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Article

Molecular and Source-Specific Profiling of Hospital *Staphylococcus Aureus* Reveal Dominance of Skin Infection and Age-Specific Selections in Pediatrics and Geriatrics

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†Ha'il College of Medicine (COM) Research Unit is a research-intensive hub that, in addition to housing advanced research initiatives, co-ordinates medical and clinical research in the college, and organizes, follows up, and facilitates Deanship Funded Research Programs such as annual "Research Groups" series, Badi, and other institutional local, national and regional program

Abstract: The changing epidemiology of *Staphylococcus aureus* has created several gaps in its population structure and emergence of strains. Two global shifts in the aftermath of the past methicillin-resistant *S. aureus* (MRSA) pandemic are: a rise in healthcare-associated infections and evolution of cutaneous and soft tissue infections with high morbidities and mortalities. Furthermore, bitter lessons from COVID-19 showed *S. aureus* necrotizing-pneumonia and skin conditions aggravating Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) and Monkeypox manifestations. Limited data and paucity of high-quality evidence exist for many key clinical questions. Using clinical microbiology, molecular characterization, hospital data on age and infection sites, and antibiograms, we have investigated *S. aureus* infection patterns. We showed that age-specific distribution in both intensive care unit (ICU) and non-ICU revealed highest infection rates (94.7%) in senior-patients >50 years; most were MRSA (81.99%). However, specific distributions of geriatric MRSA and MSSA rates were 46.5% and 4.6% in ICU and 35.48% and 8.065% in non-ICU, respectively. Intriguingly, age groups 0-20 years showed uniquely similar MRSA patterns in ICU and non-ICU patients (13.9%, 9.7%, respectively) and MSSA in ICU (11.6%). In age groups 20-50 years, MRSA were 2-fold in non-ICU (35%) than ICU (18.6%). Interestingly, highly significant association was found between infection-site and age-groups (*P*-value .000). Skin infections remained higher in all ages; pediatrics 32.14%, adults 56%, and seniors 25% while respiratory infections were lower in pediatrics (14.3%) and adult 17%), and highest in seniors (38%). Blood and "other" sites in pediatrics recorded (28.6%; 25%, respectively), slightly lower in adults (18.6%; 8.6%) and seniors (14%); 22.8%), respectively. Further, significant association existed between infection-site and MRSA (Chi-Square Test, *P*-value .002). The common cutaneous infections across all age-groups and the significant association of MRSA to geriatric-respiratory infections have a high potential for skin-carriage as reservoir for endogenous infection. The similar frequencies of both lineages in youth in all settings imply MSSA-carriage as potential evolutionary origins for MRSA. These findings have important clinical implications for strategic planning in patient management and *S. aureus* control particularly in age-specific infections and vigilance for potential viral coinfections.

Keywords: clinical *S.aureus*; skin carriage; geriatric-MRSA pneumonia; endogenous-*S.aureus*

1. Introduction

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Despite significant advances in medicine and healthcare systems, the world is witnessing an era of serious pandemic emergences. One of the most difficult types of skins respiratory bacterial outbreaks are those infections caused by virulent lineages of invasive *Staphylococcus aureus.* By the virtue of their predilection to skin and respiratory infections, these strains potentially aggravate virus pandemics. The recent devastating pandemics, the Covid-19 syndrome and the monkeypox skin syndrome [1], are the typical skin and soft tissue infections aggravated by the virulent *S. aureus* lineages. The SARS-CoV2, the third consecutive outbreak of coronavirus, has rapidly evolved into an extremely elusive virus with advanced mechanisms of pathogenicity and epidemicity. The SARS-CoV2 infection is highly prone to be aggravated by clinical manifestations such as skin, necrotizing pneumonia, and cytokine storm by the re-emerging methicillin resistant *Staphylococcus aureus* (MRSA). The MRSA necrotizing pneumonia is known to elicit influenza-like symptoms requiring admission to the intensive care unit and mechanical ventilation. In extreme cases, necrotizing pneumonia and disseminated intravascular coagulation can induce Acute Respiratory Distress Syndrome (ARDS) shock leading multi-organ failure[2. How MRSA virulence operates in pneumonia is still far beyond clear; how this superantigen induces cytokine storm while impairing neutrophil chemotactic activity-the core mechanism of the storm generation, is only one puzzling scenario [2]. Interestingly, while MRSA has been found to cause gastroenteritis and unilobar infiltrates, methicillin sensitive *S. aureus* (MSSA) was involved in airway hemorrhage, multilobar infiltrates and ARDS [3]. However, reports on the potential implications of MRSA in monkypox and SARS-CoV2 pneumonia is limited. In addition, profiles of *S. auareus* infections and their clinical characteristics in non-COVID non-monkeypox patients is not understood in the region. Thus, this study aims to determine the rates of MRSA and MSSA infection and disease profiles in non-COVID, non-monkeypox patients in Ha'il, Saudi Arabia.

The changing epidemiology of *S. aureus* lineages in humans have created several gaps in our understanding of this species adaptive mechanisms of infection. For instance, during the last decade, emergence of *pvl* positive community acquired (CA)-MRSA, MRSA, and MSSA lineages have paralysed global healthcare systems with mortality rates similar to that of AIDS, tuberculosis, and viral hepatitis combined [4; 5–9]. *Staphylococcus aureus* is an centuries-old pathogen that has been evolving and emerging through decades, not in spite of, but surprisingly as a consequence of the advances in medical progress [10. Thus, the history explains a continuous evolutionary pattern of the original pandemic strains. A single lineage caused more invasive diseases than the combined rates of those caused by bacterial species with transformable genomes; namely, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Neisseria meningitidis*, and *Haemophilus influenzae*. Nevertheless, estimates of *S. aureus* mortality rates well exceeded that of the HIV [6,11]. Referring to overuse of anti-MRSA drugs, and fear of history repeating itself in MRSA pandemics, Chalmer et al., [12] reported under the title: "Those Who Do Not Remember the Past Are Doomed to Repeat It". Although global MRSA outbreaks have declined, high morbidities and mortalities are still being reported in the United States and elsewhere globally [13. A comprehensive review of 15 clinical investigations showed that up to 74% of worldwide *S. aureus* infections were caused by different MRSA lineages in Europe [14, and another pandemic with an annual economic burden of $3.3 billion in the USA alone, was proposed. Thus, more insights into *S. aureus* strategies encompassing mechanisms of acute nosocomial resistances, carriage, adaptation, and evolution of virulence in different lineages have become imperative in the context of the emerging pandemics. Specifically, profiles resistant strains circulating in hospitals and communities, antimicrobial resistance patterns, and carriage rates is not well defined in the region.

