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Trend determination of methicillin-resistant *Staphylococcus aureus* infections with statistical modeling

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ABSTRACT

OBJECTIVE: The purpose of this study is to analyze the situation of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in TRNC by examining the prevalence of Community-acquired Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA)/Hospital-acquired Methicillin-Resistant *Staphylococcus aureus* (HA-MRSA) cases, the acceleration of CA-MRSA/HA-MRSA patients from past to present, the impact of pandemic, gender and age on MRSA cases.

METHODS: In order to analyze the trend of MRSA cases and the effects of selected parameters on MRSA cases, statistical tests are employed to the obtained data including ANOVA test, regression analysis tests and Post-hoc Tukey test.

RESULTS: Incidence rate of MRSA carriage in the community has increased over the years to 45.6%. p-value of the relationship between community-acquired MRSA cases and hospital-acquired MRSA cases is less than 0.05. The results also revealed that the p-value of both the relationship between COVID-19 and MRSA cases and the relationship between 50+ age and MRSA cases are significant. On the other hand, p-value of the relationship between gender and MRSA cases is found to be greater than 0.05.

CONCLUSION: It is concluded that MRSA carriage in the community has increased over the years and CA-MRSA and HA-MRSA cases are related since $p<0.05$. Moreover, it's revealed that there is an effect of COVID-19 pandemic and 50+ age (66% of the cases) on MRSA cases because of $p<0.05$ while the gender is not an effect for MRSA cases in the country, since p-value for gender comparison is greater than 0.05.

Keywords: Bacteria; hospital infection; methicillin-resistant staphylococcus aureus; statistical modeling; trend.

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Acquired antimicrobial resistance emerges as a result of unconscious and incorrect use of antimicrobial agents. This situation occurs due to the mutation or uptake of resistance genes which causes treatment failures and the spread of resistant microorganisms in the community [1]. Nowadays, increase in resistance to antibiotics that are used in the treatment of bacterial infections is an alarming trend observed worldwide [2].

Staphylococci are bacteria that can be found as a normal flora element on the skin and mucosal surfaces of hu-

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mans. Approximately 15% of healthy adults carry *Staphylococcus aureus* (*S. aureus*) continuously. Unfortunately, this rate is even higher in hospitalized patients, healthcare personnel, eczema patients and drug-addicted individuals. Approximately 60% of the individuals in society carry *S. aureus* bacteria in their noses at certain periods of their lives [3, 4]. Due to these and more, *S. aureus* is considered as one of the most important bacteria causing both community- and hospital-acquired infections. According to recent health reports, there is an increase in methicillin-resistant *Staphylococcus aureus* (MRSA) infections, especially in intensive care units [5, 6].

Various infections such as bacteremia, pneumonia, meningitis, endocarditis, skin and soft tissue, surgical site, urinary tract, bone and joint infections and toxic shock syndrome caused by MRSAs are important public health problems in many countries around the world. It has been reported that in hospital-acquired MRSA infections, recent hospitalization, dialysis, surgical intervention, long hospital stays, and a history of comorbidity predispose to the emergence of infections. Community-acquired MRSA outbreaks can generally occur in care centers, contact sports athletes, injecting drug users, military personnel, and individuals in prisons. In addition, infections caused by MRSAs are strongly associated with morbidity, mortality, and healthcare costs [1, 7, 8].

Various studies that have been conducted to examine the antimicrobial resistance model of *S. aureus* have shown that this bacterium can be resistant to β-lactam, aminoglycoside and macrolide antibiotics. The increased resistance to β-lactam antibiotics in *S. aureus* is due to the production of the enzyme β-lactamase, which hydrolyzes the β-lactam ring and destroys the antibiotic. This has rapidly reduced the efficacy and use of penicillin for severe staphylococcal infections, especially among hospitalized patients [9–11]. Because of these resistance properties developed by the bacteria, difficulties are experienced in many staphylococcal infections and glycopeptide antibiotics such as vancomycin are used as a treatment option [2].

In recent years, significant changes have started to be observed in the epidemiology of MRSA infections. The first of these is the increase in methicillin resistance rates observed in *S. aureus* isolates. Another change is the decrease in vancomycin susceptibility in MRSA isolates that are reported from some centers. As a result, *S. aureus* moderately susceptible to vancomycin (VISA), heterogeneous VISA (hVISA), and vancomycin-resistant *S. aureus* (VRSA) emerged among MRSA isolates. The third important change is the emergence of MRSA strains not only in hospitals but also in the community [12, 13].

