

## SHORT REPORT

## Clinical validation for oxacillin susceptibility testing of coagulase negative staphylococci

N Cimolai, J E Carter

Arch Dis Child 2002;86:446-448

Oxacillin susceptibility of coagulase negative staphylococci as assessed by conventional methods was confirmed by a modified Etest method, extended to detect heteroresistance. Verification of susceptibility was followed by successful treatment for six consecutive children with deep seated infections. Physicians' trust in such a validated method will contribute to the appropriate use of antibiotics.

Coagulase negative staphylococci (CNS) are an uncommon cause of deep seated infection in children; most such infections occur among children in hospital, those with complicated medical disorders, or those with prosthetic devices.<sup>1</sup> The treatment of invasive CNS infections is complicated as these bacteria are often resistant to  $\beta$  lactamase resistant penicillins. Hence, vancomycin is usually administered before susceptibility testing is available when such infections are suspected, and prosthetic devices are often removed or revised in order to secure a good outcome.<sup>2,3</sup> In addition, it has been recognised that patients who suffer from infections with seemingly susceptible CNS, as assessed by conventional in vitro susceptibility testing methods, may not have good bacteriological or clinical outcomes as such methods do not reliably detect in vivo resistance in the bacterial population.<sup>4</sup>

Although staphylococci may produce  $\beta$  lactamase enzyme which degrades penicillin, it is not active against antistaphylococcal semisynthetic  $\beta$  lactam agents. The mechanisms of resistance to the latter antibiotics involve alterations to penicillin binding proteins, which renders the antibiotic unable to interfere with cell wall synthesis. In a population of resistant bacteria, a variable proportion may express such resistance, and hence are "heteroresistant", posing problems for resistance determination.

In order to encourage appropriate use of  $\beta$  lactam antibiotics for CNS infections, several approaches have been proposed to improve in vitro susceptibility testing performance.<sup>5-9</sup> These techniques aim to show heteroresistance by phenotyping or finding resistance genes by molecular techniques. In this report, we illustrate the application of one such technique to the successful treatment of six patients with invasive CNS infections.<sup>7</sup>

## METHODS AND RESULTS

Bacterial isolates were obtained from a variety of sources (table 1). Each infection was of an invasive nature which would commonly merit prolonged antibiotic treatment. Bacterial isolates, identified initially only as CNS, were examined for purity before being subjected to susceptibility testing. Subsequent speciation with APISstaph (bioMerieux, France) revealed that all were *Staphylococcus epidermidis* except for one that was *Staphylococcus lugdunensis* (patient 6). Initial assessment included critical agar dilution testing with oxacillin (6

mg/l oxacillin in Mueller-Hinton agar with 2% NaCl supplementation and 24 hours incubation). This assessment was undertaken with standard methods and control organisms.<sup>10</sup> All the isolates were susceptible in this assay. For confirmatory testing, the isolates were assessed by Etest (on Mueller-Hinton agar with 2% added NaCl) according to the manufacturer's instructions (AB BIODISK); minimum inhibitory concentrations (MICs) were determined according to standard criteria as recommended by the manufacturer. Oxacillin MICs ranged from 0.2 to 2.0 mg/l. In order to determine the presence of oxacillin heteroresistance, the zone of bacterial inhibition from Etest was examined carefully at 24 and 48 hours. Any suspect colony was transferred to confirm bacterial growth and then reassessed by Etest.<sup>7</sup> Resistant CNS colonies are scant and usually pin-point; the repeat Etest thereafter can identify whether the isolate has an MIC greater than 6 mg/l. No such colonies were found after Etest screening of the isolates from these patients, thus reconfirming the susceptibility as initially determined by critical agar dilution.

Table 1 details some characteristics of these six patients and their infections. All patients had serious and invasive infections, and all patients had preceding or existing illnesses which either complicated the treatment course or put them at risk of CNS infection. Given the clinical presentations, these patients all received an initial course of antibiotics, usually as an empirical regimen for presumed infection. Each went on to complete long term treatment with cloxacillin. Patient 1 continued to yield positive cultures for CNS from the external ventricular drain until intravenous cloxacillin was initiated. Patient 3 was treated with intravenous and subsequently oral cloxacillin; oral use was followed by demonstration of peak serum bactericidal activity of 1/256. Patient 4 continued to have positive blood cultures throughout the time that intravenous vancomycin was being administered despite adequate serum peak and trough levels (34-35 mg/l and 10-17 mg/l respectively). Blood cultures became negative only after intravenous cloxacillin was administered. Patient 5 also had consistently positive blood cultures despite receiving intravenous vancomycin, but therapy was complicated by the intermittent dosing required for intermittent haemodialysis and dose adjustment during treatment of active and advanced renal failure. A specific lumen of a multilumen vascular access device was thought to be colonised by and acting as the source of CNS infection. Blood cultures became negative within two days of commencing intravenous cloxacillin treatment despite leaving the central venous catheter in situ throughout the treatment period.

