SHORT ARTICLE

Evaluation of a new selective medium for methicillin-resistant *Staphylococcus aureus*

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The aims of this study were to evaluate the performance of a new medium, desferrioxamine oxacillin tellurite egg-yolk mannitol salt agar (DOTEMSA) in detecting methicillin-resistant *Staphylococcus aureus* (MRSA) and then to compare this medium against the Public Health Laboratory Service (PHLS) recommendation of mannitol salt agar (Oxoid) with oxacillin (OMSA) and Baird-Parker medium with ciprofloxacin (BPC) for the isolation of MRSA. The individual selective agents contained in DOTEMSA were tested against isolates of coagulase-negative staphylococci (CNS) and the medium with all constituents was challenged with various bacteria. Routine screening specimens were plated out on OMSA, BPC and DOTEMSA and the plates were incubated and examined at 24 and 48 h. Tellurite, desferrioxamine and oxacillin each inhibited the majority of CNS isolates; only three (of 103) grew in the presence of all three agents. Sixty-two of 63 isolates of MRSA grew on DOTEMSA and 59 produced lipase. Most other bacteria were inhibited. In all, 184 MRSA isolates were isolated from 540 screening specimens. The sensitivity of OMSA, BPC and DOTEMSA was 42%, 81% and 51% at 24 h, and 60%, 89% and 89% at 48 h. At 48 h, the combination of BPC and DOTEMSA detected 99% of MRSA isolates. Seventy, 49 and one non-MRSA isolates needed investigation for each of the three media respectively. A proposed strategy for MRSA screening would use BPC and DOTEMSA, examining BPC at 24 h and both media at 48 h. Provisional reports could then be issued at 24 h on the basis of rapid agglutination tests to confirm isolates as *S. aureus* from BPC and at 48 h on the basis of typical colonies from DOTEMSA.

Introduction

Serious infections caused by *Staphylococcus aureus* resistant to β -lactamase stable β -lactams (methicillin-resistant *S. aureus*, MRSA) are now common in England and Wales [1] and are associated with significant morbidity and cost [2]. Current control strategies rely in part on the detection of infection and colonisation, and the isolation of affected patients [2]. The sensitivity and speed of detection of MRSA by culture have been considered recently [3] and the Public Health Laboratory Service (PHLS) has issued a standard operating procedure recommending the use of mannitol salt agar (MSA) with oxacillin (OMSA) [4] together with Baird-Parker medium with ciprofloxacin (BPC) [3] as an additional medium where quinolone-resistant strains are prevalent.

Although BPC performs well it will not detect those strains, a minority at present, which are susceptible to ciprofloxacin. We have formulated a new selective medium for MRSA, desferrioxamine oxacillin egg-yolk tellurite mannitol salt agar (DOTEMSA), which relies on egg-yolk to detect lipase and with potassium tellurite [5] and desferrioxamine [6] to suppress coagulase-negative staphylococci (CNS), which was partly developed from previous formulations [4, 5, 7–9]. This study was undertaken to examine the effects of these selective agents against CNS, to assess the growth of other common bacteria on DOTEMSA and to compare this medium against the standard combination of OMSA and BPC.

Materials and methods

Effect of selective agents on CNS

Potassium tellurite (Sigma) 30 mg/L, desferrioxamine mesylate (DesferalTM, CIBA) 100 mg/L and oxacillin (SV29, Mast Selectavial) 2 mg/L were added sepa-

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rately to MSA (CM85, Oxoid). A total of 105 isolates of CNS was selected randomly from routine clinical isolates. Isolates were inoculated into glucose broth (CM67, Oxoid Nutrient Broth No. 2 with additional glucose 1%), incubated for 6 h at 37°C and then applied to the medium with a 36-pin multipoint inoculator. The plates were examined for growth after 42 h at 37°C in air. The inoculum was quantified, with four isolates of CNS, by performing serial 10-fold dilutions of the 6-h broth cultures in glucose broth and applying these dilutions to blood agar with the multipoint inoculator. Colonies were counted after overnight incubation at 37°C in air.