Highlight key points

- Both the effect of HA-MRSA patients on CA-MRSA patients and the effect of CA-MRSA patients on HA-MRSA is approximately 60-70%. This emphasizes the prevalence of MRSA in the community as well as in hospitals and the infection is not only associated with the HA-MRSA patients.
- The percentage of positive cases was 38% before pandemic while during pandemic this percentage is changed as 56%. Hence, an increase is seen in the cases during pandemic.
- There is no significance between MRSA cases and gender in the country.
- There exists an important significance between the age group 50+ and the other age groups. Thus, it can be concluded that 50+ age group can be a potential MRSA patient in our country.

The preparation of public health programs to investigate and combat infectious diseases is a very difficult and complex issue. A better understanding of important health issues and their ability to combine our knowledge of a topic with data are indicative of the important ability of statistical models. In addition, statistical modeling and epidemiological prediction of infections are good methods to understand why and how pathogens spread and how this spread/occurrence can be prevented [14, 15]. In this type of modeling, many distributions and models can be applied to the obtained data. Distributions may differ according to the aim and type of data/data groups.

The purpose of this study is to analyze the situation of MRSA in our country by examining the prevalence of CA-MRSA/HA-MRSA cases, the acceleration of CA-MRSA/HA-MRSA patients from past to present, the impact of pandemic on MRSA cases, the impact of gender on MRSA cases and the age group of MRSA cases. Hence, by applying the results, necessary measures can be taken to prevent the spread of resistant bacteria both in the community and in health institutions.

MATERIALS AND METHODS

In this paper, the data is obtained from the Near East University Hospital. The ethical approval for the study was obtained from the Near East University Scientific Research Ethics Committee with the project number

Table 1. The effect of HA-MRSA patients on CA-MRSA

Dependent variable: CA-MRSA				
Equation	Model summary			
	R	R^2	F	p
Linear	0.566	0.32	2.359	0.185
Logarithmic	0.688	0.473	4.496	0.087
Inverse	0.758**	0.575	5.767	0.059
Quadratic	0.855**	0.731	5.442	0.072
Cubic	0.860**	0.739	2.832	0.208
Power	0.784**	0.615	7.996	0.037*
S	0.858**	0.737	14.001	0.013*
Growth	0.657	0.432	3.806	0.109
Exponential	0.657	0.432	3.806	0.109
Independent variable: HA-MRSA				

CA-MRSA: Community-acquired Methicillin-Resistant *Staphylococcus aureus*; HA-MRSA: Hospital-acquired Methicillin-Resistant *Staphylococcus aureus*; *: P<0.05; **: R>0.7.

Table 2. The effect of CA-MRSA patients on HA-MRSA

Dependent variable: HA-MRSA				
Equation	Model summary			
	R	R^2	F	p
Linear	0.566	0.32	2.359	0.185
Logarithmic	0.657	0.432	3.806	0.109
Inverse	0.718**	0.516	5.330	0.069
Quadratic	0.737**	0.543	2.381	0.208
Cubic	0.737**	0.543	2.381	0.208
Power	0.784**	0.615	7.996	0.037*
S	0.884**	0.713	12.418	0.017*
Growth	0.688	0.473	4.496	0.087
Exponential	0.688	0.473	4.496	0.087
Independent Variable: CA-MRSA				

CA-MRSA: Community-acquired Methicillin-Resistant *Staphylococcus aureus*; HA-MRSA: Hospital-acquired Methicillin-Resistant *Staphylococcus aureus*; *: P<0.05; **: R>0.7.

YDU/2022/108-1653 on 30.11.2022. This study is conducted according to the Declaration of Helsinki and it captures the data of 48,835 patients retrospectively. In the samples of 13,350 of these patients, microorganisms are detected. *S. aureus* is detected in 612 (out of 13,350) patients and 279 of these patients are diagnosed as MRSA positive. For the analysis, data is separated into groups according to their types (CA-MRSA/HA-MRSA), years (before pandemic/after pandemic) and ages. Suitable statistical models and tests are applied to the data groups to examine whether any significance exists or not between these groups by using SPSS Statistics 24 (IBM Corp.) package program.

