

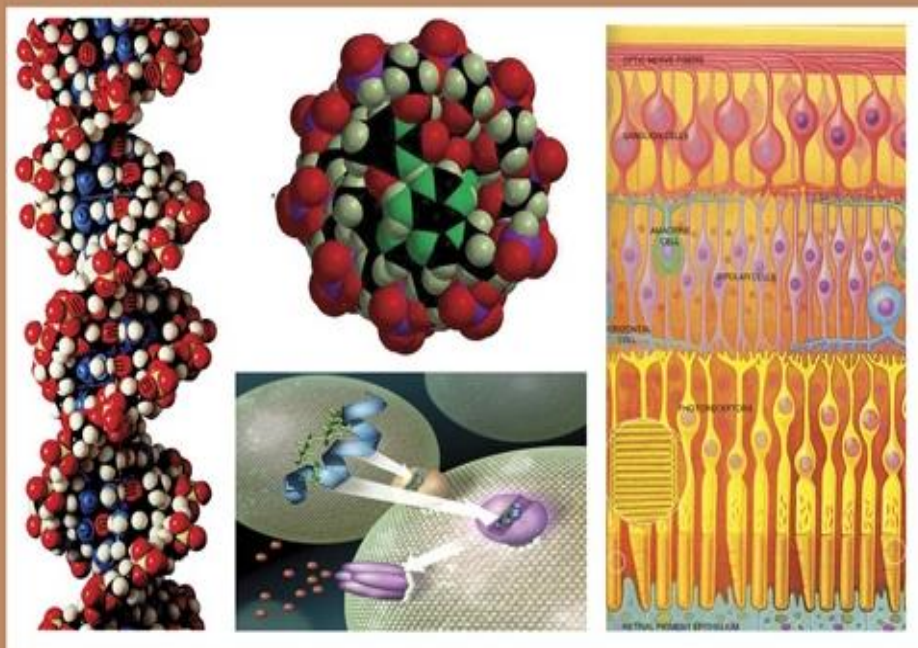


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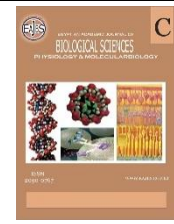
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Prevalence of *Staphylococcus aureus* and Community-Associated Methicillin-Resistant Strains On Doorknobs in Albaha Region, Saudi Arabia

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ABSTRACT

Staphylococcus aureus can cause food poisoning, folliculitis, furuncles, carbuncles, abscesses, cellulitis, fasciitis, endocarditis, endovascular infections, pneumonia, septic arthritis, osteomyelitis, and sepsis. Methicillin-resistant *S. aureus* (MRSA) isolates were originally limited to hospitals, healthcare settings and laboratories. However, the frequency of community-acquired MRSA infections has alarmingly increased in the last two decades. The present study determined the prevalence of methicillin-sensitive *S. aureus* (MSSA) and community-acquired methicillin-resistant *S. aureus* (CA-MRSA) on doorknobs/handles at the campus of Albaha University in Saudi. Samples from the doorknobs and handles collected between May 2021 and January 2022 were grown on mannitol salt agar and identified using conventional and molecular methods. A total of 35 (17.6%) and 12 samples (6%) were found positive for MSSA and MRSA, respectively. Current findings suggested that university staff and students were potential colonizers of MSSA and CA-MRSA and that fomites potentially transmitted infections to staff and students. Current findings strongly suggest the implementation of effective prevention measures as an essential step to decrease the risk of acquiring CA-MRSA infections in a public setting.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) infection is considered a global threat to public health. Fomites (non-living objects) such as doorknobs and handles are high-risk common contact surfaces, which facilitate transmission of various pathogens including MRSA within localized areas (Xiao *et al.*, 2019). The recent COVID-19 pandemic has reinforced hand hygiene practice which is considered the most essential and effective measure of preventing pathogens transmission among healthy individuals (Demaria *et al.*, 2022; Prajapati *et al.*, 2022).

Staphylococcus aureus (SA), a gram-positive coccus (GPC) colonizing 20-30% of the human population along with other mammals, is a frequent cause of infections in both the hospital and community worldwide (Al-Humaidan *et al.*, 2015) SA often colonizes the mucosa, skin, the anterior nares of the nose along with multiple body sites in humans (Li *et al.*, 2021). However, resistance to antimicrobial agents is rapidly transforming an earlier harmless infection into a serious threat (Li *et al.*, 2021; Marzec and Bessesen 2016).

