

The Prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) Nasal Passage among Medical and Non-Medical Students at Al-Jouf University

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ABSTRACT

This study aimed to compare the occurrence of potentially nasal Staphylococcus aureus (S. aureus) of both female medical students and non-medical students at Al-Jouf University and its resistance & sensitivity of methicillin antibiotic. A cross-sectional study was conducted on 30 females' volunteers. Students including 15 medical students and 15 non-medical students. Specimens were taken from the subjects by a sterile moistened swab, which inserted into each nostril in turn, to a depth of approximately 1 cm, and rotated five times. Then the specimens were subjected to bacteriological examination, isolated S. aureus were subjected for further identification by biochemical tests. Then methicillin antibiotic test sensitivity and resistant were performed on isolates. We found that S. aureus in both samples medical and non-medical with 60%, and methicillin resistant S. aureus in each samples medical and non-medical with 66.7% as Statistical analysis demonstrated.

Key words: *Staphylococcus aureus, methicillin resistance, questionnaire, medical students, agar.*

INTRODUCTION

S. aureus a bacterium commonly isolated from humans is an important causative agent (Lowy et al., 1998). *Staphylococcus* to cause staph infection and is a successful pathogen due to a combination of nasal carriage and bacterial immuno- evasive strategies (Kluytmans et al., 1997 & Cole et al., 2001). The ability of the nasal passages to harbor *S. aureus* result from

a combination of a weakened or defective host immunity and the bacterial ability to evade host innate immunity (Clin et al., 1997).

S. aureus is a Gram-positive coccal bacterium that is a member of the Firmicutes, and is frequently found in the human respiratory tract and on the skin (Cole et al., 2001).

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S. aureus is a common cause of skin infections (e.g. boils), respiratory disease (e.g. sinusitis), and food poisoning. Disease-associated strains often promote infections by producing potent protein toxins, and expressing cell-surface proteins that bind and inactivate antibodies (Ogston et al., 1984). The emergence of antibiotic-resistant forms of pathogenic *S. aureus* (e.g. MRSA) is a worldwide problem in clinical medicine. It is still one of the five most common causes of nosocomial infections and is often the cause of postsurgical wound infections (Kluytmans et al., 1997). Methicillin, introduced in 1961, was the first of the semi synthetic penicillinase-resistant penicillin. Its introduction was rapidly followed by reports of methicillin-resistant isolates (Jevons et al., 1961). The spread of these methicillin-resistant strains has been a critical one for clinicians. The therapeutic occurs of infections that result from methicillin-resistant *S. aureus* (MRSA), is worse than the outcome of those that result from methicillin-sensitive strains (Cosgrove et al., 2003).

The aim of this study is to compare the occurrence of potentially *Staphylococcus aureus* of female medical students and female non-medical students in Al-Jouf University and its resistance & sensitivity to methicillin antibiotic.

MATERIALS AND METHODS

Subjects and study protocol:

The study was carried out at Al – Jouf University. 30 students including 15 medical students and 15 non-medical students. Two-nose swab for each volunteer were taken. Then the samples cultivated on Nutrient agar and Mannitol Salt Agar. Then the Müller Hinton agar prepared, after that methicillin antibiotic added on it and sensitivity and resistant tested.

Collection of sample:

Specimens were taken from the subjects in the following way: a sterile moistened Cotton swab was inserted into each nostril in turn, to a depth of approximately 1 cm, and rotated five times.

Cultivation of samples:

Sample was inoculated onto Mannitol Salt Agar. Plates Inoculation the media, (incubation at 37°C for 18-24 h). Suspend 11g of powder in 1L Of Distilled water in flask 1000 ml and sterilization in autoclave at 121°C for 15 minutes) (Abilo et al., 2006).

Isolation of microorganism is in pure culture:

Samples were inoculated onto nutrient agar plate Inoculation the media with sample using streak method and incubation at 37°C for 18-24 h. to uses later in other test. Suspend 23g of powder in 1L of Distilled water in flask 1000 ml and sterilization in autoclave at 121°C for 15 minutes (Jawetz et al., 2010).

Gram staining:

Gram's stain was done according to the method described by Cruickshank et al. (1975). Bacteria colored red were labeled as Gram-negative organisms and violet colored were labeled as Gram-positive organisms.