The declaration of COVID-19 as new respiratory pandemic in early March 2020 brought about significant changes in respiratory management of infections and implementation of various measures and restrictions globally [15. Microbial coinfections during SARS-CoV2 as well as monkey box and other outbreaks, always have important roles in aggravating diseases, increasing mortality, and morbidity rates 16] Viral, bacterial, and

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fungal coinfections alter the pathophysiology of the disease and the patient recovery outcome [17,18].. Among microbiological coinfections, bacterial pathogens are considered more important than other organisms based on experience with previous viral pandemics [19. In earlier viral outbreaks, bacterial coinfection resulted in high mortality rates especially in critically ill patients in intensive care units as they are at higher risk of coinfection compared to regular hospitalization [20. However, the role of bacterial coinfection before COVID-19 and monkey box were not well defined making it difficult to speculate during the pandemic. Of particular importance are the respiratory and skin pathogens such as MRSA. Susceptibility to infections by one of the MRSA lineages such as pneumonias, skin, and soft tissue is ideally measured by rates of colonization of the nares and skin surfaces where their absence is a known negative indicator of the disease. Albeit nasal screening is used for infection control and prevention purposes. These screening procedures are not well described in non-COVID-19 patients in the region. This is important for future studies in understanding MRSA virulence mechanisms and their potential aggravation of viral pandemics. In has been recently highlighted that MRSA nasal screening in non-COVID-19 patient is a useful antimicrobial stewardship to avoid unnecessary empiric MRSA therapy such as vancomycin [21,22]. Current guidelines for the treatment of pneumonia as per the American Thoracic Society (ATS) and Infectious Diseases Society of America (IDSA) recommended empiric MRSA coverage in patient at-risk [23,24. While initiating appropriate empiric antibiotics in a timely manner is critical, identifying nasal and skin carriage status is equally important for specific initiation and de-escalation timings of anti-MRSA coverage therapy.

Infectious community-acquired pneumonia (CAP) is a most severe infection acquired in communities and/or extended home-cares leading to mortality and hospitalizations worldwide due to necrotizing pneumonia [12,25–27. The implications of this species in hospital pulmonary sepsis have been postulated to correlate with ARDS for years until a recent study confirmed the MRSA direct involvement and the role of FTY720 S-phosphonate in endothelial cell protection [28. The risk is that case fatality rates of MRSA in sepsis patients is dramatically high globally. Estimated 30 million cases of lung sepsis annually have led to more than eight million deaths, i.e.,15–30% in high-income countries and 50% or higher in low-and middle-income countries [29]. This is particularly serious when *pvl*-positive *S. aureus* (CA-MRSA lineage) infections are involved in ARDS conditions. More importantly, clinical management is potentially devastating when the etiologic scenarios in ARDS and CA-MRSA pneumonias are further complicated by similar respiratory Covid-19 and/or skin monkey box syndromes. Therefore, rapid differential diagnosis for MRSA pneumonias is imperative. However, exclusion of the infectious pneumonia requires much needed time on antibiotics or antivirals. In addition, despite the links between infectious pneumonia ARDS, continuous surveillance of additional cases is required for substantial evidence in such patients. Thus, multi-point surveillance of *S. aureus* lineages including MRSA and PVL- positive *S. aureus* in nosocomial setting is important for control of necrotizing pneumonia, ARDS, and potential aggravation of monkey box or Covid-19. Data is limited in these new aspect of *S. aureus* research.

In recent years, with the increase in global population dynamics a significant increase in community associated lung infections have occurred. Despite the remarkable progress made in advancing healthcare systems, pneumonia associated to lung sepsis remains a burdensome in global public health [30,31. Furthermore, the high complexity and costs associated to lung care complicates cases leading to high morbidity and mortality. For instance, an annual estimate of 30 million cases of sepsis have led to more than 8 million deaths; i.e.,15–30% in high-income countries and 50% or higher in low-and middle-income countries [29]. Particularly, the clinical and economic burden of CAP is staggering, far-reaching, and expected to increase as new antibiotic resistance mechanisms emerge and the world's population ages [32]. In the US alone the annual hospitalization rate for CAP is more than USD$ 2.6 million, ranking second only to childbirth for hospital admissions [available at: https://www.hcup-us.ahrq.gov/db/nation/nis/NIS_Introduction_2017.jsp]

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accessed May 24 2022, the Agency for Healthcare Research and Quality National: regional estimates on hospital use for all patients from the HCUP National Inpatient Survey (NIS 2017)]. Therefore, a leading cause of death worldwide is sepsis especially when developed as a dysregulated immune response to infectious pneumonia [33,34].

