Innovare
Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences
Vol 6, Issue 4, 2014 ISSN- 0975-1491

Original Article

DISC DIFFUSION METHOD VERSUS PCR FOR MECA GENE IN DETECTION OF OXACILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN UNIVERSITY CHILDREN'S HOSPITAL IN DAMASCUS, SYRIA

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Received: 04 Mar 2014 Revised and Accepted: 19 Mar 2014

ABSTRACT

Objective: To compare the performance of oxacillin disc agar diffusion(ODD) test with polymerase chain reaction(PCR)-based mecA gene to detect ORSA.

Methods: In the present study, 61 isolates of S.aureus were isolated and were discriminated by the University Children's Hospital laboratory team, from different sources after collecting them from ill children aged from one day to 13 years. Antimicrobial susceptibility testing to oxacillin(OXA) was conducted by disc-diffusion method. Then, mecA and PVL genes were discriminated by PCR.

Results: Our study showed that 21% of isolates were resistant to OXA by ODD method, while 29.5% (18% ORSA and 11.5% OSSA) of isolates possessed mecA gene by PCR-based mecA detection. Two isolates were resistant to OXA based on ODD method, although they were lacked mecA gene. The proportion of PVL-positive isolates among OSSA was high (10%), while among ORSA was 5%.

Conclusion: Use of ODD and PCR-based mecA detection methods together provided useful information on antibiotic resistance and molecular diversity of S. aureus. There is need to reduce the spread and endemic of ORSA and PVL-positive OSSA clones in the hospital.

Keywords: S.aureus, ORSA, PCR, mecA, PVL.

INTRODUCTION

Oxacillin-Resistant Staphylococcus aureus (ORSA) is among the most common causes of hospital-acquired and community-acquired infections worldwide [1-5]. It is a major cause of wide variety of infections ranging from minor infections of the skin and soft tissue (SSTIs) to severe infections such as endocarditis, osteomyelitis and sepsis [6-8]. Although, the resistance of S.aureus to OXA is due to the acquisition of the mecA gene that encodes a new penicillin binding protein(PBP) termed PBP2a or PBP2'[3, 5, 9], non-mecA mechanisms such as increased β-lactamase production or changed in native PBPs can cause low-level OXA resistance[10-12].

Some isolates with mecA are heteroresistant and may be phenotypically susceptible to OXA[10,11], as well as, susceptible strains carrying a non-functional or non-expressed mecA, may be detected.The presence of mecA is generally considered to indicate a potential for resistance and is used as a marker to identify ORSA [13].The most common of CA-ORSA contains the Panton–Valentine Leukocidin (PVL) genes, that absent in HA-ORSA, and they have the ability to lyse leukocytes. CA-MRSA strains that contain PVL genes are frequently associated with SSTIs, as well as with life-threatening necrotizing pneumonia. A large multinational clinical trial conducted outside the United States showed that PVL-positive S.aureus isolates are more likely to be Oxacillin-sensitive S.aureus(OSSA) than ORSA[14-18]. Detection of the mecA gene with PCR is the reference method to determine oxacillin resistance[19]. Antibiotic susceptibility testing of S.aureus in Syria is based on phenotypic testing particularly the disk diffusion method, but recent studies in another settings of the world rely on the PCR to detect the mecA gene for the identification and confirmation of ORSA[20]. Since no information is available on the nature of antibiotic resistance genes of S.aureus in Syria, we conducted this study to provide with the baseline information on antibiotic resistance and molecular epidemiology of OSSA and ORSA in the University Children's Hospital in Damascus,Syria.

Materials and Methods

Bacterial isolates

A total number of 61 S.aureus isolates collected from August 2012 to August 2013, from different clinical specimens(pus, blood, urine, CSF, bronchial secretions….etc.) at the University Children's Hospital in Damascus, Syria were studied. The isolates were collected from ill children aged from one day to 13 years and were discriminated by the University Children's Hospital laboratory team according to conventional bacteriological tests, which included Gram staining, catalase test, and coagulase production… etc.

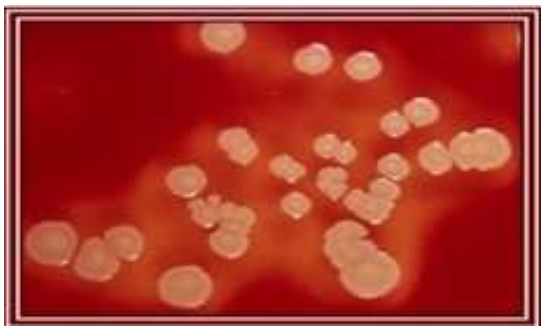

Fig. 1: It shows S.aureus colonies on blood agar

Oxacillin Disk Agar Diffusion (ODD) technique

Antimicrobial susceptibility testing to oxacillin was conducted by disc-diffusion method (Kirby-Bauer method). A sterile swab was dipped in a suspension of S.aureus and was plated onto Muller-Hinton agar(Abtek Biologicals Ltd, UK). Oxacillin disks (10 µg; Abtek Biologicals Ltd, UK), were applied using sterile tweezers.The agar

plates were incubated at 35˚C for 24 hours. Zones of inhibition were measured, which an inhibition zone ≤10 mm indicated oxacillin-resistance, ≥13 mm oxacillin-sensitivity and 11-12 mm intermediate resistance.