Antibiotic susceptibility test:

Disk diffusion; (Kirby-Bauer technique)

Müller Hinton broth :__Using aseptic technique, pick 3-5 isolated colonies of the same organism from an 18- to 24-hour Nutrient Agar plate and inoculate into 5 mL of Müller Hinton Broth and Incubate 2-6 hours at 35°C. Periodically check turbidity against the 0.5 McFarland turbidity standards. If too turbid, dilute aseptically with additional Müller Hinton Broth and repeat turbidity check. If not turbid enough, continue incubation. When turbidity is comparable to the standard.

Place the appropriate methicillin discs onto the Müller Hinton agar a bacteria suspension of the organism from Müller Hinton broth is swabbed over the entire surface of the medium using lawn method. Deposit methicillin antibiotic disc in centers so that they are not less than 10 mm from the edge of the Petri dish, and their centers are at least 30 mm apart. Incubate plates aerobically at 35 ± 2°C within 15 min after the discs are applied. Examine Mueller Hinton Agar plates after 16-18h. The diameters of all zones of

inhibition are measured by the CLSI (Abilo et al., 2006).

Statistical Methods:

This study used SPSS ver. 16 to analyze the quantitative data gathered from the participants. The data analysis summarized the results in statistical form, providing the means and the ranges of the data collected. The main purpose of the quantitative analysis was to identify the statistical significance of the numbers involved.

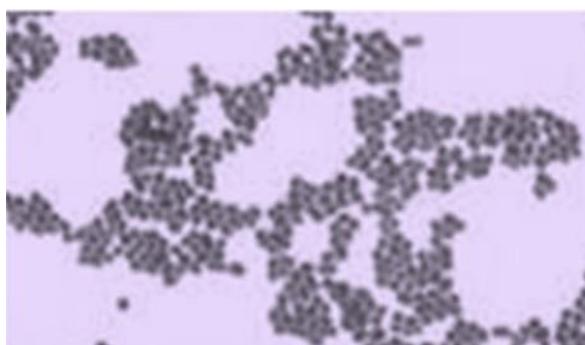


Fig. 1: Clusters of *S.aureus* by Gram stain.

RESULTS

Microscopic examination of isolates revealed Gram positive cocci (single, in pairs, or irregular grape-like clusters) , 0.5-1.5µm in diameter as shown in Fig. 1

Virtually all strains of *S. aureus* ferment Mannitol. Bright yellow colonies on yellow background indicate fermentation of Mannitol on Mannitol Salt Agar. Fig. 2.



Fig 2: Mannitol fermentation on Mannitol Salt Agar

Antibiotic susceptibility testing:

Susceptibility test was done by Disk diffusion method. Thirty strains of *S. aureus* were isolated and tested for their antimicrobial sensitivity patterns. Majority of the isolates showed high resistance against Methicillin. The percentages was (66.7%) of the both

medical students and non-medical students. In contrast, (33.3%) of the both medical students and non-medical students were sensitive against Methicillin.

Specimens of Medical students:

The percentages of resistance and sensitivity to antibiotic are shown in (Table: 1 & Fig: 3).

Table (1): showed percentages of (positive and negative) for Gram stain (Gram-positive cocci in cluster):

	Gram stain (Gram positive cocci in cluster)		Growth on MSA (Ferment yellow colonies		Growth on N.A		Coagulase test		S.T (Resistances or sensitive)	
	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	R	S
F	15	0	8	7	15	0	9	6	10	5
%	100	0	53.3	46.7	100	0	60	40	66.7	33.3

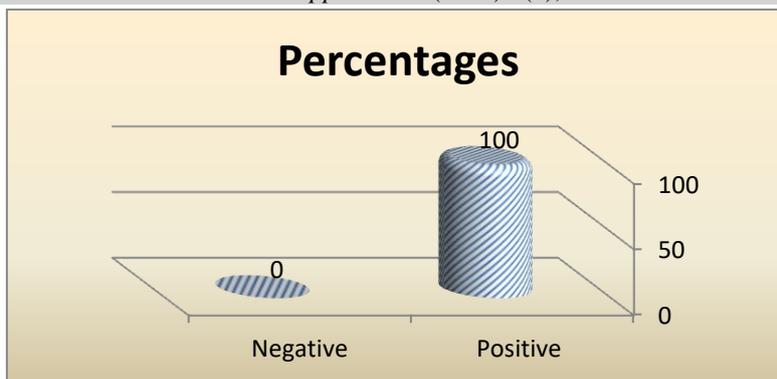


Figure (3): graph showing the percentages of (positive and negative) for Gram stain (Gram-positive cocci in cluster)