Although decades of pandemics of necrotizing infectious pneumonia caused by MRSA have slightly declined, high morbidities and mortalities are still being reported [13]. For instance, up to 74% of global *S. aureus* infections are caused by evolving new lineages [14 (Köck et al., 2010)Worth [mentioning that evolution of invasive MSSA strains causing bloodstreams infections in healthy communities are being increasingly reported [35]. Emergence of a single MSSA clone in Greece carrying high level resistances primarily to mupirocin (99%), has caused significant staphylococcal scaled skin syndromes in a setting; 85% of the cases were impetigo and 4.8% [36]. Furthermore, the recent emergences of a new and the previously unreported clonal complexes of methicillin-resistant MRSA strains in the region is of concern [37–40].

Although *S. aureus* is a medically important pathogen, significant portion of human populations carry the species asymptomatically in their nasopharynx and skin. This goes largely unnoticed when the host-pathogen natural ecosystem is in balance. However, this carriage status is often prone to localized or disseminated serious systemic invasive infection when this balance is shaken. It is still not clear how *S. aureus* switches from commensal to life threatening pathogen and from humans to animal associated lineages or vice versa, despite evidence of its highly clonal genome. This evidence has been long established from intrinsic and highly polymorphic accessory genes [41–45]. The common genomic background of the species in humans and animals opens a new aspect in *S. aureus* epidemiology, the serious zoonotic transfer of MRSA from pets and dairy animals into human-human-human transmissions. For these reasons, regular hospital surveillance of *S. aureus* infections for local strain profiles, sources of transmissions, and antimicrobial resistance is important. We have previously used surveillance programs to understand the rates of *S. aureus* infections followed by molecular differentiations of MRSA and MSSA lineages to identify dominant clonal lines in North America and Middle East [46–49].

Unfortunately, significant variations occur in the rates of *S. aureus* surveillance programs in the Middle Eastern and African countries (MENA) that made it difficult to develop an effective MRSA management program. A recent comprehensive report from January 2005 to December 2019 revealed great heterogeneity in MRSA rates. For instance, nasal MRSA colonization ranged from 2%–16% in Gulf Cooperation Council (GCC), 1–9% in the Levant, and 0.2%–9% in North African Arab states. Clinical isolates of MRSA ranged from 9%–38% in GCC, 28%–67% in the Levant, and 28%–57% in North African states. Studies demonstrated a wide clonal diversity in the MENA. In addition, significant diversities in clonal complexes and antimicrobial resistances were also seen in the region with variation in patterns depending on location and clonal type [50]. Thus, comprehensive, and accurate prevalence rates of *S. aureus* in the region requires to be established in each country first before vertical genotyping of dominant clones for local strain profiling. This study was intended to understand the antibiogram patterns, disease profiles, and types of circulating MRSA and MSSA lineages while assessing the performance of MRSA screening protocol in-place.

2. Materials and Methods

2.1. Bacteriological analysis, patients' demographics, and antimicrobial susceptibility testing

In this work, we analyzed all positive specimens for non-duplicate isolates of methicillin-resistant *S. aureus* (MRSA) and methicillin sensitive *S. aureus* (MSSA) obtained from clinical samples recovered from hospitals in Ha'il in the first quarter year of 2021. All data was collected from microbiology laboratory records, hospital medical records, various sources within hospitals. The data included but not limited to antibiotic sensitivity data, specimen types and collection sites, intensive care unit (ICU), and non-ICU ward, and age

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differences from King Khalid hospital. Specimens were analyzed by routine bacteriology and standard antimicrobial sensitivity testing. Briefly, they were cultured for primary isolations and preparations of cultures for automated machines. Isolates were identified by standard bacteriological methods and ID and susceptibility testing using automated systems. For non-automated procedures, specimens that were aseptically collected in suitable transport media and swabs to the lab, processed immediately, and cultured using standard conditions and media under 37C incubations for 18 hours. Bacterial isolates were kept in broth cultures at -80°C for future reference and vertical studies. However, most of the work was done on automated systems including BD Phoenix system (BD Biosciences, Franklin Lakes, NJ, USA) and MicroScan plus (Beckman Coulter, Brea, CA, USA) were used for the identification and antimicrobial sensitivity analysis of microorganisms. Susceptibility was confirmed by culture and agar diffusions experiments as necessary. The susceptibility testing and breakpoint interpretive standards were carried out accordance to the recommendations of Clinical and Laboratory Standard Institute (CLSI document M100S-26) [51]. Molecular kits to confirm lineages of *S. aureus* species along with primary identifications were used. The advanced build-in realtime GeneXpert systems for direct molecular diagnostics and characterizations and strain and species confirmations directly from specimens was used as explained in molecular characterization section below in detail

2.2. Classifications as Multi-, Extremely- and Pan-Drug Resistant Bacteria (MDR, XDR, and PDR)

In standard definitions of acquired resistances classifications, *S. aureus* isolates classified as multi drug-resistant (MDR) by the virtue of their methicillin resistance. Furthermore, extensive drug-resistant (XDR), and pan drug-resistant (PDR) are usually applied based on recommendations of European Centre for Disease Control. The MDR criteria for acquired resistance states non-susceptibility to at least one agent in three or more antimicrobial categories, XDR was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) and PDR was defined as non-susceptibility to all agents in all antimicrobial categories as reported by Magiorakos et al., [52]. Intrinsic resistances to particular drugs were not included. Criteria for defining *S.. aureus* MDR classifications include one or more of the following have to apply): 1. MRSA is always considered MDR by virtue of being an MRSA.**2.** (ii) non-susceptible to ≥1 agent in >3 antimicrobial categories.

http://www.ecdc.europa.eu/en/activities/diseaseprogrammes/ARHAI/Pages/public_consultation_clinical_microbiology_infection_article.aspx

2.3. Molecular Detection of S. aureus, (MRSA and MSSA lineages using GeneXpert Spa, mecA and mec (SCCmec) build-in primers