Growth of bacteria on DOTEMSA

Isolates, other than MRSA, were selected randomly from routine clinical isolates: 63 isolates of MRSA, 95 methicillin-sensitive *S. aureus*, 156 CNS, 26 enterococci, 55 streptococci and 68 *Escherichia coli* or other Enterobacteriaceae were tested. Of the MRSA, 22 were sequential clinical isolates and 41 came from this laboratory's collection of past isolates and were selected to represent a wide range of phage types and antibiograms. DOTEMSA was prepared with Oxoid MSA with oxacillin 2 mg/L, desferrioxamine mesylate 100 mg/L, potassium tellurite 30 mg/L and egg-yolk 5% suspension (50% v/v in sterile saline). Isolates were inoculated into glucose broth which was incubated for 6 h at 37°C and then applied to DOTEMSA with a 36-pin multipoint inoculator. The plates were examined after 18 h and after 42 h at 37°C in air.

Study of clinical specimens

Sequential specimens submitted for detection of MRSA during 13 working days between Nov. 1999 and Feb. 2000 were examined. Cotton-tipped swabs were received in Amies transport medium and were broken off into 1-ml volumes of sterile saline and vortex mixed. The media were then inoculated from this with a new swab, which was recharged between plates. After every 100 specimens the order of inoculation was rotated.

OMSA was prepared from Oxoid MSA medium with oxacillin 2 mg/L, BPC from Baird-Parker medium (CM275, Oxoid) with ciprofloxacin (CiproxinTM infusion, Bayer) 8 mg/L and DOTEMSA was prepared as described above. The plates were incubated at 37°C in air and were examined at 24 and 48 h.

On DOTEMSA colonies of MRSA are black and are typically surrounded by an opaque zone due to lipase production. At 48 h colonies are *c.* 1 mm in diameter with an opaque halo of *c.* 3 mm diameter.

Suspect colonies were subcultured on to blood agar which was incubated overnight at 37°C. Colonies that were found positive by PROLEXTM Latex Agglutina-

tion System Staph aureus (PL.081, Prolabs) were confirmed as MRSA by inoculating on to DNAase agar (CM321, Oxoid), DST agar (CM261, Oxoid) with oxacillin 2 mg/L and salt 2% (incubated at 37°C) and DST agar with methicillin 4 mg/L and no additional salt (30°C). Discrepancies were resolved by the tube coagulase test with rabbit plasma. Oxacillin/methicillin plates were read at 24 h.

Strains were classified as EMRSA 15 or EMRSA 16 by the resistance pattern to erythromycin, gentamicin, ciprofloxacin and tobramycin or by phage typing at the Staphylococcal Reference Unit, PHLS Central Public Health Laboratory, Colindale Avenue, London.

Analysis of comparison of isolation rates was done by the two paired proportion method [10] in which the numbers of specimens positive by one test only were compared with the numbers positive by the other test only.

Results

Table 1 shows the effect of desferrioxamine, tellurite and oxacillin on 105 isolates of CNS. The geometric mean of the inoculum derived from testing four isolates of CNS was 3.8×10^4 cfu/spot. Only three isolates were able to grow in the presence of all three agents and none of these was lipase positive.

The number of different bacterial isolates able to grow on DOTEMSA is shown in Table 2. All isolates of methicillin-susceptible *S. aureus*, streptococci and Enterobacteriaceae were inhibited, as were 149 of 156 CNS isolates (seven that grew were lipase negative) and 12 of 26 enterococci (14 grew poorly and were lipase negative). Sixty-two of 63 MRSA isolates grew on the medium. Three of the MRSA isolates were

Table 1. Ability of 105 isolates of CNS to grow on mannitol salt agar with and without selective agents

Medium	Growth at 42 h (%)
Mannitol salt agar (MSA)	103 (98)
MSA + desferrioxamine	35 (33)
MSA + tellurite	7 (7)
MSA + oxacillin	33 (31)

Table 2. Growth of different bacteria on DOTEMSA

Bacteria	Number tested	Growth on DOTEMSA (%)
<i>S. aureus</i> (methicillin-resistant)	63	62 (99)*
<i>S. aureus</i> (methicillin-sensitive)	95	0 (0)
CNS	156	7 (4)
Enterococci	26	14 (54) [†]
Streptococci	55	0 (0)
Enterobacteriaceae	68	0 (0)

*Sixty-one isolates grew well; 59 were lipase positive.

