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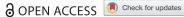
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# Promising anti-microbial effect of apple vinegar as a natural decolonizing agent in healthcare workers

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#### **ABSTRACT**

Introduction: Colonized Healthcare workers (HCWs) are an essential reservoir of nosocomial infections. This study aims to determine the prevalence of methicillin-resistant Staphylococcus aureus (MRSA) carriage rate among HCWs, to evaluate at Fayoum University Hospital the susceptibility of isolates to mupirocin and Chlorhexidine and to investigate the antimicrobial effect of different vinegars on MRSA as a natural decolonizing agent.

Methods: Nasal and hand swabs were collected from 124 HCWs at Fayoum University Surgical Hospital. Isolates were identified using the standard microbiological methods. Susceptibilities to mupirocin and Chlorhexidine were determined by disk diffusion and broth micro-dilution. Screening antimicrobial effect of commercial vinegars was determined by agar well-diffusion method and microdilution method.

Results: About one tenth 11.3% (14/124) of HCWs showed nasal carriage of MRSA. Workers were the predominant carriers (P = 0.013). The overall non-nasal carriage rate of MRSA was 6.5% (8/124). Among MRSA isolates Low-level Mupirocin resistance (LLMR) showed in (36.4%, 8/22). MICs ranged from 0.25 to 32  $\mu$ g/ml. Also, (13.6 %, 3/22) showed Chlorhexidine resistance, MICs ranged from 0.039 to 5 μg/ml. Apple vinegar showed the highest susceptibility among vinegars (p < 0.0001) with MIC values varied from 0.058 to 1.87 µg/ml

Discussion: The emergence of mupirocin (36.4%) and Chlorhexidine (13.6%) resistant Staphylococcus aureus among HCWs should be of excessive concern. Apple vinegar has a promising antimicrobial effect against MRSA isolates and could be used as a decolonizing agent.

#### **ARTICLE HISTORY**

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#### **KEYWORDS**

Apple vinegar; mupirocin; methicillin-resistant Staphylococcus aureus: healthcare workers

# 1. Introduction

Staphylococcus aureus (S. aureus) has been documented as a chief pathogen in human diseases [1]. They are becoming problems especially while dealing with the postoperative wound infection. Nasal carriage of S. aureus plays a main role in the spread of infection [2]. Many studies have identified the importance of nasal MRSA screening and subsequent decolonization to reduce surgical site infection caused by (MRSA) [3]. It is estimated that between 0.8% and 6% of people in the U.S.A are asymptomatically colonized with MRSA [4]. With the increased occurrence of MRSA, the consumption of vancomycin has also enlarged. So, Vancomycin-Resistant S. aureus (VRSA) has started to develop [5]. Of the medications available for decolonization, mupirocin, Povidone-iodine, bacitracin, chlorhexidine. Mupirocin binds to bacterial isoleucyl-tRNA synthetase (IRS) and inhibits protein synthesis [6]. Mupirocin was introduced in 1985, with mupirocin-resistant S. aureus (MupRSA) first reported in 1987. The resistance of MRSA to mupirocin is categorized into two types: Low-level or intermediate resistance (MupL or MupI) with minimum inhibitory concentration (MICs) of 8-256 µg/ml, and high-level resistance (MupH) with MICs  $\geq$ 512 µg/ml.

LLMR is associated with changes in the native IRS. A plasmid-mediated MupA gene connected to high-level resistance. Those plasmids carry resistance determinants to other antibiotics, such as macrolides, tetracycline, and trimethoprim [7]. Antiseptic bathing of patients with Chlorhexidine is often employed with nasal mupirocin. Chlorhexidine binds to the bacterial cell membrane, causing depolarization and cell death. Reduced susceptibility to Chlorhexidine in MRSA occurs by plasmid-mediated genes, such as qacA and qacB that encode multidrug efflux pumps [8]. The acceleration in these resistant circumstances needs new alternative antimicrobials. Vinegar has been produced as a commercial product for consumption and for use in healing. Apple Cider Vinegar (ACV) is produced from cider that has undergone acetous bioconversion and has relatively low acidity (5% acetic acid). It also contains organic acids, flavonoids, polyphenols, minerals, and vitamins [9]. The present study was undertaken to assess the carriage rate of MRSA among HCWs working at operation rooms (ORs), to evaluate the susceptibility of isolates to mupirocin and Chlorhexidine, and to investigate the antimicrobial activity of different types of vinegars against MRSA isolates to be used as a decolonizing agent.

