and types of nose-borne bacteria. Respiratory tract infection, otitis media, and allergy (rhinitis) were considered risk factors, and they were reported in 31, 9, and 27, respectively (Table 4). Based on that, 67 participants were treated as high-risk groups. Of 67 participants 43 (64%) have been reported to have increasing rates of carrying MRSA than other bacteria types. A statistical analysis was performed for all groups based on the chi-square test. There were no statistically significant differences between the occurrence of a certain type of bacteria and the subjected groups ($\chi^2 = 17.8$ and $P=0.063$) (Table 4). Further comparison between the healthy group and the high-risk group has also been addressed, and there were no statistically significant differences between the groups ($\chi^2 = 6.9$ and $P=0.14$). In both groups, nose-borne MRSA was

Table 2: Types of culture media (pure versus mixed) obtained after incubation of the collected specimens.

Category	Number of cases (156)	Types of isolates	Number of cases (156)
Pure culture	83 (53%)	MRSA*	61 (39%)
		MRSE**	19 (12%)
		<i>Enterobacter</i> spp	3 (2%)
Mixed culture	73 (47%)	<i>Pseudomonas</i> spp	20 (13%)
		MRSA* + <i>Klebsilla</i> spp	17 (11%)
		<i>Enterobacter</i> spp	15 (10%)
		<i>Klebsilla</i> spp	8 (5%)
		<i>Pseudomonas</i> spp	7 (4.5%)
		<i>Citrobacter</i> spp	4 (2.5%)
		<i>Serratia</i> spp	2 (1%)

* Methicillin-resistant *Staphylococcus aureus*

** Methicillin-resistant *Staphylococcus epidermis*

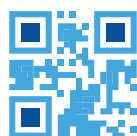


Table 3: Prevalence of bacterial strains among pharmacy students in Tripoli

Bacterial isolates (229)	Strains	Number (229)
Gram-Positive Bacteria 153 (67%)	MRSA*	113 (49%)
	MRSE**	40 (17.6%)
	<i>Klebsiella spp</i>	25 (11%)
Gram-negative bacteria 76 (33%)	<i>Enterobacter spp</i>	18 (8%)
	<i>Citrobacter spp</i>	4 (1.8%)
	<i>Serratia spp</i>	2 (0.9%)
	<i>Pseudomonas spp</i>	27 (11.7%)

* Methicillin-resistant *Staphylococcus aureus*

** Methicillin-resistant *Staphylococcus epidermis*

predominant over the other bacterial types followed by MRSE and *Klebsiella* spp (Table 5).

Antibiotic susceptibility profiles

Antibiotic susceptibility tests were performed during the time of sample collection and targeted the positive 229 colonization isolates. Almost all confirmed isolates exhibited 100% resistance rates to Augmentin, Clindamycin, and Rifampin. The only effective antibiotics in this study against the isolates were Imipenem and Ciprofloxacin (100% susceptibility for both). Resistance to Ceftriaxone

was identified only against *S. epidermidis* (53%), *S. aureus* (44%), and *Enterobacter* spp (33%) (Table 4).

On the other hand, Trimethoprim/sulfamethoxazole exhibited inconsistent results and the results were significantly different among colonization isolates. While it was very effective against *Klebsiella* spp and *Serratia* spp (100% susceptibility), *S. Epidermidis* (79% resistance) and *Pseudomonas* spp (50% resistance) were highly resistant. Moreover, the resistance profile of *S. aureus*, *Enterobacter* spp, and *Citrobacter* spp were 33%, 43%, and 40%, respectively (Table 3).