On Mannitol salt agar the percentages of (positive and negative) for Growth on Mannitol Salt Agar (Ferment Mannitol, produce yellow colonies and Media turns

yellow or non-fermenting Mannitol and Media does not change color), most of frequencies are (+ve) with (53.3%) and (-ve) with (46.7%), figure 4 shows this percentages:

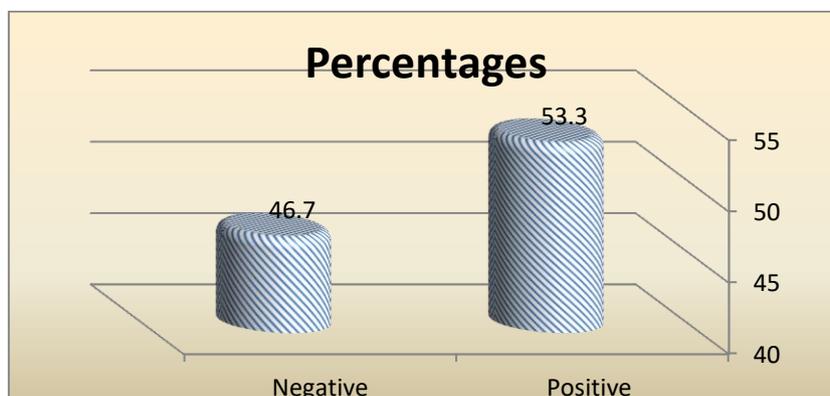


Figure (4): shows percentages of (positive and negative) for Growth on MSA

On Nutrient agar the percentages of (positive and negative) for the Growth all of frequencies

are (+ve) with (100%) and (-ve) with (0%) this shows in figure 5.

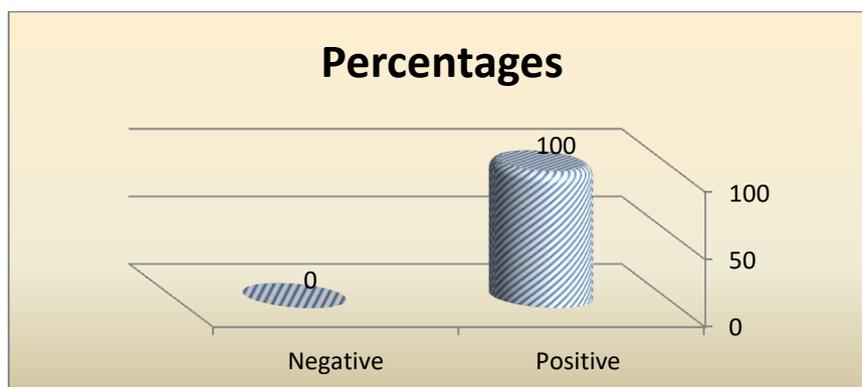


Figure (5) percentages of (positive and negative) for (Growth on N.A)

Specimens of non-Medical students:

Table (2) percentages of (positive and negative) for Gram stain (Gram-positive cocci

in cluster), all of frequencies are (+ve) with (100%) the figure. 6 shows this percentages.

Table (2): percentages of (positive and negative) for Gram stain (Gram-positive cocci in cluster):

	Gram stain (Gram positive cocci in cluster)		Growth on MSA (Ferment yellow colonies)		Growth on N.A		Coagulase test		S.T (Resistances or sensitive)	
	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	R	S
F	15	0	11	4	15	0	9	6	10	5
%	100	0	73.3	26.7	100	0	60	40	66.7	33.3