#### 2. Materials and methods

# 2.1. Study design

This study had two approaches; a cross-sectional approach related to the carriage rate of MRSA among HCWs and experimental approach concerned with the investigation of the antimicrobial activity of different types of vinegars against MRSA isolates. The current study was conducted for 6 months from (March 2018 till September 2018).

All HCWs (Doctors, nurses, workers, and technicians) at (ORs), who did not have underlying diseases and were not taking antibiotics for 2 weeks prior to sampling, were included in the study with the only exclusion criteria is the refusal of the participants to include in the study.

A total of 124 HCWs from (ORs), who accepted to share in the present study, at our Surgical University Hospital (SUH) were included in the study. Fayoum University Hospital (Surgical, Internal medicine) is a teaching hospital. It has 365 staff members (176 nurse staff, and 189 physicians), and 500 beds.

#### 2.2. Bacterial isolation and identification

Nasal and non-nasal, i.e., (hand) swabs (the palms, web space between the fingers) were collected from 124 participants (two swabs for each) using a flexible sterile cotton swab, moistened with sterile saline. The nasal swab was inserted to approximately a 1 cm depth and gently rotated five times [10]. Then, the samples were transferred quickly to the laboratory to and inoculated onto nutrient, blood, and MacConkey agar plates.

(Oxoid LTD, Basingstoke, England). All the inoculated plates were incubated at 37°C for 48 hours. Isolates were identified according to standard microbiological methods [11]. Suspected colonies of S. aureus were sub-cultured on Mannitol salt agar (Oxoid LTD, Basingstoke, England). If no growth occurred, they were reincubated at 35°C for another 24 hours, with a maximum incubation period of 72 hours. S. aureus isolates were further confirmed as MRSA by studying their resistance to Cefoxitin using Cefoxitin disc (30 mg) (Oxoid, Lenexa, KS). Automated identification and antimicrobial susceptibility system Vitek-2 Compact system (Biomerieux, India) were used to identify presences of mec gene or not [12].

The sensitivity of MRSA isolates to mupirocin and bacitracin was performed by Kirby-Bauer disc diffusion method on Mueller Hinton agar (Oxoid, Lenexa, KS) using discs of antibiotics: Mupirocin (5 µg) and Bacitracin (0.04 units) (Hi-Media, India). The zone of inhibition was measured and interpreted based on CLSI guidelines. And S. aureus ATCC 25,923 was used as a control strain for mupirocin sensitivity. Zone size of ≤10 mm for bacitracin disc was considered resistant [13]. For Mupirocin susceptibility: inhibition zone

≥14 mm was considered sensitive, while zone ≤13 mm was resistant [14]. Isolates also tested for Chlorhexidine (4%) and Povidone-iodine (7.5%) susceptibility as described previously by Shino et al. [15].

# 2.2.1. Vinegar samples

The Apple cider vinegar, Grape vinegar, and Lemon vinegar samples used in this study are purchased from the local supermarket, which are natural and additive-

Three kinds of Vinegars were tested against MRSA isolates: Agar well diffusion method was performed as described by Okeke et al. [16], with slight modification. Briefly, freshly prepared inoculum (10<sup>5</sup> CFU/ml) of isolates was streaked all over the surface of Muller Hinton, wells were made in the medium with the help of sterile cork-borer having 6-mm diameter, 100 µl of each vinegar was added to each well, 50 µl of sterile broth was added to one well as a negative control. The experiment was conducted in triplicate. The plates were allowed to be incubated at 37 Co for 24 h. The diameters of the zones of inhibition were measured and reported.

