Full Length Research Paper

Comparison of clinical methods for the phenotypic detection of Methicillin resistant *Staphylococcus aureus*: Disc diffusion methods with Brilliance™ MRSA agar

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This study was based on the comparison of revised guidelines of Clinical and Laboratory Standard Institute (CLSI), British Society for Antimicrobial Chemotherapy (BSAC) and Brilliance™ MRSA agar (Oxoid) for the phenotypic identification of methicillin resistant *Staphylococcus aureus* (MRSA). A total of 133 clinical isolates of *S. aureus* were tested using new CLSI, BSAC guidelines and Brilliance™ MRSA Agar. All the strains were tested according to prescribed guidelines of CLSI (M100–S20, 2010) and BSAC (10.2, 2011). The 30 µg and 10 µg cefoxitin (FOX) performed best for the detection of MRSA and gave 100% sensitivity and specificity. While 1 µg oxacillin (OX) showed less effective results showing sensitivity and specificity of 96.8% and 100% respectively. The cefoxitin disc diffusion method appears to be a good option for the identification of MRSA. Brilliance™ MRSA agar when used for the detection of MRSA, showed sensitivity and specificity of 98.4% and 100% respectively. In conclusion the efficiency of FOX is very high and FOX disc diffusion method is the most efficient method for the identification of MRSA phenotypically and could be a second choice for the detection of MRSA.

Key words: *Staphylococcus aureus*, CLSI, BSAC, Chromogenic media, MRSA.

INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA) is responsible for serious infections worldwide. In both hospital and community settings, MRSA is an increasing threat to immunocompromised and to the normal healthy individuals. The accurate identification of MRSA is the most important step in effective management of the infected patient. This can also help to minimize risk of its transmission (Appelbaum, 2007; Berger-Bachi and Rohrer, 2002).

In MRSA PBP2 protein (PBP2’ or PBP2a) is encoded by mecA gene which is present in MRSA strains. These proteins have low tendency for methicillin and all other β-lactam antibiotics. Factors, such as growth conditions including temperature and osmolarity of the medium, may change the phenotypic identification of methicillin resistance in *S. aureus*. The accuracy of identification methods may be affected by these factors (Chambers, 1997).

Various methods are used for the phenotypic detection of MRSA including disc diffusion method. In disc diffusion
method, CLSI (2007) recommends the utility of cefoxitin instead of oxacillin for the detection of MRSA (CLSI, 2007). Cefoxitin disc is superior to oxacillin for two reasons, firstly the interpretation of zone is easy and secondly, it is more effective for the detection of mecA-mediated resistance (Felten et al., 2002; Mimica et al., 2007; Pottrumathy et al., 2005; Swenson and Tenover, 2007; Velasco et al., 2005; Witte et al., 2007). It is difficult to prove the phenotypic detection of MRSA with disc diffusion method using special media, minimum inhibitory concentration (MIC) determination or agar break point methods. The heterogeneous methicillin resistance expression in many S. aureus strains has been described (Chambers, 1997). The number and heterogeneity level of these strains are increasing. The identification of such strains is a challenge even if special phenotypic methods are used (Brown, 2001). Therefore, for the determination of methicillin resistance in Staphylococci, mecA or PBP2 detection is considered to be a gold standard (Brown, 2001; Dufour et al., 2002; Gosbell et al., 2001; Swenson et al., 2001). However, in Pakistan most of the laboratories do not have the economic and technical capabilities for applying all these tests on the S. aureus isolates recovered in microbiology laboratories. Therefore, oxacillin disc diffusion method is used for the routine screening of MRSA.

Chromogenic media have been used for the last few years for the quick detection of microorganisms from clinical specimens (Felten et al., 2002). These media contain “chromogenic” substrate which is integrated into a solid-agar-based matrix. This substrate detects special enzyme(s) produced by the microorganism(s) which are the identification markers for microbes (Perry and Freydiere, 2007). Therefore, in contrast to other conventional methods, chromogenic media identify the pathogen by direct colony colour from the first culture. This is a time saving method which minimizes further sub-culturing for further biochemical testing until a result is obtained (Kumar et al., 2010).

This study aims to compare the sensitivity and specificity of oxacillin and cefoxitin disc diffusion methods by the revised CLSI (2010), BSAC (2011) guidelines and Brilliance™ MRSA (Oxoid) agar for phenotypic identification of MRSA.