SA can cause skin and soft tissue infections ranging from mild to life-threatening sepsis, pneumonia, and toxic shock syndrome (Sada *et al.*, 2017). Increasing MRSA infections having a mortality rate of 20-30% pose grave challenges to public health sectors (Zacharioudakis *et al.*, 2014) People with comorbidities such as diabetes mellitus type 2, chronic renal disease, stomach and small intestine ulcers, or dementia are reported to suffer high morbidity and mortality after MRSA infection (Hassoun *et al.*, 2017; Wertheim *et al.*, 2005).

Recent studies showed that SA and coagulase-negative staphylococci and fungi are the most frequent organisms associated with metastatic or complicated infections such as infective endocarditis (Albloshi and Alqumber 2021; Hassoun *et al.*, 2017). Incidence of MRSA infections, especially bacteremia is variable globally indicating an influence of geographical location over the prevalence of MRSA. It is reported at 60 to 70% in certain areas of the USA and Shanghai, China, respectively. However large geographic variations are seen in Europe with the percentages varying from 2% in the Netherlands to 54% in Portugal (Albloshi and Alqumber 2021; Yousef *et al.*, 2013). The prevalence of MRSA is also increasing in Saudi Arabia with an average rate of 38% (Yousef *et al.*, 2013).

Despite the long-term use of broad-spectrum antimicrobials, the frequency rate of infections is often difficult to manage (Yousef *et al.*, 2013). Treatment options available currently for MRSA include daptomycin and vancomycin. Nevertheless, these antimicrobial agents have limitations including low penetrance, low antimicrobial activity and even therapeutic failure (Hassoun *et al.*, 2017).

Few studies have investigated the contamination of nonporous objects such as doorknobs/handles and other points of contact in a hospital setting. An early report

showed a high multi-drug-resistant SA presence in a hospital setting (Saba *et al.*, 2017). This current study examined the prevalence of MRSA on frequently touched surfaces inside the Albaha university campus to determine whether MSSA/MRSA from doorknobs could be a risk factor in the area and to emphasize the necessity for effective and routine cleaning of door handles.

MATERIALS AND METHODS

Biological Samples:

Between May 2021 and January 2022, stainless-steel doorknobs at 198 sites located at university facilities were examined for SA contamination. Amies transport medium cotton swab moistened with sterile phosphate buffer saline (Sigma-Aldrich, UK) was used to swab the knobs/handles of the door located at locations including lecture theaters, teaching laboratories, and staff offices. The samples were all kept at 4°C and immediately transported to the microbiology laboratory and cultured for the isolation of aerobic mesophilic bacteria using the serial dilution technique to determine colony-forming unit. Moreover, the samples were streaked onto mannitol salt phenol red agar (Sigma-Aldrich, UK) before incubation at 37 °C for 48 hours. For confirmation purposes, presumptive SA-positive colonies were streaked again on Baird Parker agar (Sigma-Aldrich, UK), and incubated at 37 ± 0.5 °C for 24 hours and by conventional (catalase and coagulase tests) and molecular methods as perviously describe (Albarrag *et al.*, 2020). Characterization of microbial antibiotics susceptibly pattern was assessed using Kirby-Bauer's disc diffusion method. The antibiotic discs used were amoxicillin (25µg), ciprofloxacin (30µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamycin (10 µg), streptomycin (30µg), tetracycline (30 µg), trimethoprim-sulfamethoxazole (20 µg) and penicillin (10 µg). The MRSA was isolated using mannitol salt agar medium

containing oxacillin. MRSA count per doorknob was estimated from the ratio of methicillin-resistant to methicillin-sensitive colonies with the E-test for vancomycin (bioMérieux industrial microbiology, USA) performed to determine the susceptibility of isolates of SA for vancomycin. Moreover, for molecular confirmation of MSSA and MRSA and detection of the *mecA* gene the diagnostic Xpert MRSA/SA SSTI test was used per manufacturer's instructions in the GeneXpert® Dx System (Cepheid, Sunnyvale, CA, USA).

Statistical Analysis:

For data analysis, the findings were statistically analyzed using GraphPad Prism software 5.0 (Dotmatics, USA). Results are

expressed as means \pm standard error of the mean (SEM) unless otherwise stated.

Data were analyzed using a non-parametric Mann-Whitney test. Additionally, results were considered significant at a *p*-value of < 0.05 , Chi-square test and Fisher's exact test were used to compare the difference between MSSA and MRSA groups with respect to different antibiotics sensitivity (nominal variables).

RESULTS

Out of 198 samples, 101 tested positive in our cross-sectional study analyzing the prevalence of SA contamination on door surfaces at Albaha university premises. Toilets harbored the highest infection rate (35%) compared to other sites examined (Fig. 1).