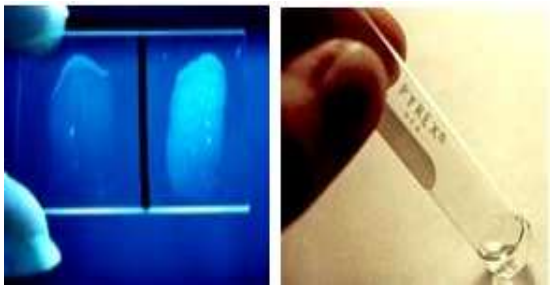

Fig. 2: It shows coagulase test by slide and by tube

Fig. 3: It shows Susceptibility test by Disc Diffusion method

Purification of DNA

DNA was isolated from 1ml of overnight culture using the Wizard® Genomic DNA Purification Kit (Promega, USA), and a mixture of 60µl of 10mg/ml lysozyme and 60µl of 10mg/ml lysostaphin (Sigma, Germany) to achieve bacterial lysis. DNA purification was performed according to the instructions of the manufacturer.The extracted DNA pellet was rehydrated in 100µl of Rehydration Solution for overnight at 4°C.

PCR amplification

PCR amplification was conducted using the GenoType MRSA kit(Hain Lifescience GmbH, Nehren, Germany). In a DNA-free room, 45µl of amplification mix(35µl PNM, 5µl 10x–polymerase incubation buffer, 3µl $MgCl_2$ solution, 0.4µl thermostable DNA polymerase, and 1.6µl water) were putted in a PCR tube. Then, 5µl extracted DNA solution was added to the amplification mix, out of the DNA- free room, to lead to a final volume of 50µl. Finally, the thermal cycler (TECHNE, TC-512, UK) was programmed for:

15 min 95ºC 1 cycle
20 sec 95ºC
30 sec 60 ºC
} 22 cycles

Hybridization

The amplification DNA product was separated using the same kit(Geno Type MRSA kit from Hain Lifescience GmbH, Nehren, Germany).

The hybridization included the following steps: chemical denaturation of the amplification DNA product, hybridization of the single-stranded, biotinlabeled amplicons to membrane-bound probes, stringent washing, addition of a streptavidin/alkaline phosphatase (AP) conjugate, and an AP mediated staining reaction. Finally, the results were read by the naked eye using an evaluation sheet (provided with the kit) that ensures the easy and fast interpretation of the banding pattern that was obtained, which each strip has a total of 6 reaction zones (figure 4).

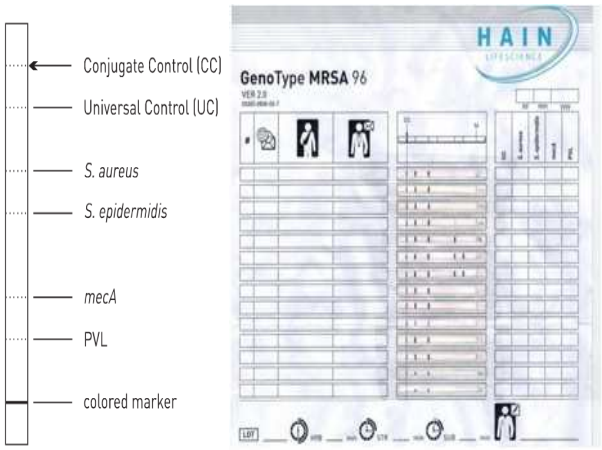

Fig. 4: It shows Strip and evaluation sheet that were used for read and interpretation of the results

Data Analysis

Frequency of the ORSA isolates from clinical specimens was calculated in percentage as total number of ORSA isolates out of total number of S.aureus isolates.

RESULTS AND DISCUSSION

Today ORSA continues to be a significant burden in hospitals but it has also emerged as a problem in the community. An accurate and rapid detection technique is essential to reduce the morbidity and mortality caused by ORSA infections. Several genotypic methods are in use but detection of mecA gene by PCR is currently considered as a gold standard. However, PCR remains beyond the capacity of most developing countries,therefore the results that were obtained by ODD method were assessed and were compared to the results that were obtained by PCR to detect the mecA gene for reliability [6, 21, 22]. In our study, 61 S.aureus isolates collected from different sources were characterized by ODD and PCR-based mecA detection methods.This study showed that 79%(48/61) of isolates were OSSA and 21%(13/61) of isolates were ORSA by using the ODD method (Table 1), while 70.5%(43/61) of isolates were mecA negative and 29.5%(18/61) of isolates were mecA positive using PCR-based mecA detection (Table 2), indicating that the compatibility between these methods in all resistance detected was weak. In fact, several resistance mechanisms mediate oxacillin resistance and phenotypic expression in many strains is heterogeneous, therefore the different between the two methods may be occurred and these strains may be very difficult to distinguish using ODD [10, 11, 22]. In our study, the mechanism of oxacillin resistance among ORSA via mecA was 61%, while a study was conducted in 2004 at two medical school hospitals, Khon Kaen and Bangkok, in Thailand found mecA accounted for 96% ORSA and the other study was conducted in Turkey found mecA accounted 94% ORSA [6, 23].

