Original Article

Prevalence of Panton–Valentine leukocidin and toxic shock syndrome toxin-1 genes in methicillin-resistant *Staphylococcus aureus* isolated from nose of restaurant workers in Kirkuk city

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Abstract

Staphylococcus aureus resides naturally in the nasal cavity of healthy individuals, including those working in restaurants, so they may be a source for spreading this bacterium to restaurant customers directly or indirectly through cooked meals. This bacterium has several virulence factors enabling it to cause many diseases in different parts of the body. It has also the capability to resist conventional antibiotics including methicillin. To investigate methicillin-resistant *S. aureus* (MRSA), 170 nasal swabs were collected from food preparation workers in 30 restaurants (5–6 workers in each restaurant) in Kirkuk city. After collection, the samples were directly transferred to the laboratory and cultured on selective media like mannitol salt agar (MSA). Microbiological examination including morphological, biochemical, and confirmatory tests showed that 24/170 of collected samples were positive for *S. aureus* with a rate of 14.12%. Among 24 isolates, 20 (83.3%) belonged to MRSA. All isolates were resistant to oxacillin and penicillin (100%), whereas sensitive to other antibiotics (gentamicin, chloramphenicol, and rifampicin). Polymerase chain reaction exhibited that 13 (65%) of MRSA isolates have toxic shock syndrome toxin-1 gene and only 4 (20%) have Panton–Valentine leukocidin gene.

Key words: Kirkuk city, methicillin-resistant *Staphylococcus aureus,* nasal carriage, Panton–Valentine leukocidin, restaurant workers, toxic shock syndrome toxin-1

INTRODUCTION

Staphylococcus aureus is considered one of the main problematic pathogens that human beings is facing. Each person in one way or another would have *S. aureus* infection during a lifetime. The severity of the infection varies from food poisoning to some dermal infections and in some cases would cause mortality.[1] The nose is the natural site of colonization of *S. aureus,* and near of 30% of people are colonized at any one time. Therefore, those people develop the risk of skin infections caused by *S. aureus*.[2] Bacterial pathogenicity is associated with the secretion of several virulence factors such as clumping factors, protein A, toxic shock syndrome (TSS), and Panton–

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Valentine leukocidin (PVL).[3] Among these exotoxins, the function of PVL in the pathogenesis is exactly unknown until it was remarked that strains that retain PVL genes were considerably associated with, sepsis, necrotizing pneumonia, and skin and soft-tissue infections. In addition, PVL consists of two components, encoded by prophage, which attack individual monocytes, phagocytic cells, and host polymorphonuclear leukocytes then lead to tissue damage. Furthermore, the existence of PVL genes was recognized as a conceivable marker of community-associated methicillin-resistant *S. aureus* (MRSA) isolates, whereas TSST-1 is correlated with acute multisystem diseases.[4] This bacteria can also be transmitted from food makers to food items through unhygienic practices such as coughing or sneezing or dealing with food before hand cleaning.[5] Food makers can act as a carrier of virulent isolates and maybe a way of their transport to food. Newly years predominance of antibiotic-resistant strains are given widespread attention, especially MRSA strains because they are not sensitive to all obtainable penicillins and beta-lactams and important reason for morbidity and mortality worldwide.[6]

Almost all previous studies in our city related to food poisoning have paid attention to screening isolates of *S. aureus* for enterotoxins in food industry workers and the resistance or sensitivity of these microorganisms to various antibiotics. Due to the deficiency of information about the presence of PVL and TSST-1 genes in methicillin-resistant isolates in these bacteria, this study aimed to the determined of antibiotic-resistant pattern and the frequency of TSST-1 and PVL genes in the nasal carriage of MRSA of restaurant workers in Kirkuk City, Northern Iraq.

MATERIALS AND METHODS

Sample collection

One hundred and seventy nasal swabs were randomly collected from workers in the kitchens of 30 restaurants, 5–6 workers in each restaurant and it was transferred directly to the Microbiology Laboratory at one educational institution for the period from December 1, 2020, to March 1, 2021.

Isolation and identification

The collected samples were placed on MSA and incubated for 18–24 h at 37°C, then phenotypic tests were performed on the growing colonies, which included the size, shape, and color of the colonies and gram stain, followed by biochemical diagnostic tests, which included catalase coagulase DNase, oxidase.[7,8] it was also cultured on the blood agar medium to know the extent of production of hemolysin, and the diagnosis was confirmed by VITEK 2 system. Some virulence factors were detected such as their secretion of the enzyme β-lactamase and the cell wall containing protein A.[9,10] To confirm isolates as methicillin-resistant, cefoxitin (30 μg) disc diffusion test was performed.