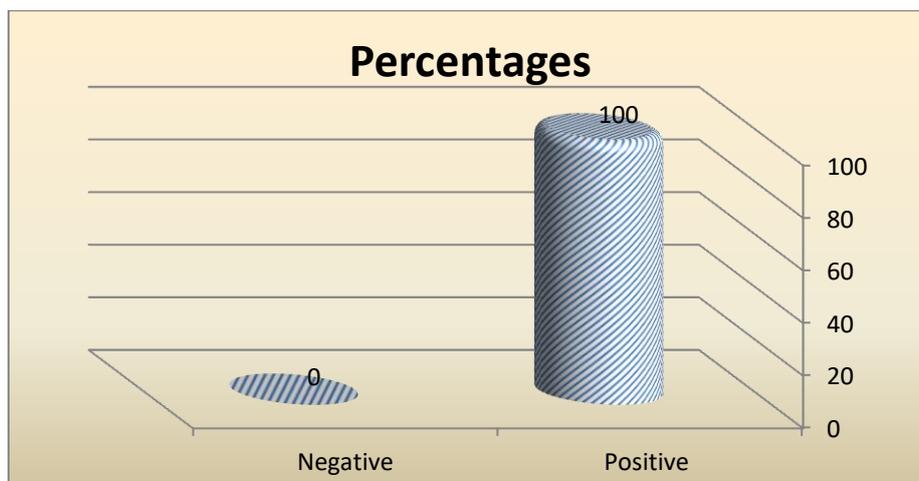


Figure (6): percentages of (positive and negative) for Gram stain

The figure below shows percentages of (positive and negative) for (Growth on MSA (Ferment yellow colonies), most of

frequencies are (+ve) with (73.3%) and (-ve) with (26.7%), the figure (7) shows these percentages:

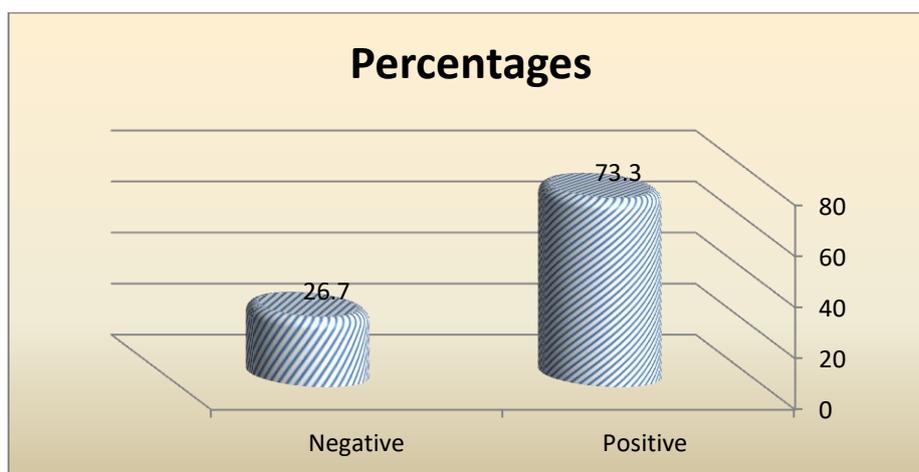


Figure (7): percentages of (positive and negative) for (Growth on MSA)

The percentages of (positive and negative) for (N.A) all of frequencies are (+ve) with (100%) and (-ve) with (0%) this illustrate in figure. 8.

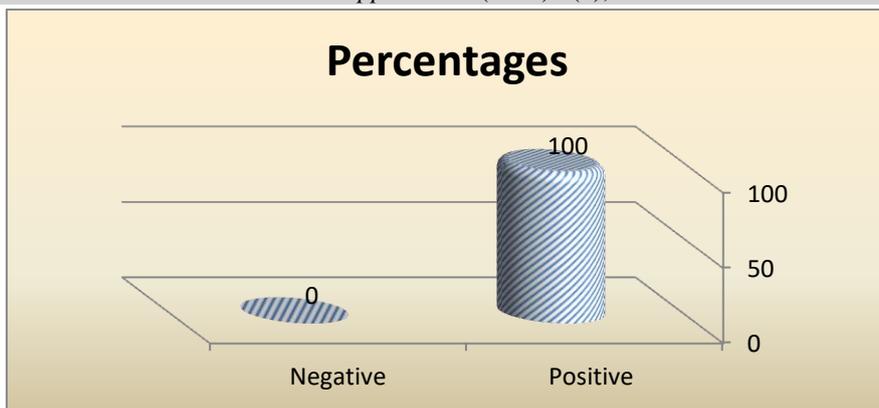


Figure (8): percentages of (positive and negative) for (N.A)

Compare between medical and non-medical:

The figure bellow shows the percentages of Gram stain (Gram-positive cocci in cluster),

there is equals between Grams stain medical or non-medical with (100%).