Direct molecular characterizations were carried out in the latest versions of the direct rapid molecular detection system using the GeneXpert-RT-PCR for *S. aureus* (SA) and MRSA. This system is equipped with mutli-gene molecular kits for robust automated detection of *S. aureus* lineages directly from specimens. Since there could be resistance genes present but not expressed for some reason, cetoxitin nasal screening ensured resistance and susceptibility were based on expression of genes and their absence, respectively The Cepheid GeneXpert SA Nasal Complete assay was used in the direct detection *S. aureus* lineage differentiation by the built-in primers for *spa*, *mecA* and the *mec* (SCC*mec*) gene sequences from specimens. The Cepheid® Xpert SA Nasal Complete assay performed in the GeneXpert® Dx System employed *in vitro* rapid detection of *S. aureus* SA and MRSA from nasal swabs of patients following manufacturers recommendations. This test utilizes automated real-time polymerase chain reaction (PCR). Culturing was also done for further susceptibility testing. The GeneXpert Dx is all-in-one system that integrates sample purification, nucleic acid amplification, and detection of the target sequence in simple or

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complex samples using real-time PCR and RT-PCR assays. It consists of an instrument, personal computer, and preloaded software for running tests and viewing the results. A single-use disposable self-contained cartridges with PCR reagents is inserted and inoculated directly with swabs. Cross-contamination between samples or during specimen collection or processing is remote since he cartridge is self-contained. A sample processing control (SPC) and a Probe Check Control (PCC) are also included. The SPC is present to control for adequate processing of the target bacteria and to monitor the presence of inhibitor(s) in the PCR reaction. The PCC verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

2.4. Statistical Analysis.

Collected data was analyzed using Statistical Package for Social Sciences software (IBM SPSS; Version 24 SPSS version 23.0 for Windows (SPSS, Inc., Chicago, IL, USA). Descriptive and stratified analysis were conducted; we present absolute numbers, proportions, and graphical distributions. We conducted exact statistical tests for proportions and show *p*-values where appropriate (a *p*-value <0.05 was considered statistically significant).

3. Results

In this study, we have collected 195 isolates of *S. aureus* for clinical disease profiling, antibiogram patterns, and rates, molecular types of circulating MRSA and MSSA lineages in different hospital settings. Of these isolates, overall, 41% (*n*=80) of the isolates were MSSA and the rest 60% (*n*=115) were MRSA. However, 167 isolates were used for comparative examination of ICU and non-ICU infections across different age groups. As shown in Figure 1, the overall MSSA infection rates were lower among different age groups of patients in ICU and non-ICU settings compared to that of MRSA. Different age groups of patients revealed different patterns of *S. aureus* lineages and disease characteristics with lowest infection rates reported in pediatric patients.

3.1. Age-specific frequencies of S. aureus lineages in ICU and non-ICU

Figure 1 and Table 1 showed that in the first group (0 to 20 years old), the overall total MRSA isolation rate in both ICU and non-ICU settings was 23.6% (*n*=39) while that of MSSA was 16% (*n*=26). However, among this age group under ICU, 14% were positive for any type of MRSA infections while 11.6% had infections with invasive MSSA lineage. On the other hand, among the same aforementioned age group under other clinical settings than ICU (inpatient and outpatient), MRSA isolation rate of 9.7% was over 2x higher than that of MSSA (4%). However, in the subsequent higher age groups, susceptibility to *S. aureus* infections substantially increased as indicated by the results obtained below. In the second age group among the young and mid-aged patients (20-50 years old), the overall MRSA and MSSA rates in all hospital settings were 53.3% and 12.7%, respectively. Patients in the above age group under ICU units reported 18.6% and 4.6% of MRSA and MSSA infections, respectively. However, the overall rate of infections by the two lineages under non-ICU "other clinical settings than ICU" was 42.7% of which 34.68% of patients were positive for MRSA infections and 8.065% of them had MSSA infections. The third age group included patients over 50 years and seniors with advanced age who had the highest frequency of overall *S. aureus* infections (94.7%). Among these, the overall total rates of the two lineages, MRSA and MSSA, in all settings were 81.99% and 12.7%, respectively. However, in ICU, 46.5% were MRSA and 4.6% were MSSA. On the other hand, under other non-ICU clinical settings, the senior patient group showed isolation rates of 35.5% and 8 % MRSA and MSSA, respectively (Figure 1, Table 1).