# 2.2.2. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) by the micro-dilution method

As Apple cider vinegar, Lemon vinegar, Mupirocin, and Chlorhexidine showed the highest antimicrobial activities against tested strains, they were chosen for further investigation. MICs for apple cider vinegar 6% and Lemon vinegar 6% were employed as described by Baldas and Altuner [17] in concentrations ranging from 60  $\mu$ g/ml to 0.05  $\mu$ g/ml (2 fold dilution). The MICs for Mupirocin were determined with pure form drug powder purchased from Hi-Media, in concentrations ranging from 512 µg/ml to 0.03 µg/ml [18]. MICs for Chlorhexidine (4%) (Sigma-Aldrich, St. Louis, MO) were done using the standard broth dilution method described by Horner et al. [19], in concentrations ranging from 40 to 0.039 µg/ml, an isolate was classified as non-susceptible to Chlorhexidine if the MIC was ≥4 µg/ml. Also, MIC, MBC of vancomycin were determined [13].

#### 2.2.3. Statistical analysis

The collected data were organized, tabulated, and statistically analyzed using statistical package for social science (SPSS Inc, version 22). For quantitative data, the median and range were calculated. Kolmogorov-Smirnov test (KS) test was performed as a test of normality; variables were not normally distributed. So, the Kruskal Wallis test was used as a test of significance. Categorical data were presented as frequencies and percentages, chi-square ( $\chi^2$ ) or Fisher's exact test, when appropriate, was used as a test of significance. For interpretation of the results of tests of



significance, significance was adopted at  $P \le 0.05$ . Adjusted P-values for multiple posthoc comparisons after classification of the individuals of different occupations into three groups (doctor, workers, and nurses) were calculated by using the Bonferroni correction method to account for the problem of multiple testing. P-value of 0.05 was divided by the number of comparisons, i.e., 3 (0.05/3). Thus, test results with Pvalues  $\leq 0.017$  were considered statistically significant.

#### 2.3. Ethical considerations

This study was approved by the Faculty of Medicine Research Ethical Committee N: R96 and University hospital director.

The study was conducted after explaining the study's aims. Verbal and written consents were obtained from all subjects included in the study and each person had the right to refuse to participate in the study.

### 3. Results

Out of 124 HCWs included in the study, 18.5% (23/ 124) were found to be carriers of S. aureus in their nasal cavities. There was no statistically significant difference in the nasal carriage of S.aureus as regards sex (P = 0.823). S. aureus nasal carriage rate was statistically higher among workers 50% (5/10) and nurses 32.4% (11/34) when compared to doctors 8.8% (7/80), (P = 0.00016 and 0.006, respectively). The study also showed that 30.6% (38/124) of HCWs were Coagulase Negative Staphylococci (CONS) carriers. Also Out of the isolated nasal S. aureus, 14/22 (63.6%) were identified as MRSA. The overall nasal carriage rate of MRSA among the studied HCWs was 11.3% (14/124).

One nurse 1/124 (0.8%) carried nasal VRSA. There was no statistically significant difference between male and female regarding the rate of nasal carriage of MRSA (P = 0.909). On the other hand, MRSA nasal carriage rate was statistically higher among workers 30% (3/10) as compared to doctors 6.3% (5/80) (P = 0.013). Although nurses had a higher MRSA carriage rate of 17.6% (6/34) than doctors, it was not statistically significant. The highest rate of MRSA nasal carriage was recognized in HCWs of the neurosurgery, general surgery, and ophthalmology (28.6%, 22.2%, and 17.2%, respectively), with no statistically significant difference (P = 0.649) (Table 1). The overall prevalence rate of S. aureus non-nasal carriage was 12.1% (15/124).

There was no statistically significant difference in the rate of non-nasal carriage of S.aureus as regards sex (P = 0.372). Non-nasal carriage rate of S. aureus was higher among workers 30% (3/10) and nurses 17.6% (6/34) when compared to doctors 7.5% (6/80), with no statistically significant difference. Out of the

isolated non-nasal S. aureus, 53.3% (8/15) were identified as MRSA. The overall non-nasal carriage rate of MRSA was 6.5% (8/124), with no statistically significant difference between male and female (P = 1.000). Non-nasal carriage rate of MRSA was higher among workers 20% (2/10) and nurses 2/34 (5.9%) as compared to doctors 5.0% (4/80), with no statistically significant difference. The highest rate of non-nasal MRSA carriage was recognized in HCWs of the anesthesia (15.8%), with no statistically significant difference (P = 0.446) (Table 2).