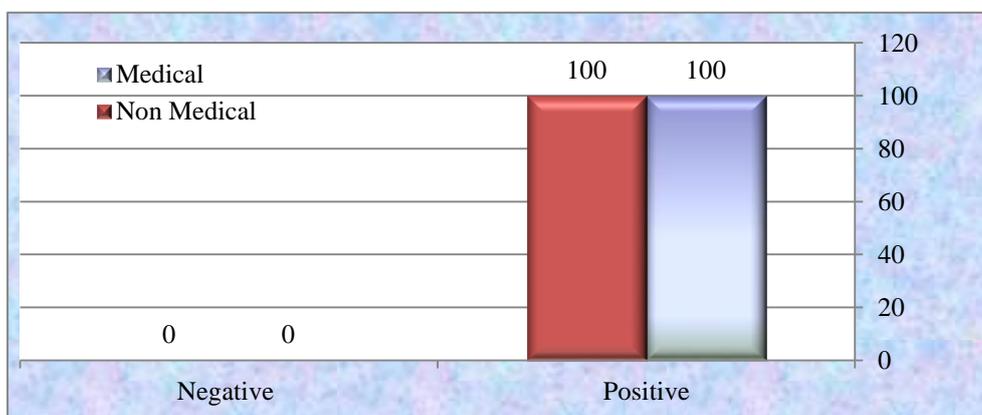


Figure (9): shows the comparing between medical and non-medical students of the percentage of Gram stain

The figure bellow shows the percentages of Growth on Mannitol Salt Agar, positive with (73.3%) in non-medical corresponding

(53.3%) medical, negative (46.7%) medical and (26.7%) non- medical.

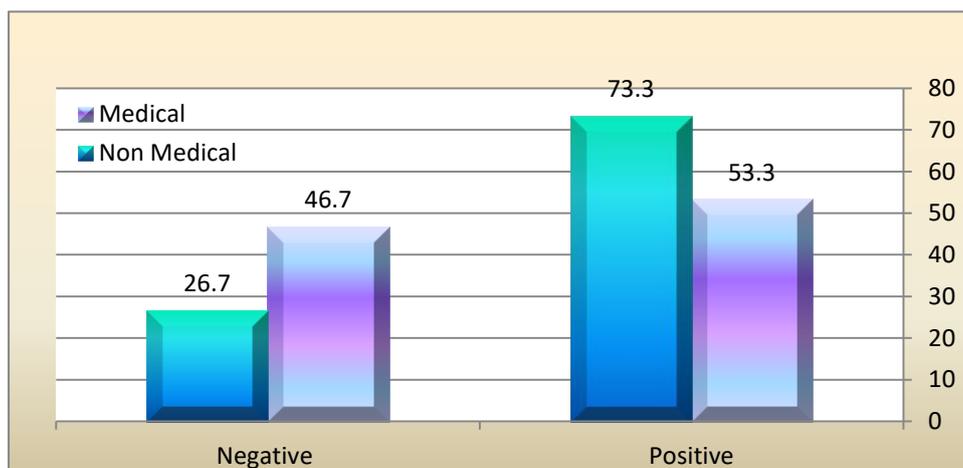


Figure (10): shows the comparing between medical and non-medical students of the percentage of growth on Mannitol Salt Agar

The figure bellow shows the percentages of (Growth on Nutrient agar), there is equals

between Gram stain (Growth on Nutrient agar) medical or non-medical with (100%).

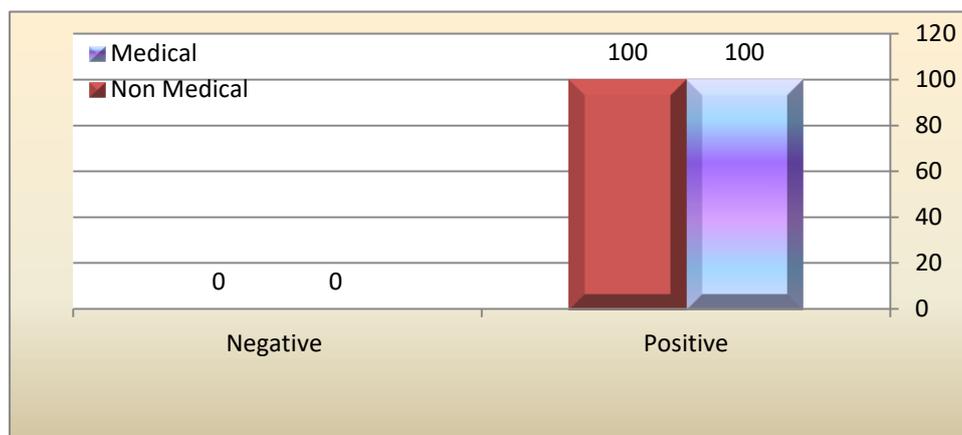


Figure (11): shows the comparing between medical and non-medical students of the percentage of growth on (Nutrient agar) **Differences between (Medical and Non –medical)**

