SHORT ARTICLE

Cefoxitin Mannitol Salt Agar for Selective Isolation of Methicilin-Resistant Staphylococcus aureus

Mohamed O. Ahmed[1], Asma K Elramalli[2], Samira G. Amri[3], Yousef M Abouzeed[1]

[1]Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya
[2]Tripoli Medical Centre, Tripoli, Libya.
[3]Burns and Plastic Surgery Centre, Tripoli, Libya.

Corresponding author: Dr Mohamed O. Ahmed Email: a.mo@live.com
Published: 07 January 2014
Ibnosina J Med BS 2014;6(1):31-33
Received: 09 September 2013
Accepted: 21 September 2013
This article is available from: http://www.ijmbs.org

Abstract
Methicillin-resistant Staphylococcus aureus (MRSA) is a major nosocomial infection that causes problems in screening testing. Cefoxitin is potent selective antibiotic agent widely recommended for the detection of MRSA. The aim is to evaluate the optimum concentration of cefoxitin incorporated into mannitol salt agar (MSA) needed to select and detect MRSA. A total of 99 MRSA isolates were tested against 4 different concentrations of cefoxitin mannitol salt agar (MSC) of: 2 µg/ml (MSC-2), 4 µg/ml (MSC-4), 6 µg/ml (MSC-6) and 8 µg/ml (MSC-8). The susceptibility of MSCs were respectively as follows: 53.5%, 53.5%, 74% and 78%. Accurate and effective laboratory screening of MRSA is an important measure control of MRSA.

Keywords: MRSA; Libya; Cefoxitin; Mannitol salt agar

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has, since the 1960s, been the most common cause of nosocomial infections and a major cause of morbidity and mortality (1). Although the Clinical Laboratory Standards Institute (CLSI) and the British Society for Antimicrobial Chemotherapy (BSAC) have published standard isolation methods for the isolation and characterization of MRSA (2), there is currently no ultimate method for screening and isolation of MRSA using solid media (2,3). As an identification method, PCR is the 'gold standard'; however, many available media have been introduced and recommended as alternative tests for accurate identification and isolation. The majority of such selective media depend on antibiotics and inhibitors to aid presumptive selection of MRSA with improved specificity and selectivity (2-6). Therefore, this experiment aims to evaluate the selectivity and the optimal concentration of cefoxitin necessary for the isolation of MRSA from Libyan sources.

Materials and methods

This work is part of a research project supported in part by the National Authority of Scientific Research (NASR), Tripoli. Ninety-nine confirmed MRSA isolates collected from different local Libyan hospitals were used in this experiment. The selected isolates used were in-house confirmed isolates previously collected from different hospitals in Tripoli, as described by Ahmed et al. (7). Mannitol salt agar (MSA, Oxoid-UK) supplemented with cefoxitin (Fox, Sigma-Aldrich) with concentrations of 2, 4, 6 and 8 µg/ml were prepared according to a modified protocol as described by Smyth and Kahlmeter (8) as follows: 900 ml of MSA media were prepared and cooled to 50°C. The cefoxitin stock solutions were prepared by dissolving quantities of 2, 4, 6 and 8 mg of cefoxitin powder respectively in 100 ml sterile water. Cefoxitin stock of each concentration was then sterilized through Whatman No. 1 filter paper; each was added to cooled MSA media, mixed gently and poured into dishes at room temperature.

Cefoxitin mannitol salt media (MSC) were stored for the next step of the experiment. The MRSA isolated strains (n=99) were grown on blood agar (Oxoid) at 30°C for 24h. From each isolate, few colonies were then streaked onto each of the MSC plates of 2, 4, 6, 8 µg/ml and incubated at 35°C. The media were first checked after 24h and results were recorded at 48h. The interpretation of results depended on the presence of any pure optimum growth of typical colonies. Plates that showed very weak growth or growth not appropriate for this experiment were excluded. Methicillin susceptible *Staphylococcus aureus* of 4µg/ml, identified previously in our laboratory were used as positive controls in this study.

Results

Fifty three isolates grew successfully on both 2 & 4µg/ml MSC. The 8 µg/ml MSC was shown to be the most effective and 77 isolates grew on this medium. Results are summarized in Table1.

Discussion

Many reports have evaluated different media that use antibiotics as selective agents. Selective media using oxacillin and methicillin are widely used, however the use of cefoxitin in such media has recently shown great reliability, superior identification of MRSA and economic value compared to others including chromogenic media (2,5,8). In our experiment the 8µg/ml MSC appears to be the optimum needed concentration for the identification of MRSA. This correlated with a previous study conducted by Willey et al 2005. The rise of concentration needed to be incorporated in the MSA media ought to be discussed. This could be related to the increased virulence of MRSA. The genetic elasticity of MRSA might have contributed in the current results (9). Other factors, such as the addition of NaCl and growing cultures at 30°C, have been indicated to be better inducers of the *mecA* genes and allow better identification of MRSA.