Statistical Analysis

In this section, statistical analysis is applied to the collected data. In this manner, the following are examined: the effect of HA-MRSA on CA-MRSA and the effect of CA-MRSA on HA-MRSA, the effect of pandemic on MRSA patients, and the effect of age on MRSA patients. The aims are determining the relationship between HA-MRSA and CA-MRSA and the significance of this relationship, specifying whether the pandemic has an effect on MRSA cases or not and deciding which age group has the highest probability to be diagnosed as MRSA positive. The results are significant if $p<0.05$ holds. Moreover, several regression model estimations and tests are used to determine which model fits better to the obtained data. Regression model is applied to see what proportion the significance of the relationship is between CA-MRSA and HA-MRSA. For the regression analysis $R>0.7$ is assumed as correlated between the corresponding groups [16]. The results are given in Table 1 and Table 2.

For determining the effect of pandemic on MRSA cases, the obtained data is divided into two groups: cases before pandemic and cases after pandemic. This effect is examined by one-way ANOVA test with dependent variables as MRSA cases. The purpose of this part is to see whether the pandemic affected MRSA infection or not. The results are given in Table 3.

Descriptive statistics and ANOVA tests are applied to the data that is divided into two groups according to the gender of MRSA-positive cases. The aim of this part is to decide whether gender has an effect on MRSA positivity or not in our country. Results of tests applied to gender groups are proposed in Table 4.

The importance of age for MRSA infection is examined with one-way ANOVA test at first. However, one-way ANOVA test only determines whether there is a significance between these groups or not; it does not indicate between which of these groups there exists

Table 3. Descriptive statistics, homogeneity of variances and One-way ANOVA test result of the cases pre-pandemic and during pandemic period

	Descriptives		
	95% CI for mean		
	LB	UB	
Cases pre-pandemic	12.5810	53.4190	
Cases during pandemic	44.1044	56.3956	
	Test of homogeneity of variances		
	Levene statistic		p
	2.541		0.162
	ANOVA		
	Sum of squares	F	p
Between groups	595.125	6.628	0.042*

CI: Confidence interval; LB: Lower bound; UB: Upper bound; ANOVA: Analysis of Variance; *: P<0.05.

Table 4. Descriptive statistics, homogeneity of variances and one-way ANOVA test result of women and men cases

	Descriptives		
	95% CI for mean		
	LB	UB	
Women	12.0020	24.8551	
Men	15.9005	26.6709	
	Test of homogeneity of variances		
	Levene statistic		p
	0.761		0.400
	ANOVA		
	Sum of squares	F	p
Between groups	28.571	0.695	0.421

CI: Confidence interval; LB: Lower bound; UB: Upper bound; ANOVA: Analysis of Variance.

a significance. Therefore, in order to determine which groups are more significant, post-hoc Tukey test is applied to the groups [17]. Age groups are divided into four: ages between 0–17 (both included), ages between 18–35 (both included), ages between 36–49 (both included) and ages greater than or equal to 50. In Table 5 and Table 6, results are proposed.

Table 5. Descriptive statistics and homogeneity of variances of age groups

	Descriptives	
	95% CI for mean	
Age	LB	UB
0–17	-1.3837	21.3837
18–35	-3.7844	65.1178
36–49	1.7856	58.2144
50+	-7.0627	137.7294
	Test of homogeneity of variances	
	Levene statistic	p
	3.728	0.061
	ANOVA	
	Sum of squares	F p
Between groups	23393.667	9.627 0.005*

CI: Confidence interval; LB: Lower bound; UB: Upper bound; ANOVA: Analysis of Variance; *: P<0.05.

Table 6. Post-hoc Tukey test results of the cases of age groups

	Multiple comparisons	
Group	Comparison group	p
0–17	18–35	0.831
	36–49	0.949
	50+	0.006*
18–35	0–17	0.831
	36–49	0.988
	50+	0.018*
36–49	0–17	0.949
	18–35	0.988
	50+	0.012*
50+	0–17	0.006*
	18–35	0.018*
	36–49	0.012*

*: P<0.05.

RESULTS

In the study, 279 MRSA-positive patients' data are used. In this study, culture results of 48,835 patients were retrospectively analyzed and microorganism growth was detected in 13,350 (27.3%) of these. *Staphylococcus aureus* was detected in 612 (4.6%) of the samples with growth and 279 (2.1%) patients were diagnosed as MRSA positive. The incidence of MRSA was 45.6% among *S. aureus*-growing culture samples. CA-MRSA patients have the percentage 31% while HA-MRSA patients have the percentage 69% in the country. 47% of MRSA-positive cases were women while 53% of them were men. The percentage of age groups were as follows: 5% between 0–17, 16% between 18–35, 13% between 36–49 and 66% for age 50 and above.