Table 4: Risk factors associated with bacterial types carried in the nose of students

Strains	RTI* (31 stu.)	Otitis media (9 stu.)	Allergy (Rhin.) (27 stu.)	Total #	Chi ²	P-value
MRSA**	21	2	20	43	17.8	0.063
MRSE***	6	1	2	9		
<i>Kelbseilla</i> spp	1	3	3	7		
<i>Enterobacter</i> spp	2	2	1	5		
<i>Citrobacter</i> spp	1	1	1	3		

*Respiratory tract infection

** Methicillin-resistant *Staphylococcus aureus*

*** Methicillin-resistant *Staphylococcus epidermis*

Table 5: Comparison between healthy and high-risk groups

Strains	High-risk group	Healthy	Ch ²	P-value
MRSA*	43	70	6.9	0.14
MRSE**	9	31		
<i>Kelbseilla</i> spp	7	18		
<i>Enterobacter</i> spp	5	13		
<i>Citrobacter</i> spp	3	1		

* Methicillin-resistant *Staphylococcus aureus*

** Methicillin-resistant *Staphylococcus epidermis*



Table 4: Resistance phenotypes of confirmed isolates to antimicrobial agents as determined by the disc diffusion susceptibility test

Bacteria	Antimicrobial resistance of confirmed isolates (%)						
	AMC	IPM	SXT	RD	CRO	CIP	CD
<i>S. aureus</i>	94%	0%	33%	72%	44%	5%	72%
<i>S. epidermidis</i>	95%	0%	79%	74%	53%	0%	43%
<i>Klebsiella spp</i>	100%	0%	0%	86%	0%	0%	100%
<i>Enterobacter spp</i>	87%	0%	43%	66%	33%	0%	68%
<i>Citrobacter spp</i>	100%	0%	40%	80%	0%	0%	100%
<i>Pseudomonas spp</i>	100%	0%	50%	50%	0%	0%	100%
<i>Serratia spp</i>	100%	0%	0%	100%	0%	0%	100%

DISCUSSION

This study provides new insight into the prevalence and antibiotic susceptibility of community-associated methicillin-resistant bacteria among female pharmacy students. According to their lifestyle, these healthy individuals may serve as vehicles to spread microbes between healthy and unhealthy individuals. As we mentioned earlier the subjects were females who spend most of their time during the day in the same area and were sharing a place for more than three hours daily. This situation makes them more susceptible to acquiring any pathogens from each other. It is well known that places with a higher proportion of people in close contact with each other had higher estimated MRSA transmission, suggesting that comorbidities are a marker of vulnerability.¹²⁻¹⁴ In addition, most of the participants have an interest in joining their jobs once they finish their studies as healthcare workers; therefore, they will be in close contact with patients in different medical settings.

Our data has shown that the percentage of healthy individuals colonized with methicillin-resistant bacteria was remarkably higher than those studies previously reported by other groups.¹⁵⁻¹⁸ The high prevalence of methicillin-resistant bacteria in the community has important implications for clinical management and hospital infection control programmers. When the incidence of methicillin-resistant bacteria increases in the community, community-associated-methicillin-resistant bacterial strains have a propensity to replace hospital-associated-methicillin-resistant bacterial strains in healthcare settings, making infection control measures less effective for reducing the prevalence of MRSA.¹⁹ We reported in this study about 88.6% of the participants were colonized with more than one type of methicillin-resistant bacteria. It is well known that different types of bacteria can be carried in the nasal area of healthy individuals without causing any problems.² However, the extending of these types of bacteria to involve some types of bacteria with methicillin-resistant properties other than *S. aureus* is what we were asking about in this study. This variation between our results and others is because the

previously cited studies have focused on the isolation and characterization of MRSA only, but in this study, we could isolate and characterize seven types of bacteria which would make a remarkable difference in the percentages yielded. It should be noted that screening for other types of bacteria was not included in the previously mentioned studies. In our study, 32% of the participants were colonized with MRSA, which was consistent with other observations from different communities.²⁰ In contrast, Abouzeed, et al., (2010) confirmed that 51% of the isolates they worked on were MRSA.²¹ However, the occurrence of community-associated *S. aureus* (CA-MRSA) infection varies by geographic area.²²⁻²⁴ In agreement with this conclusion, Borg *et al.*, (2006) have reported that the Mediterranean region indeed constitutes a high prevalence region for MRSA.²⁵ Further interesting observation in our study was the isolation and characterization of *S. epidermidis*, *Klebsiella spp*, and *Citrobacter spp* from the participants as methicillin-resistant strains in high percentages, 34%, 12.5%, and 9%, respectively. Also, other types of bacteria such as *Enterobacter spp*, *Serratia spp*, and *Pseudomonas spp* were isolated from the nasal swabs. Our observation demonstrates emerging new types of bacteria with methicillin resistance, which may be due to the acquisition of the *Staphylococcal cassette* chromosome (SCC) carrying *mecA*, the gene encoding the methicillin-resistant penicillin-binding protein.²⁶