Out of 124 doctors, nurses and workers, 22 MRSA and one isolate VRSA were isolated and found to be sensitive to linezolid, ciprofloxacin, gentamycin, and resistance among MRSA was 63.4% (14/22) to Bacitracin, 36.4% (8/22) to erythromycin, 31.8% (7/ 22) to mupirocin. Also, VRSA isolate was found resistant to Bacitracin, erythromycin, and mupirocin (Table 3). No CoNS isolates showed mupirocin resistance in our study.

Our isolates showed inhibition zone diameters of Chlorhexidine ranged from 0 mm to 38 mm, while Povidone-iodine was from 0 mm to 22 mm. The studied strains of bacteria were inhibited by Apple vinegar which produced inhibition zone diameters ranged from 20 mm to 45 mm within compere to antibiotic (Ciprofloxacin) used as a positive control. There was a statistically significant difference in sensitivity between the different substances p < 0.0001 (Table 4).

The present study found: for MRSA isolates, MICs of mupirocin ranged from 0.25 to 32 µg/ml, and 36.4% (8/ 22) isolates showed LLMR, MBCs between 1 and 128 μg/ml. Also, 13.6% (3/22) showed Chlorhexidine resistance. MIC values of Chlorhexidine were 0.039-5 μg/ml, MBCs between 0.078 and 10 μg/ml. The MIC values of the Apple vinegar varied from 0.058 µg/ml to 1.87 µg/ml. The Apple vinegar was bactericidal at MBCs ranging from 0.234 to 7.5  $\mu$ g/ml (P < 0.0001). MIC against vancomycin was in the susceptible range except for one strain where the MIC was 32 µg/ml. The MIC50 and MIC90 for MRSA were shown in (Table 5).

#### 4. Discussion

Healthcare workers may be at high risk for harboring MRSA strains resistant to topical agents [20,21]. In the present study, 18.5% of our HCWs colonized S.aureus in their nasal cavities. These results were much less than those reported by *Pourramezan* et al. [22] who found that: S. aureus was observed in nasal cavities of (39.8%) individuals. Also, Rongpharpi et al. [1] stated prevalence of *S. aureus* as 52.8%, and *Kakhandki* et al. found a prevalence of 43.6% for S. aureus [23]. In our HCWs, lower prevalence of *S. aureus* as compared to other studies could be since the hospital infection control committee of our hospital performs screening for MRSA and stringent measures are taken. The

**Table 1.** Prevalence of nasal carriage *S. aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), and Vancomycin-Resistant *Staphylococcus aureus* (VRSA) in relation to different characteristics.

		S. aureus		MRSA		VRSA
		N				
	Number of nasal samples (%)	(%)	P-value	N(%)	P-value	N(%)
Participants	124	23		14		1
	(100%)	(18.5%)		(11.3%)		(0.8%)
Sex						
Male	78	14	0.823 (NS)	9	0.909 (NS)	0
	(62.9%)	(17.9%)		(11.5%)		(0.0%)
Female	46	9		5		1
	(37.1%)	(19.6%)		(10.9%)		(2.2%)
Job						
Physician	80	7		5		0
•	(64.5%)	(8.8%)		(6.3%)		(0.0%)
Nurse	34	11	0.0016* (S)	6		1
	(27.4%)	(32.4%)		(17.6%)		(2.9%)
Worker	10	5	0.006* (S)	3	0.013* (S)	0
	(8.1%)	(50%)	(,,	(30%)	(1)	(0.0%)
Ward						
Gynecology	15	0	0.649 <sup># (NS)</sup>	0	0.839 <sup>## (NS)</sup>	0
, 3,	(12.1%)	(0.0%)		(0.0%)		(0.0%)
Anesthesia	19	3		3		0
	(15.3%)	(15.8%)		(15.8%)		(0.0%)
Neurosurgery	7	2		2		0
,	(5.6%)	(28.6%)		(28.6%)		(0.0%)
Orthopedics	6	0		0		0
•	(4.8%)	(0.0%)		(0.0%)		(0.0%)
General. surgery	9	2		2		0
	(7.3%)	(22.2%)		(22.2%)		(0.0%)
Urology	15	1		0		0
	(12.1%)	(6.7%)		(0.0%)		(0.0%)
ENT	5	1		0		0
	(4.0%)	(20.0%)		(0.0%)		(0.0%)
OR	19	5		2		0
	(15.3%)	(26.3%)		(10.5%)		(0.0%)
Ophthalmology	29	9		5		1
- 1	(23.4%)	(31.0%)		(17.2%)		(3.4%)