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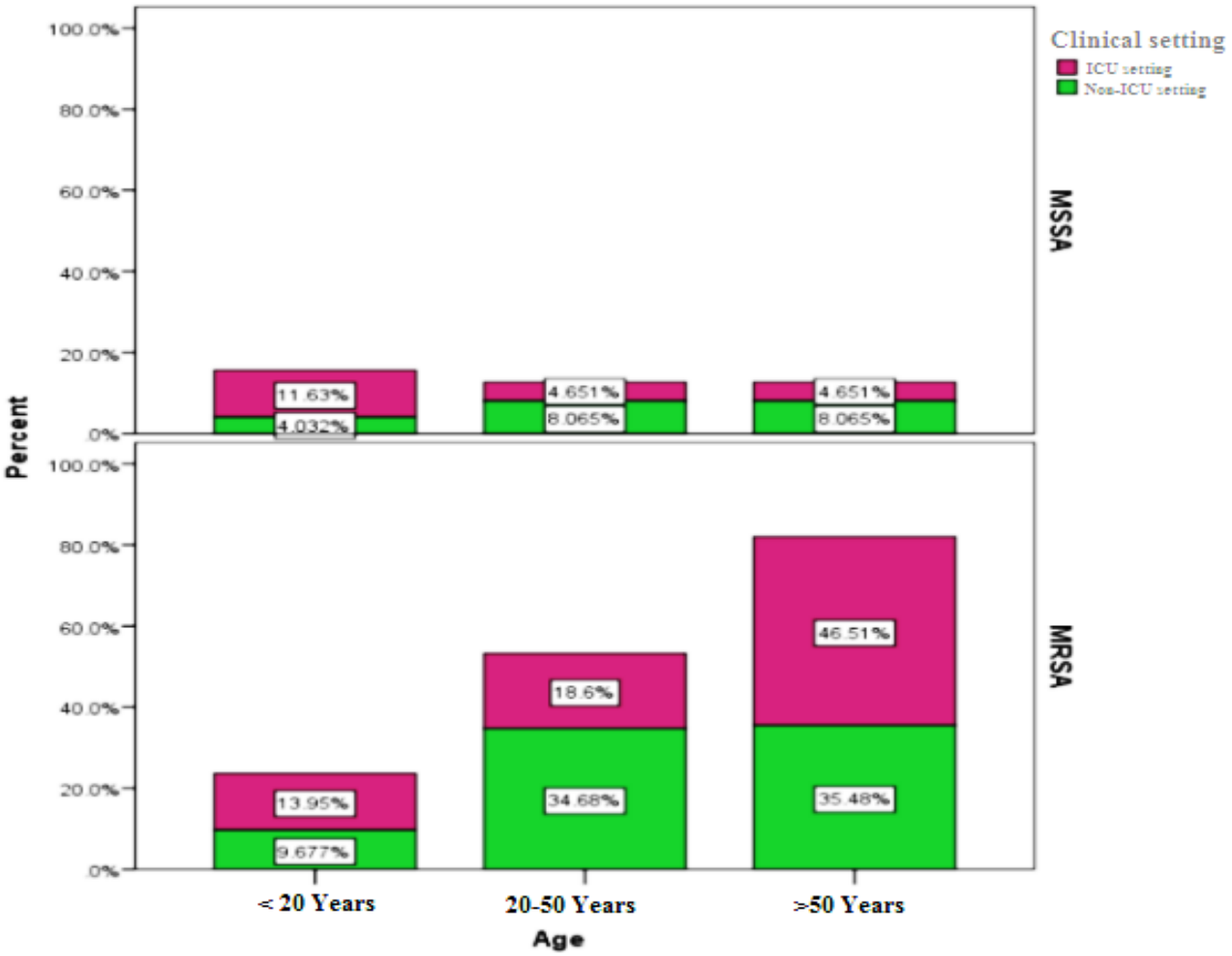

Figure 1. Age-group specific distributions of MRSA and MSSA isolates recovered from clinical specimens in ICUs, and other non-ICUs (inpatient and outpatient) settings in Ha'il hospitals, Saudi Arabia. Abbreviations: MRSA – Methicillin-resistant *Staphylococcus aureus*; MSSA– Methicillin-sensitive *Staphylococcus aureus*; ICU-intensive care unit; non-ICU-non-intensive care unit.

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Table 1. Profiles of nosocomial MRSA and MSSA clinical isolates among different age-groups of patients at ICUs, and other non-ICUs settings in Ha'il region, Saudi Arabia.

***Staphylococcus aureus* isolates**			**Age groups**		
			<20	**20-50**	**>50**
MSSA	**Setting**	ICU	5	2	9
		Non-ICU	5	10	25
	Total		10	12	34
MRSA	**Setting**	ICU	6	8	34
		Non-ICU	12	43	99
	Total		18	51	133
Total	**Setting**	ICU	11	10	43
		Non-ICU	17	53	124
	Total		28	63	167

Abbreviations/A- not available; MRSA – Methicillin-resistant *Staphylococcus aureus*; MSSA– Methicillin-sensitive *Staphylococcus aureus*.

3.2. Organ-specific distribution of clinical S. aureus infections among different age groups

Since *S. aureus* is widely assumed to cause mostly endogenous infections seeded from specific reservoir sites. We intended to examine this hypothesis and provide proof of concept on age- and organ-specific associations. We also wanted to understand the potential influence of *S. aureus* carriage sites i.e., skin and respiratory sites, as a reservoir for endogenous infections. For these purposes, we selected 177 patients in different age groups. As shown in Table 2, statistical analysis (Pearson Chi-Square, Likelihood Ratio Linear-by-Linear Association) revealed significant association between age groups and infection sites. In this study, skin infections remained higher in all age groups as followed: pediatrics 32.14%, adults 56%, and seniors 25% (Figure 2). However, respiratory infections remained relatively lower in pediatrics (14.3%) and adult 17%), while seniors showed the highest infection rates (38%). Blood and "other" (organ sources other than specified) infections were higher in pediatrics (28.6% and 25%, respectively) and slightly lower in adults (18.6%) and (8.6%) and seniors (14%) and (22.8%), respectively.

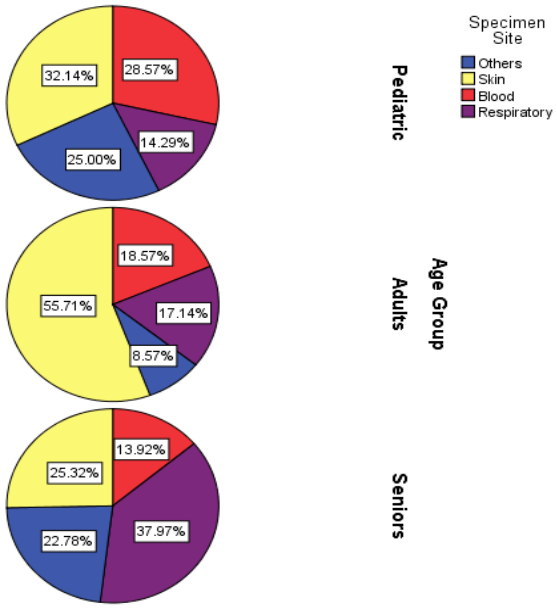

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Figure 2. Skin and other organ-specific distribution of clinical *Staphylococcus aureus* isolates among different age-groups of patients in Ha'il region, Saudi Arabia.