Most of the specimens examined yielded the same results in terms of antibiotic susceptibilities and bacterial type. This might be since about 25% of the participants were suffering from respiratory tract infection which would ease or facilitate the pathogen transmission. Recent observations have revealed that infection with MRSA can happen in hospitals and communities as well due to environmental settings.²¹ These results suggest that the characteristics of the environments of participants were similar. Our observation was consistent with other studies that showed clearly that when risk factors increased the pathogen transmission among individuals increased.²⁷ Moreover, Tinelli, M., et al., (2007) concluded in a review study that the outbreak of highly resistant community-associated or community-acquired MRSA among



routinely “closed” populations, such as Alaskan natives, American Indians, children, participants in team sports, military personnel, and correctional facility inmates, were remarkably high⁵. Our findings indicate the urgent need for making full and periodical assessments in terms of microbe carriers for people especially students who are being prepared to be healthcare workers

The interesting observation that we have shown here is that the predominant colonization strains were *S. aureus* and *S. epidermidis* followed by *Klebsiella* spp. Additionally, due to the established transmissibility of colonization isolates, the high prevalence of nasal carriage of *S. aureus*, *S. epidermidis*, and *Klebsiella* spp among healthy female pharmacy students may indicate the accelerated spread of these strains in the community.

The results of the present study extend the findings of earlier studies showing that methicillin resistance was associated with resistance to other antibiotics.⁶ As a traditional technique, we utilized the disc diffusion susceptibility test which is a valuable method for the accurate, reliable detection of MRSA and for monitoring resistance trends.²⁸⁻³⁰ In the present study, we found that community-associated methicillin-resistant bacteria had higher levels of resistance to Augmentin, Rifampicin, and Clindamycin. Importantly, a small percentage of CA-MRSA predominantly carry small size *SCCmec* type IV gene, which allows for more efficient transfer of resistance among different bacteria³¹, a factor that may account for the rapid emergence of CA-MRSA. On the other hand, the results indicated that the vast majority of the isolates were susceptible to Imipenem, ciprofloxacin, and Ceftriaxone and intermediate susceptibility trend to cotrimoxazole. Therefore, these antibiotics could be used when there are risk factors for CA-MRSA and to avoid any problem(s) that could lead to the overuse of various classes of antibiotics and contribute to increased resistance. Resistance to CA-MRSA is typically limited to beta-lactams and erythromycin, although multidrug resistance can occur. However, our results were in contrast to observation by Falagas, M.E., et al., (2007) showed that CA-MRSA had higher levels of resistance to Ciprofloxacin and cotrimoxazole.³ Furthermore, clindamycin-resistant patterns of CA-MRSA in our results were remarkably high when compared with results by Mandela *et al.*, 2012.³² It has been documented that clindamycin is a valuable curative option for MRSA infections, however, antibiotic treatment failures have been reported in patients with MRSA infections caused by inducible clindamycin resistance (MRSA-MLSBi) strains. Since evolving in the 1960s, many MRSA-resistant phenotypes with multi-resistance characteristics have been described and reported worldwide including in developing countries. The MRSA-MLSB phenotype is one of these phenotypes that confer multiple resistance to many antibiotic classes (i.e. macrolides, lincosamides, and Streptogramins B).^{33,34} However, antibiotic susceptibility trends of CA-MRSA vary considerably in different parts of the world.³⁵ In our study, most community-associated-methicillin-resistant

bacteria were similar in their antibiotic-p susceptible patterns. Therefore, it is possible that the strains were predominant in the area of study and circulating among female pharmacy students.

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