Susceptibility test to antibiotics

A sensitivity test was conducted for MRSA isolates to 13 antibiotics commonly used in Kirkuk city. These are penicillin 10 unit, rifampicin 5 μg, oxacillin 1 μg, clindamycin 2 μg, gentamicin 10 μg, erythromycin 15 μg, tetracycline 30 μg, ciprofloxacin 5 μg, and levofloxacin 5 μg. Chloramphenicol 3 μg, clindomycin 10 μg, trimethoprim. sulfamethoxazole (1.25/23.75) μg, trimethoprim 5 μg, and vancomycin 10 μg, all suppliers were from Oxford UK. The sensitivity and resistance of isolates to the antibiotics done by the disk diffusion method were estimated as reported by Kirby-Bayer.[11] and based on what was mentioned by the clinical laboratory standard institute.[12]

DNA extraction and toxic shock syndrome toxin-1 and Panton–Valentine leukocidin genes detection

Bacterial DNA was extracted from 20 MRSA isolates using a DNA extraction kit supplied by Promega Company. The concentration of extracted DNA was also estimated using a BioDrop Spectrophotometer (Cambridge CB4 England) and the purity of the product was assessed at a wavelength of 260/280 nm. Polymerase chain reaction (PCR) and gel electrophoresis were performed according to the manufacturer's agreement. The primers were used in the present study to a determination TSS-1 and PVL genes according to Khosravi *et al*. and Koosha *et al*.[13,14] as shown in Table 1. PCR was performed based on the following: basic denaturation for 5 minutes at 95°C, pursued by 30 cycles for the 30 s at 95°C, and 72°C for 30 s with a finale extension at 72°C for 7 min. PCR results were detected using gel electrophoresis at 100 V/mA for 75 min. The bands were detected by ultraviolet rays and photocopied.

RESULTS AND DISCUSSION

To investigate the MRSA, 170 swabs were taken from the noses of cooks in 30 restaurants, 5–6 workers at each restaurant in Kirkuk, for the period from January 1, 2020, to May 1, 2020. Samples were immediately transferred to

Table 1: Primer sequences and lengths of amplified DNA products

Gene primer		Primer sequence	Size
PVL	F	ATCATTAGGTAAAATGTCTGCACATGATCCA-3 -'5	433bp
	R	GCATCAACTGTATTGGATAGCCAAAAGC-3-' 5	
TSST-1	F	CTGGTATAGTAGTGGGTCTG'3'5	587bp
	R	5'AGGTAGTTCTATTGGAGTAGG'3	

PVL: Panton–Valentine leukocidin, TSST-1: Toxic shock syndrome toxin-1 gene

the laboratory and cultured on MSA and incubated for 18–24 h at 37°C.

Twenty-four of the cultured media gave positive growth at a rate of 14.11%, they were yellow, meaning they were fermented mannitol. Isolates that fermented with mannitol were diagnosed based on phenotypic and microscopic characteristics and biochemical tests. Microscopic examinations showed that these isolates were spherical and gram-positive. All of them were positive for catalase, coagulase, DNase, and protein A, whereas they were negative for oxidase. These results were confirmed by VITEK 2. Among the 24 isolates, 20 (83.3%) belong to MRSA. Inconsistent to Al-Tamim *et al*. and Vatansever *et al*.[15,16] and it was similar to the results found by Rasheed and Hussein.[17] The reason for the similarities and differences may be due to geographical location, environmental conditions, and some social customs. Culturing on blood agar showed that 9/20 (45%) alpha hemolytic isolates, whereas 12 (60%) were positive for the beta-lactamase test.

Sensitivity tests were conducted for MRSA isolates, by disk diffusion method using 13 antibiotics, which were divided according to the effect of these antibiotics into cell wall inhibitors represented by penicillin and oxacillin. Moreover, the protein synthesis inhibitors represented by clindamycin, tetracycline, erythromycin, and chloramphenicol, whereas the nucleic acid inhibitors were levofloxacin, ciprofloxacin, and rifampicin. The results mentioned in Figure 1 showed the response of the bacterial isolates to the different antibiotics used in the experiment. The highest resistance was observed against oxacillin and penicillin with percentage of 100%. The results are close to what was found by Ahmadi *et al*.[18] and similar to the results of Abie *et al*. and Zhu *et al*.,[19,20] whereas inconsistent with Gushiken *et al*.[21]

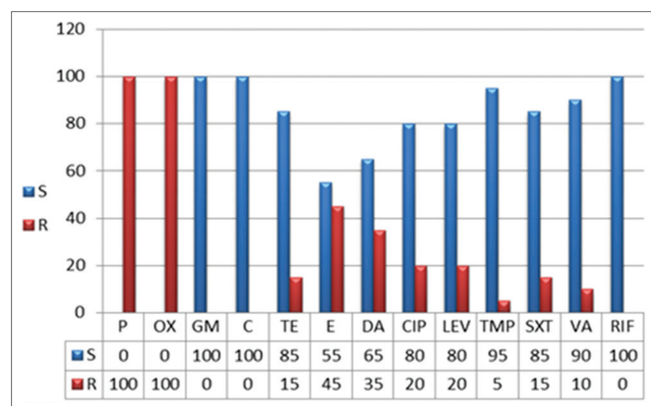

Figure 1: Antibiotic-resistant pattern of MRSA isolates. P: Penicillin, OX: Oxacillin, GM: Gentamicin, C: Chloramphenicol, TE: Tetracycline, E: Erythromycin, DA: Clindamycin, Cip: Ciprofloxacin, LEV: Levofloxacin, TMP: Trimethoprim, SXT: Trimethoprim/ sulfamethoxazole, Va: Vancomycin, RIF: Rifampicin, MRSA: Methicillin-Resistant *Staphylococcus aureus*