Table (3): shows differences between two groups (Medical and Non –medical) Mann-Whitney (U) according to (Significant p- value) of this test (1.0) greater than (0.05) this indicate there is no differences between two groups at (0.05) level

Type	N	Mean Rank	Sum of Ranks	U	Significant- value
Medical	10	10.50	105.00	50	1.0
Non –medical	10	10.50	105.00		
Total	20				

DISCUSSION

Staphylococcus aureus is a major cause of community and healthcare infection, and methicillin-resistant *S. aureus* (MRSA) is currently the most commonly identified antibiotic-resistant pathogen in many part of the world (Ayliffe et al., 1997).

The present study, 30 female students, 15 of those students Trainees in medical field and 15 students on Non-Medical field. The overall nasal carriage rate of *Staphylococcus aureus* (S.A.) was 60% in each sample medical and non-medical convergent result was recorded by Chaherjee et al. (2009) on the nasal swabs from Indian community carried out 52.3% of *S.aureus*.

However, these percentage is higher than other study conducted in Dessie hospital with 28.8 % (Agumas et al., 2013) moreover Martinez et al. (2009) compared nasal carriage rates of *S. aureus* from population across the USA and found 21.4% colonization rate of

S.aureus. Previous study done by Moellering, (1998) reported 36% of *S.aureus* in nares of Japanese adults, 32.4% in nasal cavity of and adults in USA and 35.8% in Gaborone hospital, Botswana (Truong et al., 2011). Recently, very low percentage of the nasal carriage rate of *S. aureus* found to be 12% as reported by Sharma et al. (2014) the same results were obtained by Shakya and co-workers (2010) rate of 12.5% among patients. The nasal carriage of MRSA among healthcare personnel and visitors/patient attendants has indicated the chances of transmission of the organism to the patients during patient-care.

The current study explores Nasal Carriage of *S. aureus* we found methicillin resistant *Staphylococcus aureus* (MRSA) in each sample medical and non-medical with 66.7%. our results disagree with these studies this is higher than study at National Medical College & Teaching Hospital and Dessie hospital with 45.5% & 44.1% respectively

were resistant to methicillin Sah et al. (2013) and Agumas et al. (2013).

Nadia and Baiu (2014) recorded a rate of 21.4% for MRSA, which was conducted among health care workers. MRSA nasal carriage prevalence was found to be 24.15% was observed in USA. 22.6% in Gaborone hospital, Botswana (Truong et al., 2011). On the other hand, Taiwan and Zaria, Nigeria reported a prevalence of 13.6% and 14.85% respectively, from anterior nares of healthy population, adults and school pupils (Onanuga & Temede 2011). The lowest percentage MRSA nasal carriage prevalence was found to be 5% Sharma, (2014). Moreover, the carrier rate was the same in persons in medical field and general people in our study with 66.7% but in study concerned in University in Jordan have higher percentage of person on medical with 6.4% but 1.5% for person

On non-medical. (Al Amiry et al., 2013). This differentiation may be due to my volunteers students are trainees in hospital that helped in transfer of MRSA among other student and may be the number of volunteer was not enough to show the variation between student trainees in medical and non-medial. MRSA is very difficult to eradicate, within hospital, MRSA spreads rapidly by hands of medical personnel. Gathering employees of hospital such as asymptomatic nasal and hand carriers acting as reservoirs are important sources for spreading this organism. Multiple, prolonged use of antibiotics and prolonged hospitalization are other important factors which make hospital an ideal place of transmission and perpetuation of MRSA.

The prevalence of *S. aureus* nasal carriage varies according to the quality of sampling, culture techniques, and the population studied.

CONCLUSION

The *Staphylococcus.aureus* develops itself until that it became resistance to all beta-lactam antibiotics for example methicillin. MRSA causes many diseases and it is become out of control. There is evidence that medical

personal have a same prevalence of MRSA colonization than the general population.

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