In Table 1, the dependent variable is taken as CA-MRSA while the independent is HA-MRSA. So, the effect of HA-MRSA patients on CA-MRSA patients is analyzed. It is obvious that the R (correlation coefficient) values of the inverse, quadratic, cubic, power and S equations are higher than 0.7 which indicates that the correlation between variables is high. The R^2 (coefficient of determination) values of the corresponding R values can be checked to decide the percentage of this effect. Significance of F is given in the column Sig. These values are interpreted as follows: the corresponding equations are significant if p-value is less than 0.05, otherwise they are not significant. When both R and p values are taken into consideration, best-fitted models for the effect of HA-MRSA patients on CA-MRSA are power and S models.

In Table 2, the dependent variable is taken as HA-MRSA while the independent is CA-MRSA. So, the effect of CA-MRSA patients on HA-MRSA patients is analyzed. It is obvious that the R (correlation coefficient) values of the inverse, quadratic, cubic, power and S equations are higher than 0.7, which indicates that the correlation between variables is high. The R^2 (coefficient of determination) values of the corresponding R values can be checked to decide the percentage of this effect. p-values are interpreted as corresponding equations being significant if <0.05 holds, otherwise they are not significant. When both R and p-values are taken into consideration, best-fitted models for the effect of CA-MRSA patients on HA-MRSA are power and S models.

From Table 1 and Table 2, it is concluded that both CA-MRSA and HA-MRSA are associated with each other. Both the effect of HA-MRSA patients on CA-MRSA patients and the effect of CA-MRSA patients on HA-MRSA is approximately 60–70%. This emphasizes the prevalence of MRSA in the community as well as in hospitals and the infection is not only associated with the HA-MRSA patients.

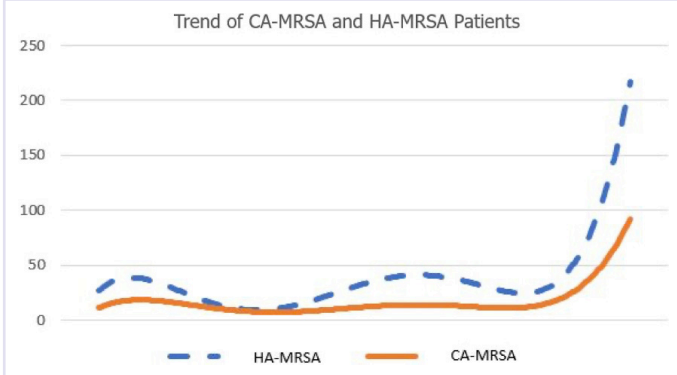

Figure 1. The pattern of HA-MRSA patients and CA-MRSA patients.

According to the tables, power model is one of the effective models for MRSA infection. In this regard power model is applied to the obtained data to determine the pattern of HA-MRSA patients and CA-MRSA patients in future. The simulation of results is given in Figure 1. As maintained in the figure, even though the picks of patients differ between HA-MRSA and CA-MRSA, an increase is expected in both of the cases. Moreover, the trend of these increases is very similar to each other. This similarity indicates that the infection is not only in hospitals; the community is also spreading the disease. The figure showed that there is an emergence about the disease in the community.

In Table 3, descriptive statistics with 95% confidence interval of cases pre-pandemic and during pandemic period is given. Also, the homogeneity of variances test's result is proposed for the cases to check whether the variance of both group is same or not in Table 3. From this table, it is concluded that the data is accurate since they are normally distributed and significance of homogeneity of variances is greater than 0.05. This accuracy allows us to apply further tests to the obtained data.

For testing the significance between groups, one-way ANOVA test is applied. Here, the dependent variable of the test is MRSA cases and groups are MRSA cases pre-pandemic and MRSA cases during pandemic period. In Table 3, the result is given. As can be seen from this table, significance is less than 0.05. So, there exists a significance between these groups.

Furthermore, the percentage of positive cases was 38% before pandemic while during pandemic this percentage changed as 56%. Hence, an increase is seen in the cases during pandemic.