Table 1: It shows comparison of prevalence rates of OSSA and ORSA in the hospital by Disc Diffusion method

Isolates	OSSA	ORSA	Total
N	48	13	61
%	79	21	100

N= number of isolates, OSSA=Oxacillin-Sensitive S.aureus, ORSA=Oxacillin-Resistant S.aureus.

Table 2: It shows comparison of prevalence rates of OSSA and ORSA in the hospital by PCR-based mecA detection method

Isolates	OSSA	ORSA	Total
N	43	18	61
%	70.5	29.5	100

N=number of isolates, OSSA=Oxacillin-Sensitive S.aureus, ORSA= Oxacillin -Resistant S.aureus.

A total of 11(18%) isolates of S.aureus were resistant to OXA and were confirmed as ORSA based on the detection of mecA gene, while 7(11.5%) isolates were OSSA possessed the mecA gene based on the detection of mecA gene (Table 3).These results indicate that PCR-based mecA detection method may be more accurate, and reliable than ODD method.

The oxacillin disc diffusion test has been found to be less reliable,with high numbers of both false-susceptible and false–resistant results. False classification was reported only for strains which are highly oxacillin-resistant due to mechanisms not based on the mecA gene[13, 22]. Two isolates were classified as ORSA based on ODD method (Table 3), although they lacked mecA gene suggesting another resistance mechanisms including β-lactamase production, modified PBPs or overexpression of existing PBPs.

The frequency of such strains is unknown but is believed to be low and without epidemiological significance.This result agreed with results of study was conducted by Hantash Abed El-Jalil and colleagues in Jordan [22, 24, 25].

Table 3: It shows comparison of ODD and mecA methods in analyzing of 61 S.aureus isolates

Isolates	ODD		mecA			
	N	%	+	%	-	%
OSSA	48	79	7	11.5	41	67.2
ORSA	13	21	11	18	2	3.3
Total	61	100	18	29.5	43	70.5

ODD = Oxacillin Disc agar Diffusion, mecA = resistance gene

Our results showed that the rate of PVL-positive isolates among OSSA was 6(10%),while among ORSA was 3(5%) (Fig. 5).These results agreed with study was conducted in China on 302 isolates of ORSA and 164 isolates of OSSA between January 1994 and December 2008, which showed that 2 isolates of ORSA and 9 isolates of OSSA were PVL positive [26]. PVL is frequently associated with severe and recurrent SSTIs. The high rate of PVL-positive OSSA that was observed in our study indicate that conditions that increase the risk of inter-individual transmission (e.g. skin-to-skin and skin-to-fomite contacts)could represent important routes of spread in the various hospital departments[14, 17, 20, 27].

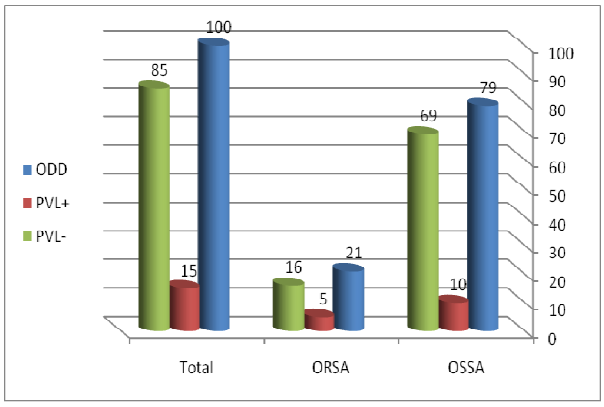

Fig. 5: It shows prevalence of PVL genes in OSSA and ORSA isolates

CONCLUSION

Our study is the first study that provides baseline information on the nature of the antibiotic resistance genes from S.aureus isolates at University Children's Hospital in Damascus, Syria. We found in our study that the PCR technique for mecA gene detection is exact and reliable, but is quite expensive and requires specialized laboratory setting and skilled persons.

Our results confirmed that ODD method for oxacillin resistance in S.aureus strains creates both false-susceptible and false –resistant results, but it can be conducted in any microbiology laboratories and is useful for multiple drug sensitivity testing. Furthermore, the ODD method based on our study was a less accurate,reliable, but it was an inexpensive.Therefore, these problems can be best avoided using a mecA gene - based detection system. The high rate of PVL-positive OSSA that was observed in our study indicate that adequate procedures are needed to reduce the spread and endemic of ORSA and PVL-positive OSSA clones in the hospital. Finally, we recommend that the combination of susceptibility testing to antibiotics and various molecular methods provide useful information on the antibiotic resistance and molecular diversity of S.aureus. Subsequently, monitoring of antibiotic sensitivity patterns, availability of genetic testing to detect antibiotic resistance genes, rational use for antibiotics and preventive measures are required to reduce prevalence of ORSA in the hospital.

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