<sup>\*</sup>P value ≤0.017 indicated a statistically significant when compared to physicians. #Gynecology and Orthopedics were excluded from the analysis. ##Gynecology, Orthopedics, urology, and ENT were excluded from the analysis.

overall nasal carriage rate of MRSA among our HCWs was 11.3%, which was comparable to the findings of other studies in different countries such as Iran (11.3%) [10], India (12%) [23], Ethiopia (12.7%) [24] Nepal (15.4%) [25], Saudi Arabia (18%) [26]. Present results were higher than described by Albrich et al. (8.1%), and *Kaminski* et al. (4.6%) in Germany [27]. However, they were lower than results reported by Navidinia et al. (77.7%) [28], ElSayed et al. (42.9%) [5], Kshetry et al. (37.6%) [29], Baral et al. (26%) [30], and Pourramezan et al. 25.5% [22]. The variation in the prevalence of MRSA among different studies may be due to differences in sample sizes, infection control policies, and antibiotic prescribing policies [29]. Our result revealed that one nurse was colonized with nasal VRSA (0.8%), a comparable finding was reported by ElSayed et al. in Egypt that only one HCW was colonized with Vancomycin-Intermediate S. aureus (VISA) (0.5%) [5]. Also, results reported by *Ploy* et al. in France, where 3.4% of HCWs were VISA nasal carriers [31]. Sassmannshausen1 et al. [27] found that: MRSA prevalence was higher in nurses (5.6%, 29/514) than in physicians (1.2%, 1/83). However, in our study: MRSA nasal carriage rate was statistically higher among workers (30%, 3/10) as compared to doctors (6.3%, 5/80)

(P = 0.013). The mechanism leading to MRSA nasal carriage is multifactorial and not properly understood, which may make that difference.

The highest rate of nasal MRSA carriage was found in HCWs of neurosurgeries (28.6%), while VRSA (3.4%) were among the ophthalmic team. *Askarian* et al. from Iran found the highest nasal carriage of MRSA was in surgical wards and emergency departments [32]. *Pourramezan* et al. [22] revealed that the highest rate of MRSA carriers (43.4%) in the Nephrology ward.

MRSA express resistance to many families of antibiotics which limit the treatment possibilities [33]. Some studies [34,35] reported high rates of resistance against erythromycin among MRSA from HCWs and which were similar to our findings. Also, we showed that all of the MRSA isolates remained susceptible to linezolid, ciprofloxacin, gentamycin. These findings corroborate the results of other previous studies from Iran, Nepal, and India [32,34,35]. Mupirocin resistance is being reported in many parts of the world in Korea 5%, India 6%, China 6.6%, Spain 11.3%, USA 13.2%, Trinidad Tobago 26.1%, and Turkey 45% [36]. Our MRSA isolates showed LL MR (36.4%), MICs of Mupirocin ranged from 0.25 to 32 μg/ml. Our findings were higher than in an Indian study that aimed to assess

Table 2. Prevalence of hand carriage (non-nasal) S. aureus and methicillin-Resistant Staphylococcus aureus (MRSA) according to different characteristics.