In Table 4, descriptive statistics and the homogeneity of variances test result of men and women cases are given. The results proposed in Table 4 guaranteed that the data is accurate since they are normally distributed and significance of homogeneity of variances is greater than 0.05. With this accuracy, further tests can be applied to the obtained data.

For testing the significance between groups, one-way ANOVA test is applied. Here, the dependent variable of the test is MRSA cases and groups are positive women MRSA cases and positive men MRSA cases. The result of ANOVA test is proposed in Table 4. Since the significance is greater than 0.05, there is no significance between these groups.

Table 5 is the result of descriptive statistics of age groups' cases and the homogeneity of variances of these groups. It is obvious from Table 5 that the data has a normal distribution and hence it is accurate. In Table 5, the one-way ANOVA test for MRSA cases of mentioned age groups is also given. Before post-hoc tests, it is meaningful to apply ANOVA test to decide the existence of significance between groups. Since p-value is less than 0.05 this significance exists.

As mentioned before, when a significant analysis is made between more than two groups, ANOVA test only tells us if the significance exists or not; it does not point out which groups are more significant. Hence, for the age groups, post-hoc Tukey is applied. Since the data is accurate and ANOVA test result is significant, Tukey test can be applied. The results of this test are proposed in Table 6. From this table, there exists an important significance between the age group 50+ and the other groups. Thus, it can be concluded that 50+ age group can be a potential MRSA patient in our country.

DISCUSSION

MRSA infections maintain to pose a major challenge to healthcare systems worldwide, including in many European countries. In literature, there exist studies which are showing that usage of antibiotics is an important risk factor in Mediterranean region as well as other regions [18, 19]. The emergence and spread of CA-MRSA clones in the community have also initiated a universal public health problem. Approximately 50% of healthy individuals carry *S. aureus* asymptomatically as permanent or intermittent. In addition to the increase in *S. aureus* reservoirs detected in recent years, the increase in multi-drug resistant-CA-MRSA strains has caused health problems to become more complex and difficult [20].

In the study of Ahmadi et al. [20], the rate of *S. aureus* carriage was found enormously high. In this study, 600 randomly selected people were examined. As a result, carriage of *S. aureus* was found at a rate of 30.17% (181/600) while the rate of MRSA was determined as 9.17% (55/600) [20]. Lack and/or poor awareness of personal hygiene (including facial, hand, and nostril cleaning habits) is accepted as a possible cause of *S. aureus* carriage [21]. The percentages of MRSA that are isolated from clinical specimens vary between countries. The prevalence of MRSA is reported as 50% in Portugal and Italy, 25% in France, Greece, and England, and 2% in Netherlands and Switzerland [20]. In the study of Dilnessa and Bitew [22], 14.3% (n=194) *S. aureus* was detected in 1360 clinical samples and 17.5% (n=34) of them were found to be MRSA. The incidence of MRSA in the examined total clinical sample was 2.5% [22]. 210 bacterial strains isolated from various clinical specimens were examined in the study of Moglad and Altayb [23] and gram-positive (+) bacteria (GPB) was detected at a rate of 30% (n=63). It was observed that 82.5% (n=52) of the isolated GPBs were *S. aureus* and 60% (n=38) were MRSA [23]. In another study, 666 clinical specimens were analyzed and 133 (19.96%) of them were confirmed to be *S. aureus*. In the same study, the incidence of MRSA among *S. aureus* was determined as 70.64% (n=94) [24]. In the light of the data that are obtained as a result of the presented study, the prevalence of MRSA in the country is 2.1% (45.6% among *S. aureus*).

Generally, older people are more susceptible to infections than younger adults. This is because aging is associated with immune dysfunction, particularly with cellular immunity. Beyond this, because the elderly commonly have a history of hospitalization, they are unfortunately exposed to multi-drug-resistant microorganisms that exist in hospital environment or contribute to the circulation of these microorganisms [25]. It has been suggested that age is associated with nasal *S. aureus* carriage. Accordingly, there are studies showing that the rate is higher in the elderly [20]. MRSA, which mostly causes nosocomial infections, is also a causative agent in infections acquired in the community today and can cause severe clinical pictures in patients, infants and the elderly with weakened immune systems. Nasal MRSA colonization is seen especially in places where hygienic conditions are not adequately provided, such as hospitals, nursing homes and dormitories [26]. In the presented study, the incidence of MRSA infections in elderly patients was found to be significantly higher than in the others.

