Abstract: A single allele-specific PCR targeting the G88A polymorphism was found to be specific for the detection of USA300 community acquired methicillin resistant Staphylococcus aureus (CA-MRSA). This study aims to determine the prevalence of USA300 in Kurdistan region-Iraq based on this method. We have access to 52 frozen CA-MRSA strains isolated from clinical samples and 10 frozen CA-MRSA strains isolated from healthy carriers. These strains have already been genotyped for SCCmec and PVL gene. These strains were examined for the nuc and mecA genes, then PCR targeting the G88A polymorphism was performed to determine the prevalence of USA300 strain in the community. Two of the clinical samples typed as USA300 CA-MRSA both typed PVL positive and SCCmec type IV. One of the strains isolated from healthy carrier typed as USA300, SCCmec type IV and PVL positive. This is the first study investigating USA300 CA-MRSA prevalence in Kurdistan region in Iraq. USA300 isolates are now epidemic in the USA and are gaining ground around the world. Therefore, actions should be taken to minimize the emergence and transmission of these strains. This method is a cost-effective, a high-throughput, and a rapid method for the detection of USA300 CA-MRSA which offers the opportunity to study such a clinically important strain especially in developing countries.

Keywords: USA300 CA-MRSA, PVL, Iraq.
1. Introduction

Community associated Methicillin-resistant *Staphylococcus aureus* (MRSA) PFGE strain type USA300 (USA300 CA-MRSA) has become not only the dominant strain in USA, but it also became the predominant in other countries (Nimmo, 2012 August). Such a strain is now considered as a major international epidemic strain, but whether it will supplant established community-associated strains in other countries remains to be investigated (Nimmo, 2012 August).

Recently, a report by Chadwick et al. described a single allele-specific PCR targeting the G88A polymorphism and was found to be 100% sensitive and specific for the detection of USA300 community acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) (Chadwick et al., 2013). Here in Iraq, studying the prevalence of USA300 through classical methods is extremely difficult hence this PCR based method offered a great opportunity to study the prevalence of such a strain in Iraq. A part from two reports of USA300 infection cases among USA military personal have come from combat support hospitals in Iraq (Huang et al., 2011, Murray et al., 2010) no studies have been conducted to investigate the prevalence of USA300 CA-MRSA in Iraqi populations.

2. Methods

We have access to 52 frozen CA-MRSA strains isolated from clinical samples. Also, we examined 10 frozen CA-MRSA samples isolated from healthy carriers in the community. These strains have already been genotyped for SCCmec and genes encoding the Panton–Valentine leucocidin (PVL) based on the previously described assays (McClure et al., 2006 March, Zhang et al., 2005). DNA was extracted from *S. aureus* isolates using the Qiagen DNA extraction kit according to the manufacturer’s instructions (Qiagen). Firstly, our samples were tested for the nuc gene (*S. aureus*-specific nuclease) and meca gene (methicillin resistance) to confirm that the used strains are MRSA. Then, allele-specific PCR assays that detect the USA300 G88A pbp3 sequences were performed and gel electrophoresis was used to detect amplifications on a 1% agarose gel.

3. Results

The prevalence of PVL gene was (10/52) 19%. Also, these strains were typed for SCCmec. Out of 52 MRSA isolates, 7.7%, 11.5% and 19.2% carried SCCmec I, II, and III, respectively. 61.5% of our isolates carried SCCmec type IV. Two strains were SCCmec type IV-bearing, PVL-positive MRSA strains. None of our strains carried SCCmec type V. All CA-MRSA typed as type IV SCCmec and one of them was PLV gene positive.

It was found that two of the clinical samples typed as USA300 CA-MRSA: both isolated from skin and soft tissue. Surprisingly, one of the strains isolated from healthy carrier typed as USA300. All the three strains typed as SCCmec type IV and were PLV gene positive.

4. Conclusion

These results are of potential importance because early detection of the USA300 clone of CA-MRSA may be of particular importance because it is highly virulent, transmissible, and is increasing in prevalence. This is the first study investigating USA300 CA-MRSA prevalence in Kurdistan region in Iraq. Using the same method, the prevalence and the trend of infection with such a strain can be studied in the future.

USA300 isolates are now epidemic in the USA and are gaining ground around the world. Therefore, actions should be taken to minimize the emergence and transmission of these strains. Vaccines are one of the ways that can halt the spread of this pathogen in the future. Furthermore, light should be shed on the improving of hygiene in homes, healthcare and communities setting and awareness should be taken of the random use of antimicrobial agents. Finally, this method is a cost-effective, a high-
throughput, and a rapid method for the detection of USA300 CA-MRSA which offers the opportunity to study such a clinically important strain especially in developing countries.

Acknowledgements

The authors reported no conflicts of interest and no funding was received for this work.

References