to different chi	iracteristics.				
		S.			
		aureus		MRSA	
	Number of				
	hand samples	N(%)	P-value	N(%)	P-value
Participants	124	15		8	
		(12.1%)		(6.5%)	
Sex					
Male	78	11	0.372	5	1.000
		(14.1%)	(NS)	(6.4%)	(NS)
Female	46	4		3	
		(8.7%)		(6.5%)	
Job					
Physician	80	6	0.168#	4	0.753#
		(7.5%)	(NS)	(5.0%)	(NS)
Nurse	34	6		2	
		(17.6%)		(5.9%)	
Worker	10	3		2	
		(30%)		(20%)	
Ward					
Gynecology	15	0	0.963 <sup>##</sup>	0	0.446 <sup>###</sup>
		(0.0%)	(143)	(0.0%)	(IV3)
Anesthesia	19	3		3	
NI	7	(15.8%)		(15.8%)	
Neurosurgery	/	0 (0.0%)		0 (0.0%)	
Orthopedics	6	0.0%)		0.0%)	
Orthopedies	o o	(0.0%)		(0.0%)	
General.	9	1		1	
surgery	_	(11.1%)		(11.1%)	
Urology	15	2		0	
<i>5,</i>		(13.3%)		(0.0%)	
ENT	5	0		0	
		(0.0%)		(0.0%)	
OR	19	4		3	
0.1.1.1.1	20	(21.1%)		(15.8%)	
Ophthalmology	29	5		1 (2.40/)	
		(17.2%)		(3.4%)	

<sup>##</sup>Gynecology, Neurosurgery, Orthopedics, and ENT were excluded from the analysis.

Table 4. Median and ranges of inhibition zone diameters of different vinegars and biocides against the studied isolates.

Substances	Median (mm)	Range (mm)	P-value
Ciprofloxacin (positive control)	3.0	2.0-4.0	<0.0001* (S)
Cholohexidine	2.0	0.0-3.8	
Povidine-iodine	1.0	.0-2.20	
Lemon vinegar	3.0	2.0-4.0	
Grape vinegar	2.5	2.0-3.2	
Apple vinegar	3.5	2.0-4.5	

<sup>\*</sup> P value  $\leq 0.05$  indicates statistically significance.

having low-level Mupirocin resistance. Mupirocin resistance is of significant concern for infection prevention strategies increasing bacterial resistance requires the discovery of new antimicrobial treatments [18]. Also, we found 13.6% (3/22) of MRSA showed Chlorhexidine resistance. MIC values of Chlorhexidine were 0.0039-5 μg/ml, Hughes and Ferguson in Australia found 10% (19/198) of S. aureus isolates showed a raised MIC to Chlorhexidine [42]. Higher findings were reported in Korea: 65% of isolates were Chlorhexidine resistant [18]. Shenget al. in Taiwan found: 72 (35.0%) MRSA isolates with Chlorhexidine MIC > or = 4  $\mu$ g/mL [43]. Because of the emergence of biocide-antibiotic resistance, it is important to evaluate different substitutes for antibiotics as natural products [44]

We have evaluated the antimicrobial activity of different vinegars as alternative decolonizing agent for nasal or non-nasal MRSA. The studied strains of bacteria were inhibited by Apple vinegar which produced inhibition zone diameters ranged from 20 mm to 45 mm. Yagnik et al. [9] investigated the antimicrobial

Table 3. Antimicrobial susceptibility of methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Staphylococcus aureus (VRSA) isolates collected from HCWs and workers.

Antibiotics	MRSA i	solates N (22)	VRSA isolates N (1)		
	Sensitive N* (%)	Resistant N* (%)	Sensitive N* (%)	Resistant N* (%)	
Mupirocin R ≤ 13 mm	15 (68.2%)	7 (31.8%)	0 (0%)	1(100%)	
Linezolid R ≤ 20 mm	22 (100%)	0 (0%)	1 (100%)	0 (0%)	
Gentamycin R ≤ 12 mm	22 (100%)	0 (0%)	1 (100%)	0 (0%)	
Erythromycin R ≤ 13 mm	14 (63.3%)	8 (36.4%)	0 (0%)	1 (100%)	
Ciprofloxacin R ≤ 15 mm	22 (100%)	0 (0%)	1 (100%)	0 (0%)	
Bacitracin R ≤ 10 mm	8 (36.4%)	14 (63.3%)	0 (0%)	1 (100%)	

<sup>\*</sup>N: number.