The increase in the frequency of CA-MRSA infections has further increased the significance of nasal *S. aureus* carriage. It is believed that the increase in the rate of nasal

carriage also has an effect on the increase in the frequency of CA-MRSA infections. These infections are also more common in individuals living in crowded environments such as athletes, military personnel, prisons, nursing homes, boarding houses and extended families. Moreover, having a pet, having a family history of frequent hospitalizations, presence of health professionals in the family, antibiotic usage in the last 6 months or a history of outpatient clinic admission are also considered as risk factors [27, 28]. The presented study shows that CA-MRSA carriage and CA-MRSA infections tend to increase over the years. It is obvious that these infections can be prevented by knowing the incidence of CA-MRSA in each region, determining the risk factors and applying appropriate empirical treatments [27]. In the presented study, the rate of detected CA-MRSA cases is 31% and the rate of detected HA-MRSA cases is 69%. As a result of a meta-analysis of 152 studies, it is emphasized that the rate of CA-MRSA varies between 0% and 23.5% [29]. Despite this, the rate of CA-MRSA detected in our study was found to be slightly higher than in the literature.

Some important studies strongly suggest that men are more likely to be carriers of MRSA. However, some studies have proven that women are worse affected by bloodstream infections from MRSA [30]. In the study of Smit et al. [31], 2638 patients with CA-MRSA infection were examined. In this study, it is reported that female patients with CA-MRSA infection had an increased 30-day mortality rate compared to men [31]. In the presented study, no relationship is found between gender and MRSA infection.

There exist studies in the literature showing that the COVID-19 pandemic affects some infectious diseases. In particular, a decrease has been detected in other respiratory tract infections caused by infectious agents that are similar to SARS-CoV-2 in terms of transmission route. As an example, there has been a significant decrease in pneumonia, influenza and RSV infections during the COVID-19 pandemic period. All the protection measures taken (such as social distancing, travel restrictions, improving personal hygiene awareness, etc.) with the pandemic are seen as the main reason for the decrease in respiratory tract infections [32–35]. On the contrary, Guvenir et al.'s [36] study pointed out that there is an increase in the animal population and the incidence of zoonotic infections such as Rickettsiae due to restricted human movements during COVID-19 pandemic. Lastinger et al.'s [37] study, on the other hand, emphasizes that MRSA bacteremia detected after the onset of the COVID-19 pandemic generally increased. In another study, a significant relationship was found between the COVID-19 pandemic and both ICU and non-ICU MRSA infections. Accordingly, it was reported that there was a 94.7% increase in total MRSA infections in the year after the start of the pandemic (until the end of 2020) [38]. In parallel, in the presented study, an 18% increase in MRSA infection and carriage is observed after the onset of the COVID-19 pandemic. It is thought that the ignorance of other infectious diseases due to the intense interest shown in COVID-19 infection and the unnecessary/wrong use of antibiotics due to the difficulties in correct diagnosis allow some other infectious agents to spread both in the community and in hospital settings.

As a result, it will be a critical move for human health to identify the infectious agents that are affected by the COVID-19 pandemic and are in an increasing trend and to plan the improvements by taking the necessary precautions by the infection control teams. In light of the data obtained from the presented study, MRSA carriage in the community has increased over the years, and both CA-MRSA and HA-MRSA infections have increased with the effects of the COVID-19 pandemic. Therefore, it is important to apply antibiotic usage strategies correctly and take necessary precautions to reduce mortality and morbidity rates caused by MRSA.

Study Limitations

The presented study captures only the patients that are administered to the Laboratory of the Near East University Hospital. However, the percentage of these patients is a remarkable amount for the whole country.

Conclusion

In this study, with the obtained results, it is concluded that MRSA carriage in the community has increased over the years and CA-MRSA and HA-MRSA cases are related to each other due to the value of p. This emphasizes the importance of control strategies due to the impact of CA-MRSA cases on HA-MRSA. Furthermore, it's demonstrated that there exist some factors affecting MRSA cases including COVID-19 pandemic and age. According to the study, COVID-19 pandemic has a significant effect on MRSA cases, which may lead to a public health problem in the future. Also, 50+ age group that captures the 66% of the cases is observed as a risk group for being an MRSA patient. On the other hand, it is found that gender has no significant effect on MRSA cases in the country.

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