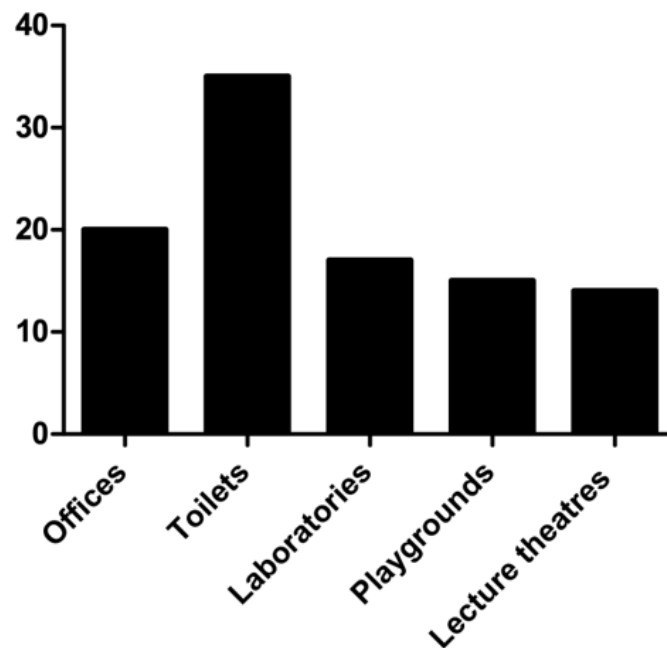


Fig. 1: Demographic distribution and percentage of contamination at each site

The doorknobs in 35 (17.6 %) of 198 rooms were contaminated by MSSA and 12 (6%) were contaminated with MRSA. A mixture of MSSA/MRSA contamination was detected at 6 doorknobs (3%). A variable contamination count was observed across the sites. Briefly, SA contamination samples drawn from 40 rooms showed an increased

count of contamination by 2.9×10^4 and 2.3×10^4 cfu MSSA/doorknob, those in seven rooms by 3.5×10^5 SA/doorknob and 6.0×10^3 cfu MRSA/doorknob, and those in three rooms by 700 cfu SA/doorknob, 200 cfu SA/doorknob, and 300 cfu MRSA/doorknobs, (Table 1).

Table 1: Doorknob* contamination by staphylococcus auras /methicillin-resistant Staphylococcus aureus (MSSA/MRSA) at Albaha university premises.

Contaminants	No. of doorknob contaminated/ No. of doorknob examined (%)	No. of room doorknobs contaminated density (cfu/doorknob)				
		1-10	10-100	100-1000	1000-10000	100000-1000000
MSSA	35/198 (36.3 %)	18	9	3	2	3
MRSA	12/198 (10%)	1	4	5	2	0
MSSA & MRSA	6/198 (3%)	0	2	1	2	1
MSSA &/or MRSA	40/198 (20.2%)	20	15	5	0	0

* Swabbed from the outside of the room only

All recovered SA isolates (101) were also tested against 10 antibiotics using the traditional disk diffusion test and E-test for vancomycin susceptibility. Our results showed that all the recovered SA isolates were susceptible (100%) to trimethoprim-sulfamethoxazole, chloramphenicol, ciprofloxacin, amoxicillin, and vancomycin. Additionally, the results indicated that out of 35 SA, the rate for MSSA was 17.6 % (35/198), which exhibited partial sensitivity to amoxicillin (94.2%), and high sensitivity to other non- β -lactam antibiotics such as gentamycin,

chloramphenicol, ciprofloxacin and trimethoprim-sulfamethoxazole (ranged between 100-75.5%). Furthermore, 74.2 and 9% MRSA strains showed antimicrobial resistance to erythromycin and tetracycline, respectively (Table 2).

The Xpert MRSA/SA SSTI (Cepheid, USA) tests confirmed that all the strains tested had typical genotypes that are matching to the identified corresponding phenotype.

Table 2: Antibiotic Susceptibility pattern of methicillin-sensitive Staphylococcus aureus (MSSA) and methicillin-resistant S. aureus (MRSA).