Table 2. Frequency of clinical *Staphylococcus aureus* isolate from skin and other organs among different age-groups of patients in Ha'il region, Saudi Arabia.

			SPECIMEN SITE				
			Others	Skin	Blood	Respiratory	Total
Age	**<20**	Count	7	9	8	4	28
		Percentage	25.0%	32.1%	28.6%	14.3%	100.0%
	20-50	Count	6	39	13	12	70
		Percentage	8.6%	55.7%	18.6%	17.1%	100.0%
	>50	Count	18	20	11	30	79
		Percentage	22.8%	25.3%	13.9%	38.0%	100.0%
Total		Count	31	68	32	46	177
		Percentage	17.5%	38.4%	18.1%	26.0%	100.0%

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	25.032[a]	6	.000
Likelihood Ratio	25.237	6	.000
Linear-by-Linear Association	2.848	1	.051
N of Valid Cases	177		

3.3. Association between site of infection and clinical S. aureus lineage

To understand the clinical patterns of MRSA and MSSA lineage infections among different age groups of patients, we have developed a strategy to validate the notion of site- and lineage-specific infection concept. For this, we have screened 195 *S. aureus* clinical isolates from different specimen types. As shown in Table 3, significant association between specimen site and MRSA detection was observed (*P*-vlaue.002). Total MRSA and MSSA infections in all sites were 74.9% and 25.1%, respectively. The frequency of MRSA infections in different specimens were 70.7%, 65.3%, 72.7%, and 95.7% in "others", skin, blood, and respiratory samples, respectively. On the other hand, MSSA infections were 29.3%, 34.7%, 27.3%, and 4.3% in "others", skin, blood, and respiratory samples, respectively (Figure 3; Table 3).

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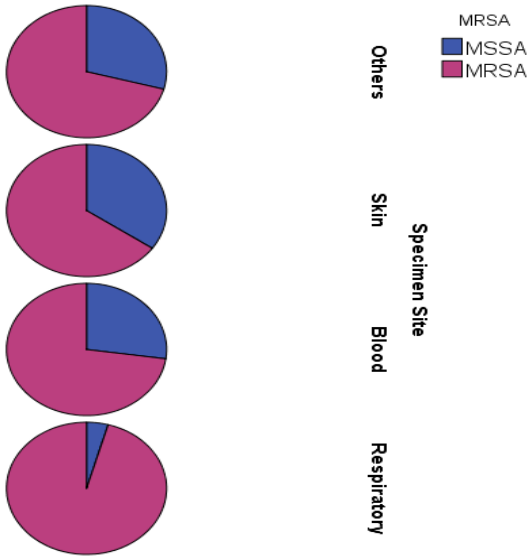

Figure 3. Frequencies of nosocomial MRSAs and MSSAs rates on the skin and different clinical specimen sites in Ha'il region, Saudi Arabia. Abbreviations/A- not available; MRSA – Methicillin-resistant Staphylococcus aureus; MSSA– Methicillin-sensitive Staphylococcus aureus.

Table 3. Association rates between site of infection on different clinical specimen and MRSA and MSSA lineages in Ha'il region, Saudi Arabia.

Specimen Site by			***Staphylococcus aureus* isolates**		
			MSSA	MRSA	Total
Specimen Site	**Others**	Count	12	29	41
		Percentage	29.3%	70.7%	100.0%
	Skin	Count	26	49	75
		Percentage	34.7%	65.3%	100.0%
	Blood	Count	9	24	33
		Percentage	27.3%	72.7%	100.0%
	Respiratory	Count	2	44	46
		Percentage	4.3%	95.7%	100.0%
Total		Count	49	146	195
		Percentage	25.1%	74.9%	100.0%

3.4. Molecular characterization and Antimicrobial susceptibility testing (AST) of clinical S. aureus lineages

All *S.aureus* isolates were characterized by the robust molecular system GeneXpert-RT-PCR using the build-in mutli-gene primers molecular kits for robust direct automated detection from specimens and cetoxitin nasal screening. The Cepheid GeneXpert *S. aureus* (SA) Nasal Complete assay was used in the direct *S. aureus* species confirmation, detection and differentiation of MRSA lineages from MSSA by *nun, spa, mecA* and the *mec* (SCCmec) gene sequences from specimens of patients following manufacturers recommendations on automated real-time polymerase chain reaction (PCR). This approach ensures accurate molecular detection and characterization of fresh lineages directly from samples minimizing the potential mutation or adaptive expressions that prone to alter clonal properties of

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isolates. Concomitant culturing was carried out from all suspected specimens for further susceptibility testing and confirmation when necessary.

The AST of clinical *S. aureus* recovered from different clinical specimens is shown Figure 4. Out of 195 *S. aureus* isolates studied, MRSA isolates were 75% (n=146) while MSSA were 25% (n=49). The antimicrobial susceptibility testing revealed significant progress and encouraging results for the MRSA and CA-MRSA management strategies introduced. Every patient, in-patient, out-patient, causal visitors, and consultees are all subjected to nasal screening for MRSA. Any positive result is immediately subjected to a quarantine isolation until cleared. In this, study, the antibigram of *S. aureus* (Figure 4,) was tested again 22 antimicrobials in different categories. We identified three different patterns of susceptibilities that were grouped into three different groups as followed: Group1 antibiotics included (Tetracycline, Teicoplanin, Daptomycin, Linezolid, Mupirocin,https://www.goodrx.com/nitrofurantoin/what-is Nitrofurantoin, Vancomycin, Moxifloxacin and Clindamycin) with a sensitivity rate of more than 97% except for Clindamycin 92%. Group2 antibiotics included (Trimethoprim / sulfamethoxazole,Ciprofloxacin,Erythromycin, and Cefotetan/Cefalexin) with a sensitivity of more than 70%. Group3 antibiotic included (Fusidic acid,Imipenem,Amoxicillin clavulanic acid,Cefotaxime,Oxacillin,Cefoxitin,Ampicillin and Pencillin G) with a resistance more than 50% even reaching up to 90%. Antimicrobial resistance classifications of *S. aureus* lineages is based on standard definitions for acquired resistance where they were MRSA lineage is classified as a multi drug-resistant (MDR) by virtue of only being an MRSA.