**RESULTS**

A total of 133 clinical isolates of S. aureus were screened by all three methods. All isolates were recovered from the wounds of both in and out door patients of two tertiary hospitals of Peshawar, Pakistan.

By using CLSI guidelines, among 133 isolates, OX 1 µg, 61(45%) were MRSA and 70(52.6%) were MSSA. Two isolates showed false negative results and gave intermediate zone diameters of 11 mm. The specificity and sensitivity of OX 1µg was 100 and 96.8%, respectively. In contrast FOX 30 µg showed 100% sensitivity and 100% specificity respectively (Table 1). All the isolates showed the zone diameter less than 21 mm.

Using BSAC recommendations, 63(47.36%) of the Staphylococci, were MRSA and 70(52.6%) were MSSA. According to this guideline, FOX 10 µg gave 100% specificity and 100% sensitivity. By using the OX 1 µg method, two isolates gave false negative results, showing zone diameter greater than 14 mm thus these two isolates were sensitive. Therefore, the OX 1 µg showed 96.8% sensitivity and 100% specificity (Table 1).

Brilliance™ MRSA agar (Oxoid) was used for the phenotypic identification of MRSA (Figure 1) as described by Kumar et al. (2010). Among 133 isolates, 61(45%) were MRSA and 72(54%) were MSSA. It gave false negative results for one isolate which did not show any growth, while it showed positive results for MRSA for both

**MATERIALS AND METHODS**

Three different methods were compared for the identification of MRSA and MSSA. The S. aureus Oxford Staph ATCC 9144 was used as a negative control and MRSA BIG 0047 (collected at Harrogate District hospital, Harrogate UK, 2007) was used as a positive control. In total, 133 clinical isolates of S. aureus were tested.

All the S. aureus isolates were collected from two tertiary care hospitals of Peshawar, Khyber Teaching Hospital and Lady Reading Hospital. These isolates were tested by using OX 1µg disc on Muller Hinton Agar (MHA) (Oxoid, Basingstoke UK) without additional sodium chloride (NaCl). A suspension of each S. aureus isolate was prepared to a 0.5 McFarland standard. All the strains were inoculated on MHA and were incubated for 24 h at 30°C in air. According to CLSI (M100-S20) guidelines, if the zone diameter was ≤10 mm, the test strain was considered as MRSA. For FOX 30 µg discs were used on MHA and incubated for 16-18 h at 33-35°C. If the zone diameter was ≤ 21 mm, then the test strain was considered as MRSA.

By using BSAC (10.2, 2011) method, all the 133 clinical isolates of S. aureus were tested by using FOX 10 µg on Iso-Sensitest Agar (ISA) (Oxoid, Basingstoke UK) and OX 1 µg on MHA (Oxoid, UK) supplemented with 2% NaCl. All the strains were inoculated on ISA for FOX and on MHA for OX and incubated at 35 and 30°C each for 18 h, respectively. According to BSAC recommendations if the zone diameter was ≤ 21 mm for FOX and ≤ 14 mm for OX, then the test isolate was considered as MRSA.

Brilliance™ MRSA Agar (Oxoid, Basingstoke UK) has been used for the phenotypic identification of MRSA. It is an opaque medium which contains new chromogen. Many staphylococci including S. aureus have phosphatase activity which gives blue color on this chromogenic medium. The growth of MSSA is inhibited by the cocktail of antibiotics present in this medium. It also contains many compounds that inhibit the phosphatase activity in other Staphylococci, thus increasing the level of sensitivity and specificity. Brilliance™ MRSA agar is supplied as pre-poured culture plates. It is used for the identification of MRSA in human specimens.

All the 133 clinical isolates of S. aureus were tested on this medium. Before inoculation, the medium was allowed to warm up to room temperature and then dried up in incubator at 37°C. The agar screening plates were inoculated directly by picking isolated colonies of test isolate and incubated for 18-24 h at 37°C. The denil blue color colonies were reported positive for MRSA.
new breakpoints for the FOX 30 µg disc test for identification of mecA in S. aureus have been validated by CLSI (Nicole et al., 2009). Their results showed that FOX disc diffusion test is the best technique and a better alternate for the OX disc diffusion test due to its easy interpretation and high accuracy. Our study findings are similar to their findings. In the present study, FOX 10 µg and 30 µg showed that they are the best for phenotypic detection of MRSA. We have also found false negative results for detection of MRSA by using OX 1 µg according to both CLSI (M100-S20) and BSAC guidelines. Skov et al. (2003) also found that for identification of MRSA, FOX disc diffusion test was usually good, with sensitivity and specificity of 100% and 99%, respectively; which is similar to this study. They used FOX 30 µg on ISA (Skov et al., 2003). In another study, Skov et al. (2005) found that using semi-confluent growth on ISA and MHA along with standard incubations conditions, performance of FOX 5 and 10 µg discs was excellent for the identification of MRSA (Skov et al., 2005). In the present study the sensitivity and specificity were both 100% for FOX 10 µg, which is similar to the findings of Skov et al. (2005).