[†]Poor growth and lipase negative.

lipase negative, but neither these nor the strain that failed to grow were UK epidemic strains.

Of the 540 screening specimens 246 were nose swabs, 151 from groin or perineum and 80 from the axilla. MRSA was isolated from 184 swabs by at least one method. These isolates were from 83 patients. Seventy-four patients carried EMRSA 15, two carried EMRSA 16 and seven had other strains or were not categorised. Two isolates only were susceptible to ciprofloxacin. Table 3 shows the performance of the three media. The lower half of the table shows the number of isolates that would have been detected if combinations of media were used. The differences between the sensitivity of BPC *versus* OMSA and DOTEMSA *versus* OMSA at 48 h were both significant ($p < 0.00001$). BPC was clearly the most sensitive medium at 24 h, but BPC and DOTEMSA performed equally well at 48 h and significantly better than OMSA. Furthermore, DOTEMSA was very specific; 70, 49 and one non-MRSA isolates required investigation from OMSA, BPC and DOTEMSA, respectively, at 48 h.

Discussion

DOTEMSA largely owes its selective effect to desferrioxamine and tellurite. Desferrioxamine inhibits *S. epidermidis* and *S. hominis*, which together make up the major component of skin staphylococci, by the chelation of soluble iron [6]. *S. aureus* and other species of staphylococci, which are not inhibited, presumably have different ways of assimilating iron. The differential effect of tellurite was first described by Ludlam [5] and has been used, along with egg-yolk as an indicator, in the formulation of Baird-Parker medium [11]. The mechanism of resistance and susceptibility of staphylococci to tellurite is poorly understood. In initial studies the potassium tellurite concentration was found to be critical, with 40 mg/L too inhibitory to MRSA and 20 mg/L not inhibitory enough to CNS. Both these agents and oxacillin were found to inhibit most CNS. This was confirmed by the small number of strains growing on DOTEMSA and is likely to contribute to the sensitivity of the medium for detecting MRSA by inhibiting the competing flora.

Table 3. Comparison of the three media, alone and in combination, to detect 184 MRSA isolates from screening specimens

Media	Number of MRSA detected (%) at	
	24 h	48 h
OMSA	77 (42)	110 (60)
BPC	149 (81)	164 (89)
DOTEMSA	93 (51)	163 (89)
OMSA and BPC	152 (83)	168 (91)
DOTEMSA and BPC	155 (84)	182 (99)
DOTEMSA and OMSA	104 (57)	165 (90)

Tellurite is inhibitory to more strains of CNS than is desferrioxamine and it may be worthwhile to repeat the study comparing this medium with and without desferrioxamine.

Mannitol salt agar with oxacillin (OMSA) did not perform as well as a methicillin-containing medium in a previous study [3] and it may be that methicillin is a better selective agent than oxacillin. However, methicillin will soon be unavailable. Other problems are that mannitol fermentation is not specific for *S. aureus*, as many coagulase-negative strains ferment mannitol, and also the medium is not inhibitory enough to CNS. Egg-yolk can be used additionally as an indicator of lipase [7] and is used in some centres [12].

DOTEMSA is very sensitive for detecting MRSA and although detection may be delayed until 48 h, its high specificity would allow provisional reporting of MRSA at that time. It is also clearly the more sensitive of the two oxacillin-containing media and would be useful for detecting ciprofloxacin-susceptible MRSA strains (2% in Sheffield at present). These are not detected by BCP, which incorporates ciprofloxacin as a selective agent. DOTEMSA is simple to prepare and the additional additives (over MSA) increase the cost by *c.* 1.5 p/agar plate.

BPC has again proved its value, especially for early detection. Provisional reports can be issued at 24 h based on rapid latex agglutination for *S. aureus* [3], taking into account the continued low rate of ciprofloxacin resistance in methicillin-susceptible *S. aureus* (around 4% in Sheffield in 1999). A useful combined strategy for speed and sensitivity would be to use both BPC and DOTEMSA, examine the BPC at 24 h to issue a preliminary report and then examine both media at 48 h. Because of the specificity of DOTEMSA this would require very little increased work over the examination of BPC alone.

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