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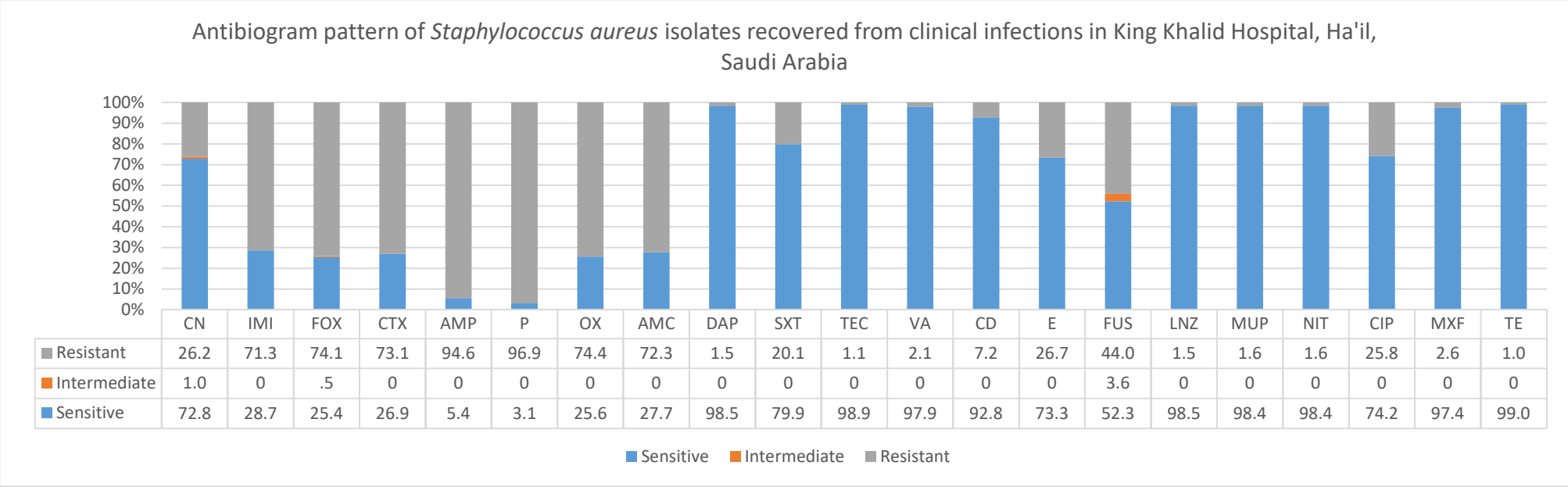

	CN	IMI	FOX	CTX	AMP	P	OX	AMC	DAP	SXT	TEC	VA	CD	E	FUS	LNZ	MUP	NIT	CIP	MXF	TE
Resistant	26.2	71.3	74.1	73.1	94.6	96.9	74.4	72.3	1.5	20.1	1.1	2.1	7.2	26.7	44.0	1.5	1.6	1.6	25.8	2.6	1.0
Intermediate	1.0	0	.5	0	0	0	0	0	0	0	0	0	0	0	3.6	0	0	0	0	0	0
Sensitive	72.8	28.7	25.4	26.9	5.4	3.1	25.6	27.7	98.5	79.9	98.9	97.9	92.8	73.3	52.3	98.5	98.4	98.4	74.2	97.4	99.0

Figure 4. Antibiogram patterns of *Staphylococcus aureus* isolates recovered from clinical infections in Ha'il regin, Saudi Arabia.

Abbreviations of 21 different antimicrobials: CN gentamicin, IMI imipenem, cefoxitin FOX, cefotaxime CTX, AMP ampicillin, P penicillin, OX oxacillin, AMC AMPICILLIN/SULBACTAM, DAP daptomycin, SXT trimethoprim/sulfamethoxazole, TEC teicoplanin, VA vancomycin, CD clindamycin, E erythromycin, FUS fusidic acid, LNZ linezolid, MUP mupirocin, NIT nitrofuran, CIP ciprofloxacin, MXF moxifloxacin, TE tetracycline,

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4. Discussion

In this study, we have investigated different aspect of nosocomial *S. aureus* infections that revealed findings with significant clinical implications for development of effective patient management strategies and MRSA and MSSA containment practices. These included the patterns and distribution of clinical isolates, rates, and frequencies of different molecular types of circulating MRSA and MSSA lineages. In addition, we studied age-specific distribution rates among groups of patients, antibiogram patterns across 22 different antimicrobials. More importantly, studies on skin carriage status for potential endogenous infections in respiratory and other sites were examined. Finally, *S. aureus* lineage-specific distributions in different organs was determined.

Age-specific infections among different age groups of patients revealed that senior patients over 50 years old had the highest *S. aureus* infection rates (94.7%); the overwhelming of these were MRSA (81.99%) and 12.7% were caused by MSSA. This finding is consistent with many other studies in geriatric infection and long term care facilities (LTCF) around the world [53–56] However, it was surprising to find lower MRSA transmissions in hospitalized seniors compared to new admission screening and shorter-term residents. These novel findings are rare except for a few studies describing similar results in Japan [55]. At present, we do not have clear explanations on these findings other than the stricter MRSA screening protocols in place since the aftermath of MRSA pandemics more than a decade ago [6,11 12]. Positive impact of active MRSA screening protocols have been found in different countries where reduction rates were significant [57]. The recent implementation of a National Infection Control Campaign in UK resulted in large decreases in ICU-acquired infections, including MRSA, occurred across the UK ICU network during the first few years [58]. Nevertheless, state mandated State-Mandated Active Surveillance was not successful in some regions in the USA due to limitations in application [59]. However, 46.5% of the geriatric MRSA infections, and only about 4.6% MSSA, we reported here were in ICU. Unfortunately, MRSA sepsis and complications remain as a significant issue in ICU in many geographic regions where prevalence has been found exceptionally high in many countries including China [60], Korea [61], India [62], as well as Saudi Arabia [63]. Thus, further surveillance programs and stricter screening procedures have become imperative in clear MRSA from critically ill patients. This should also include non-ICU MRSA where the rates showed 35.48%.