Cefoxitin also has been largely observed to have a strong inducing effect of PBP2a and improves the identification of positive *mecA* gene *Staphylococcus aureus* (10). Accurate and quick laboratory diagnosis, susceptibility testing and infection control measures are critical in treating, managing and therefore preventing MRSA infections (11,12). This is important for the accuracy of isolation of MRSA in local Libyan laboratories and great caution must be considered when screening and reporting MRSA or related virulent resistant phenotypes, e.g. VRSA. Reports and research have identified such misidentification and screening concerns in Libya (13-15). Proper efficient screening programs and transparent prevention control schemes are extremely important in the screening of MRSA and thus limiting the complication of its virulence and complications.

Table1. Proportion of MRSA growth against cefoxitin-mannitol salt agar

MRSA	Bacterial growth on MSC			
	2 µg/ml	4 µg/ml	6 µg/ml	8 µg/ml
99	53 (54%)	53 (54%)	73 (74%)	77 (78%)

References

1. Appelbaum PC. MRSA–the tip of the iceberg. Clin Microbiol Infect 2006;2:3-10.
2. Brown DF, Edwards DI, Hawkey PM, Morrison D, Ridgway GL, Towner KJ et al. Infection Control Nurses Association. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA). J Antimicrob Chemother 2005; 56: 1000-18.
3. Bou G. Minimum inhibitory concentration (MIC) analysis and susceptibility testing of MRSA. Methods Mol Biol 2007;391:29-49.
4. Smyth RW, Kahlmeter G, Olsson Liljequist B, Hoffman B. Methods for identifying methicillin resistance in *Staphylococcus aureus*. J Hosp Infect 2001; 48:103-7.
5. Louie L, Soares D, Meaney H, Vearncombe M, Simor AE. Evaluation of a new chromogenic medium, MRSA Select, for detection of methicillin-resistant Staphylococcus aureus. J Clin Microbiol 2006;44:4561-3.
6. Willey BM, Mazzulli T, McGeer A, Poutanen S, Skulnick M. Evaluation of oxoid mannitol salt agar with cefoxitin for the detection of methicillin-resistant Staphylococcus aureus (MRSA) from surveillance specimens. American J Infec Cont 2005;33:184-5.
7. Ahmed MO, Elramalli AK, Amri SG, Abuzweda AR, Abouzeed YM. Isolation and screening of methicillin-resistant Staphylococcus aureus from health care workers in Libyan hospitals. East Mediterr Health J 2012;8:37-42.
8. Smyth RW and Kahlmeter G. Mannitol salt agar-cefoxitin combination as a screening medium for methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 2005; 43: 3797-9.
9. Holden MT, Feil EJ, Lindsay JA, Peacock SJ, Day NP, Enright MC, et al. Complete genomes of two clinical *Staphylococcus aureus* strains: evidence for the rapid evolution of virulence and drug resistance. Proc Natl Acad Sci 2004;101:9786-91.
10. Anand KB, Agrawal P, Kumar S, Kapila K. Comparison of cefoxitin disc diffusion test, oxacillin screen agar and PCR for *mec-A* gene for detection of MRSA. Indian J Med Microbiol 2009;27:27–9.
11. Marchese A, Gualco L, Maioli E, Debbia E. Molecular analysis and susceptibility patterns of meticillin-resistant Staphylococcus aureus (MRSA) strains circulating in the community in the Ligurian area, a northern region of Italy: emergence of USA300 and EMRSA-15 clones. Int J Antimicrob Agents 2009;34:424-8.
12. Barrett SP, Teare EL, Sage R Methicillin resistant Staphylococcus aureus in three adjacent health districts of south-east England 1986-91. J Hosp Infect 1993;24:313-25.
13. Ghenghesh K, Rahouma A, Tawil K, Zorgani A, Franka E. Antimicrobial resistance in Libya:1970-2011. Libyan J Med 2013;8:20567.
14. Zorgani A and Ziglam H. Injured Libyan combatant patients: both vectors and victims of multiresistance bacteria?. Libyan J Med 2013;8:20325.
15. Ahmed MO, Abuzweda A, Alghazali MH, Elramalli AK, Amri SG, Aghila E. Misidentification of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals in Tripoli, Libya. Libyan J Med 2010;5:5230.