Antibiotics	MSSA (n=35)			MRSA (n=58) *		
	Sensitive	Intermediate	Resistance	Sensitive	Intermediate	Resistance
Amoxicillin	33 (94.2)	2 (5.7)	0 (0.0)	0 (0.0)	1 (0.1)	57 (99.9)
Ciprofloxacin	32 (91.4)	3 (8.5)	0 (0.0)	51 (87.9)	7 (12.4)	0 (0.0)
Chloramphenicol	35 (100)	0 (0.0)	0 (0.0)	52 (89.7)	6 (10)	0 (0.0)
Ciprofloxacin	29 (82.2)	6 (17.1)	0 (0.0)	50 (86.8)	6 (10.3)	2(3.5)
Erythromycin	27 (77.1)	8 (22.8)	0 (0.0)	3 (5.5)	12 (20.2)	49 (84.4)
Gentamycin	25 (71.4)	10 (28.5)	0 (0.0)	2 (5)	5 (8)	50 (75.7)
Streptomycin	20 (57.1)	15 (42.8)	0 (0.0)	2 (3)	6 (15.15)	52 (78.7)
Tetracycline	27 (77.1)	3 (8.5)	5 (14.2)	50 (75.5)	8 (13.15)	0 (0.0)
Penicillin	0 (0.0)	0 (0.0)	35 (100.0)	0 (0.0)	0 (0.0)	58 (100.0)
Trimethoprim-sulfamethoxazole	32(91.4)	3 (8.5)	0 (0.0)	50 (86.2)	8 (13.7)	0 (0.0)

DISCUSSION

Bacterial colonization of instruments/objects in hospital settings is considered a major risk factor for increased incidences of nosocomial infections (Bhatta *et al.*, 2022). The transfer of pathogenic bacteria via public fomites is well documented as a source of

infection outbreaks (Furnkranz and Walochnik 2021; Suleyman *et al.*, 2018). MRSA survival for long periods in hospital environments is likely due to infection transmission from fomites (Makison and Swan 2006) and surfaces like doorknobs/ handles. CA-MRSA is MRSA strain isolated from individuals not exposed to

healthcare settings having patients with MRSA infections (Wong *et al.*, 2018). Recent studies showed MRSA isolation from public fomites in contact with patients, along with staff's personal items and subsequent CA-MRSA transmission (Jaradat *et al.*, 2020).

Our study revealed that of all the doorknobs contaminated with MSSA/MRSA pathogens, the highest number was observed in toilet doorknobs with an infection rate of 35% followed by offices (20%), playgrounds (15%), and lecture theaters (14%). Our results are in agreement with a recent report showing the highest rate of MRSA contamination in restroom sinks and doorknobs (Jaradat *et al.*, 2020). Infections associated with MRSA are often difficult to treat due to limited therapeutic antibiotics and the presence of resistance to them. Our results showed that MSSA exhibited a low multidrug-resistant bacteria (MDR) pattern while MRSA isolates showed 100% resistance to Amoxicillin and Penicillin. Our findings are consistent with those of Nataraj (Nataraj and Mallappa 2021) report showing MRSA resistance against Amoxicillin (99.9 %) and Penicillin (100%), with at least 80% of MRSA strains (Cong *et al.*, 2020) found resistant to Penicillin and Ampicillin. MRSA isolates resistant to vancomycin likely emerged due to the misuse or unnecessary use of antibiotics. The findings suggest strict control over antibiotic use to reduce the likelihood of the emergence and spread of MRSA vancomycin resistance. In addition, Acquired VRSA is most prevalent among Enterococcus and is still rare in other pathogenic bacteria (Khan *et al.*, 2018). Gentamicin can be used in cases of staphylococcal infections (Wu *et al.*, 2021). Our genotyping results are in agreement with early studies (Albarrag *et al.*, 2020). All the

MRSA isolates were *mecA* positive with *SCCmec* III as the most common *SCCmec* type followed by *SCCmec* V/IVa. The remaining isolates were non-typeable. The emergence of *SCCmec* Type IV is the most common type associated with MRSA as high as 50% (Baig *et al.*, 2018; Kondo *et al.*, 2022). Effective approaches such as surveillance assessments of MRSA classification are necessary to control MRSA spread which could be accomplished via the detection of *mecA* gene carriers and most of

the *SCCmec* types associated with SA. The PCR technique is considered a specific and sensitive method to identify MRSA carrier status. However, it is expensive and not easily adapted in most laboratories.

Conclusion:

MRSA infections cause a large economic burden due to illness and productivity loss. Our findings suggest that hand hygiene can significantly reduce the infection rate and decrease respiratory and gastrointestinal infections. Further, Fomites disinfection can decrease surface contamination and interfere with the spread of disease in and around Albaha university. Future experiments are needed to assess the role of faucets, towel dispensers, doorknobs, and handles in the microbial exchange between fomites and humans and their contributions to SA infections and adverse health outcomes. The public needs to observe hand hygiene and use quality disinfection procedures in order to prevent CA-MRSA contamination and cross-contamination (spread).

Conflict of Interest: The authors declare no conflict of interest.

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