Profiles of *S. aureus* infections in children and young adults (age groups 0 to 20 years old), showed unique distribution pattern despite their lower rates than those of older age groups. While MRSA lineage showed similar frequency in both ICU and non-ICU patients (13.9%, 9.7%, respectively), MSSA infection rates in ICU (11.6%) were similar to those of MRSA, but lower in non-ICU (4%). Since geriatric and pediatric ICUs are separate, and that MRSA screening is equally applied in all patients, the similar frequency of MRSA in ICU and non-ICU is potentially a host-organ-, and age-specific factor in colonization and/or infection rather than underlying cause for hospitalization. Relevant findings in this context have been historically established. For example, significant association between *S. aureus* types and age as well as host-and-tissue specificity has been found long ago in human and animal lineages [64,65]. This is further supported by our results in this study where Chi-Square Tests revealed significant association between age groups and infection sites (p-value .000) and that skin infections remained higher in all age groups as followed: pediatrics 32.14%, adults 56%, and seniors 25% implying carriage and genetic transfer of virulence. Intriguingly, this interesting picture also reflects reservoir and carriage status for evolutionary origins of these lineages. It is plausible that in a confined age group under a single setting the common genetic background allowed for gene transfer and evolution of virulence between MSSA and MRSA lineages. We have previously established that the host- and organ-specificity of related

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S. aureus lineages drives gene transfer and evolution of virulence.[47]. This occurs only between related lineages due to a novel lineage-specific type-I restriction-modification system that blocks distant gene transfer into *S. aureus* [66]. Future vertical genomic profiling for gene content would provide valuable clues to the multifactorial mechanisms involved. This is important since MRSA rates were 2-fold higher in non-ICU (35%) than that of ICU (18.6%) among mid-aged patients (20-50 years old) while MSSA rates remained relatively lower in both settings.

The lineages specificity and infection profiles to different sites revealed further inside into the distribution of clinical isolates of the MRSA and MSSA infections. We report on significant association between specimen site and MRSA detection as indicated by the Chi-Square Tests value (.002). The likely reason for this could be explained in terms of the natural resident sites on human skin and respiratory regions (anterior nares and nasopharyngeal sites) and their transmission routes therefrom. Con, MSSA infections rates were higher on skin implying potential carriage owing to the fact that skin is a normal ecological niche. Since MRSA detection in respiratory sites including all nasopharyngeal and lung regions as well as in blood is common, its high frequency on skin among all age groups might imply endogenous seeding from reservoir carriage sites. This particularly strengthened the contagious cutaneous mode of transmission route in nosocomial MRSA dynamics. Future molecular genotyping and genomic analysis will provide more insights into MRSA evolutionary origins and MSSA profiles on carriage sites.

The patterns of *S.aureus* antibiogram shown by the antimicrobial susceptibility testing (AST) indicated effectiveness of the MRSA management strategies introduced. All isolates were tested again 22 antimicrobials in different categories. By test results as being considered MRSA, isolates were assigned a MDR definition based on standard classifications of MRSA [52]. We identified three different patterns of susceptibilities that were grouped into three different groups as followed: Group1 antibiotics included (Tetracycline, Teicoplanin, Daptomycin, Linezolid, Mupirocin, Nitrofurantoin, Vancomycin, Moxifloxacin and Clindamycin) with a sensitivity rate of more than 97% except for Clindamycin 92%. Group2 antibiotics included (Trimethoprim / sulfamethoxazole, Ciprofloxacin, Erythromycin, and Cefotetan/Cefalexin) with a sensitivity of more than 70%. Group3 antibiotic included (Fusidic acid, Imipenem, Amoxicillin clavulanic acid, Cefotaxime, Oxacillin, Cefoxitin, Ampicillin and Pencillin G) with a resistance more than 50% even reaching up to 90%. Susceptibility to Group 1 is much higher prompting to CA-MRSA phenotypes. As a widely accepted property, these lineages are normally susceptible to non beta lactam antibiotics. Future studies on genotyping and detection of gene contemts and cassette types would reveal more information.

5. Conclusions

Thus, in this study we provide significant insight into the rates of nosocomial *S. aureus* distributions relative to specimen, site, age, units of hospital, disease patterns and associated lineage types. The highest prevalence of MRSA in senior patients at ICU is worrisome. However, the intriguingly similar MRSA patterns in both ICU and non-ICU patients to that of MSSA rates in ICU, strongly imply potential age-specific selection processes amongst young patients < 20 years. More importantly, the highly significant association to infection site and all age groups, particularly, dominance of skin infections in all patients and the significant association of MRSA to respiratory infections have a high potential for skin-carriage as a reservoir. These findings have important clinical implications for strategic planning in patient management and *S. aureus* control in sources and transmission dynamics ; particularly in geriatric and pediatric settings. Future vertical studies will reveal more insights into lineage/types gene content and evolutionary history. The study is limited by being a single-centered; large scale multi-center approach is likely to gain more insights into the *S. aureus* infection profiles in the region.

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Data Availability Statement: There is no additional data deposited in any other site other than those in this manuscript.

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