All isolates were also sensitive to gentamicin and chloramphenicol at 100%, whereas they were resistant to clindamycin, erythromycin, and tetracycline with percentages of 35%, 45%, and 15%, respectively, our results match the results obtained by Najafi Olya *et al*.[22] Furthermore, all isolates were sensitive to rifampin, an inhibitor of RNA synthesis by 100%. Moreover, they were resistant by 20% to ciprofloxacin and levofloxacin. As for vancomycin, most of them were sensitive to this antibiotic by 90%, whereas the percentage of sensitivity to inhibitors of basic metabolic substances was trimethoprim sulfamethoxazole and trimethoprim 95% incompatible with Rostamzad and Rostamneia.[23] The reason for the sensitivity of some bacterial isolates to the antibiotics under study is that they were able to reach their targets efficiently and thus affect the target site according to the mechanism of action of each antibiotic.

S. aureus pursues several antibiotic resistance strategies including the acquisition of spontaneous point mutations in the original target that reduce antibiotic binding, acquisition of a gene that encodes an enzyme that inactivates the antibiotic, and acquisition of an antibiotic insensitive target that can bypass the effect of the antibiotic by substituting the function of the antibiotic. The original target, expressing a protein or proteins that transport the drug from the cytoplasm, thereby reducing its cytoplasmic concentration, and acquiring mutations that alter the surface properties of the bacteria that, for example, reduce the interaction of the antibiotic with the bacteria.[24]

The results of the polymerase chain reaction for the MRSA strains as in Figure 2, showed that through 20 isolated MRSA, 13 (65%) were affirmative only for the TSST-1 gene and 4 (20%) were positive only for PVL gene [Figure 3], This was similar to the results of Goudarzi *et al*.[25] who described a prevalence of the PVL encoded gene of 21.4% within MRSA isolates in Iran. While our results were contrary to what was mentioned by Udo *et al*.[26] whom isolated MRSA in Kuwait's capital City restaurants from food handlers. The presence of PVL genes coincided with SST-1 in three isolates (15%), whereas 11 isolates contained only the TSST-1 gene. This result is inconsistent with Sato *et al*.[27]

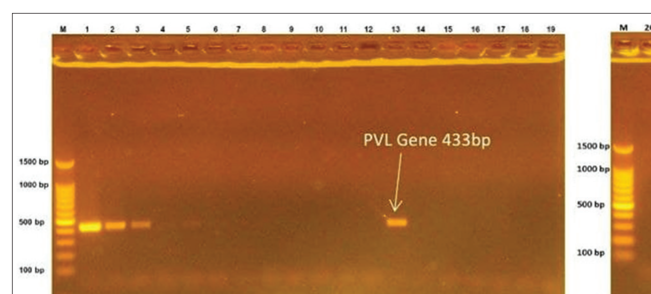

Figure 2: PCR agarose gel electrophoresis for the detection of the PVL gene (433bp) in MRSA strains. PVL: Panton–Valentine leukocidin, MRSA: Methicillin-resistant *Staphylococcus aureus*

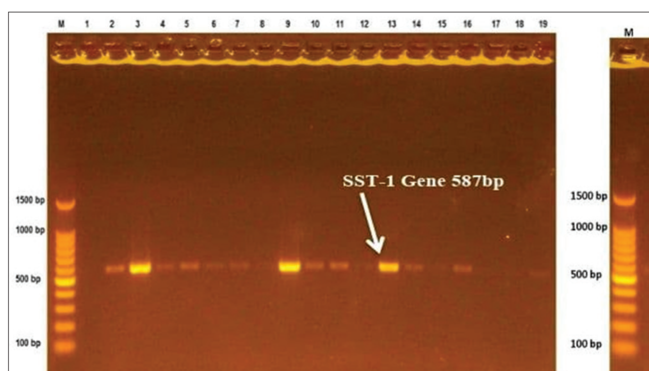

Figure 3: PCR agarose gel electrophoresis for the detection of the SST-1 gene (587bp) in MRSA strains. MRSA: Methicillin-Resistant *Staphylococcus aureus*, SST-1: Shock syndrome toxin, PCR: Polymerase chain reaction

The recent alarming development is the emergence of strains of Community acquired MRSA (CA-MRSA) with multiantibiotic resistance and containing several important virulence factors such as PVL and TSST that cause many severe and life-threatening diseases. As the name implies, CA-MRSA strains are not restricted to hospitals and are obtained from contact in various education centers such as schools and universities, restaurants, and other community settings. Carriers of nasal MRSA are more susceptible to infection than noncarriers and serve as important reservoirs of infection so the existence of these strains in the food chain means presents a potential health risk to consumers and deserves further monitoring, and we believe that the nasal MRSA colonization screening is essential in infection protection and control and may provide beneficial information to lead antibacterial therapy also there is a need for appropriate food safety training among food handlers and to improve food quality.

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Conflicts of interest

There are no conflicts of interest.

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