Mupirocin resistance in S.aureus nasal isolates from HCWs. It revealed that 25.71% of isolated S. aureus were Mupirocin-resistant MRSA. This could be due to the increased use of Mupirocin [37]. However, in other studies relative lower Mupirocin resistance rates among HCWs were reported (2%, 2.9%, and 10%, respectively) [38,39, and 18]. In another, the study conducted in Egypt that detected mupirocin-resistant isolates from wound postoperatively found 38.5% of MRSA showed LLMR [40]. Also in Navidinia et al. [41] study in Iran showed that 40 (62.5%) isolates were categorized as capacity of apple cider vinegar against E. coli, S. aureus, and C. albicans, and found that the Zone of inhibition of AV was between 20 and 25 mm against S. aureus. Bornemeier et al. [45] also tested vinegar against S. aureus and L.monocytogenes. They observed that vinegar inhibits the growth of these two bacteria. The MIC values of the Apple vinegar varied from 0.05 µg/ml to 1.87 µg/ml. The Apple vinegar was bactericidal at MBCs ranging from 0.234 to 7.5  $\mu$ g/ml (p < 0.0001). Baldas and Altuner [17] observed that apple cider vinegar affected S. aureus with MIC value: 25 µg/ml. Finally,

<sup>###</sup>Gynecology, Neurosurgery, Orthopedics, urology, and ENT were excluded from the analysis.

**Table 5.** Distribution of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) values, MIC50, MIC90, MBC50, and MBC9 for the studied isolates to different vinegars and biocides.

Substances	MICs 50 (µg/ml)	MICs 90 (µg/ml)	MICs Range (μg/ml)	P-value	MBC 50 (µg/ml)	MBC 90 (μg/ml)	MBCs range (µg/ml)	P-value
Vancomycin	0.5	4	0.125-32.0	<0.0001* (S)	2.0	8	0.25-64.0	<0.0001* (S)
Cholohexidine	0.078	0.125	0.039-5.0		0.156	5	0.078-10.0	
Mupirocin	2.0	16	0.25-32.0		4	32	1–128	
Grape vinegar	.4680	0.937	0.234-3.75		1.875	7.5	0.468-30.0	
Apple vinegar	0.468	1.87	0.058-1.87		1.875	3.75	0.234-7.5	

\*P value ≤0.05 indicates statistically significance.

MIC - Minimum inhibitory concentration,

MBC – Minimum bactericidal concentration.

MIC 50: MIC, which inhibits 50% of isolates,

MIC 90: MIC which inhibits 90% of the isolates.

MBC 50: MBC which kill 50% of isolates, MBC 90: MBC which kill 90% of isolates.

we support the use of Apple vinegar as decolonizing agent for MRSA, VRSA among HCWs as a strategy to prevent surgical site infections.

Unfortunately, there were some limitations to the current study. First, the genetic analysis of mupirocin and Chlorhexidine resistance pattern was not done due to limited resources and small sample from HCWs.

# 5. Conclusion

Nasal and non-nasal carriage of MRSA among HCWs was fairly high in our University Hospital mostly among workers and nurses. Among them, 36.4% of our MRSA isolates were resistant to mupirocin, and 13.6% were resistant to Chlorhexidine so periodic surveillance of antiseptic susceptibility among MRSA isolates is important for the control of nosocomial infections. This remains an important area for research to identify and study alternative agents for reducing MRSA colonization. We can conclude that the Apple vinegar being cheap and safe, used in the decolonization of MRSA, VRSA strains.

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# **Authors' contribution**

**Sylvana N. Gaber**: Share in microbiological laboratory tests searching the internet database, and the writing of the manuscript.

**Rasha H. Bassyouni**: Invent the idea, searching the internet database, and final editing of the manuscript.

**Fatma AboElnaga**: Share in microbiological laboratory tests, and searching the internet database and the writing of the manuscript.

**Muhammad Masoud**: Statistical analysis, and searching the internet database and the writing of the manuscript.

# **Disclosure statement**

All authors report no conflicts of interest relevant to this article.

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