Several chromogenic media and other deferential MRSA selective agars have been used to identify MRSA within 18 to 24 h (Flayhart et al., 2005; Nahimana et al., 2006; Stoakes et al., 2006; Diederen et al., 2005; Nguyen Van et al., 2006). In this study we used Brilliance™ MRSA agar (Oxoid) for the detection of MRSA. The sensitivity and specificity were 98.4% and 100%, respectively. In a study by Kumar et al. (2010) the sensitivity for Brilliance™ MRSA (Oxoid) was 97%, which is similar to this study, and the specificity was 86% which was different from this study. This change in the specificity may be due to the difference in the collection of samples, clones circulating in different parts of the world and prevalence of MRSA (Van Loo et al., 2007). Another study modelled the effect of direct identification of MRSA carriage using a real-time PCR or chromogenic media on capture of patient isolation days (PIDs). The analysis showed that real time PCR captured 22.7% more PIDs and these more undesired PIDs were because of slightly low specificity as compared to chromogenic medium (Robicsek et al., 2008). Similar performance of a chromogenic medium and RT-PCR has also been reported in

**DISCUSSION**

It is important to identify MRSA accurately because it will help in proper management of S. aureus born diseases. Many methods for the identification of MRSA have been developed but most of them are costly, time consuming and not useful enough for the correct detection of MRSA. (Kumar et al., 2010; Tiwari et al 2009).

The most reliable method for detecting MRSA isolates is the detection of mecA, however in routine clinical practice; most of the laboratories do not use molecular techniques for the detection of MRSA. Therefore, those phenotypic techniques are very important, which are capable to identify MRSA strains quickly and accurately. This will also ensure the appropriate antimicrobial therapy at appropriate time and prevent the spreading of such nasty MRSA strains in both health care and community settings. S. aureus with a mecA mediated resistance can be detected by cefoxitin (Swenson et al., 2007). In 2009,FOX (10 and 30 µg) discs. The sensitivity and specificity of Brilliance™ MRSA agar were 98% and 100% respectively (Table 1).
another study (Bischof et al., 2009). However, yet another study has reported that molecular techniques give quick results with high sensitivity (Paule et al., 2009).

Brilliance™ MRSA agar (Oxoid) is also a good, cost effective medium for the detection of MRSA because cost of screening on conventional culture medium is similar to the cost of chromogenic medium. When downstream processing time and cost of the technician is considered, variations range from 1.4 - 1.7 days, excluding time required for confirmatory test(s) (Nahimana et al., 2006).

On the basis of advantages of chromogenic media, it should be encouraged in the laboratories for rapid screening of MRSA (Kumar et al., 2010). Similarly, in a study conducted by Verkade et al. (2009) Brilliance™ MRSA (Oxoid) has been found to be the best for the identification of MRSA. They used 788 isolates of staphylococci and after 20 h of incubation, the sensitivity and specificity were 99.6% and 97.3% respectively. For the screening of MRSA in less time, this new medium is highly sensitive. Our results are similar to those reported by Vekade et al. (2009). By using Brilliance™ MRSA (Oxoid), results can be obtained within one day. Further studies should be performed to find out the usefulness of Brilliance™ MRSA (Oxoid) for the screening of MRSA directly from clinical specimens (Verkade et al., 2009).

In conclusion, FOX 10 µg and 30 µg were the best for the phenotypic detection of MRSA because their sensitivity and specificity were better than oxacillin. In most of the Pakistani labs oxacillin disc are still used for the detection of MRSA. It is therefore recommended that oxacillin discs should be replaced by the FOX discs test method because of its effectiveness. Brilliance™ MRSA agar is also effective and could be used as a second option for the direct inoculation of specimens in the laboratories.

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