## DISCUSSION

The mechanisms of oxacillin resistance in CNS are similar to those in methicillin resistant *Staphylococcus aureus* (MRSA)<sup>11,12</sup>

**Abbreviations:** CNS, coagulase negative staphylococci; MIC, minimal inhibitory concentration; MRSA, methicillin resistant *S aureus*

**Table 1** Characteristics of patients and salient feature of their infections

Patient	Age	Gender	Isolate source	Infection	Cofactors	Initial therapy	Completion therapy	Outcome
1	4 y	M	CSF	CSF and extraventricular drain	Previous cranial surgery; recent intraventricular bleed	IV cefotaxime and IV vancomycin for 2 days	IV cloxacillin for 6 days; extraventricular drain removed	Well
2	18 y	F	Renal abscess	Post-surgical abscess (renal transplant)	Renal transplant; immunosuppression; SLE; abscess also yielded viridans streptococci	IV ampicillin and IV ceftazolin for 2-3 days	IV cloxacillin for 8 days; oral cephalixin for 2 weeks; external drain left in place until drainage subsided	Well; no further drainage
3	10 wk	M	Blood and mitral valve	Endocarditis	Congenital heart and other multiple physical anomalies	IV cefotaxime for 2 days and IV vancomycin for 10 days	IV cloxacillin and gentamicin for 10 days; oral cloxacillin for 3 weeks	Well
4	18 y	F	Blood	? endovascular infection but unknown focus	Spastic quadriplegia	IV vancomycin for 7 days	IV cloxacillin for 4 weeks	Well
5	12 y	M	Blood	Haemodialysis line	Chronic renal failure; Henoch-Schönlein purpura	2 doses IV vancomycin	IV cloxacillin for 10 days	Well
6	6 mth	M	CSF and ventriculo-peritoneal shunt	CSF	Central nervous system shunt; previous arachnoid cyst	IV vancomycin for 2 days; IV cefotaxime for 3 days	IV cloxacillin for 11 days; shunt revised near completion of cloxacillin therapy	Well

CSF, cerebrospinal fluid; IV, intravenous; SLE, systemic lupus erythematosus.

and involve alteration in penicillin binding protein structure. Most resistant CNS have a resistance gene (*mecA*) which is analogous to the most common resistance gene among MRSA.<sup>13</sup> When either CNS or MRSA are isolated, the bacteria may be heteroresistant, and the phenotypic expression of such resistance may be variable. Various improvements have been made in laboratory methods for detecting such heteroresistance, especially among MRSA.<sup>14</sup>

The frequency of oxacillin resistance in CNS is high (up to 60–80% or more), especially among CNS, causing hospital infections.<sup>7</sup> The empirical use of vancomycin for suspected CNS infections is commonplace, and most physicians are hesitant to use  $\beta$  lactam agents for such infections because of the treatment failures that occur when  $\beta$  lactam agents are used, even when conventional susceptibility testing indicates that the bacteria are susceptible. Many CNS infections may be complicated by factors which promote the infection and its persistence, such as underlying illnesses and implanted foreign medical devices. Many CNS infections are treated with short term antibiotics and removal of any medical device or vascular access line. Vancomycin may be appropriate for such therapy. CNS infection that is susceptible to  $\beta$  lactam agents and which requires lengthy treatment is relatively uncommon. Vancomycin is expensive, relatively more toxic, and requires drug level monitoring. It should be used as little as possible to discourage emergence of resistance.  $\beta$  Lactam therapy appeared to be more efficacious in several of these cases, and oral therapy may be used in some circumstances, as illustrated by patient 3, allowing for more ambulatory care.

Although recognised as a dilemma for many years, the accurate determination of oxacillin resistance in CNS has received special interest in the past decade. US authorities have modified guidelines for susceptibility testing of CNS, decreasing the MIC breakpoint for definition of resistance considerably.<sup>15</sup> This strategy will accurately define susceptible bacteria, but may exclude some CNS that are actually susceptible.<sup>15</sup> Similar to MRSA, multiple resistance to other antibiotics is a marker for CNS that are likely to be genuinely oxacillin resistant, but this association is not consistent.<sup>16</sup> Thus, a reliable direct determination of oxacillin resistance is still desirable.

Given that the major dilemma with oxacillin resistance determination among CNS is the phenomenon of heteroresistance, we have used a laboratory method which will determine heteroresistance with a high degree of accuracy. The number of patients in our study is small, and we believe that a larger study is warranted to confirm these findings. The application of a reliable technique which is associated with a reasonable frequency of cure would allow physicians and medical microbiologists to be more confident treating chronic CNS infections.

#### Authors' affiliations

**N Cimolai**, Program of Microbiology, Virology, and Infection Control, Department of Pathology and Laboratory Medicine, Children's and Women's Health Centre of British Columbia, Canada

**J E Carter**, Division of Paediatric Nephrology, Department of Paediatrics, Children's and Women's Health Centre of British Columbia, Canada

#### REFERENCES

- Huebner J, Goldmann DA. Coagulase-negative staphylococci: role as pathogens. *Annu Rev Med* 1999;**50**:223–36.
- Rupp ME, Archer GL. Coagulase-negative staphylococci: pathogens associated with medical progress. *Clin Infect Dis* 1994;**19**:231–43.
- Daschner FD, Kropec A. Glycopeptides in the treatment of staphylococcal infections. *Eur J Clin Microbiol Infect Dis* 1995;**14**:S12–17.
- Predari SC, Ligozzi M, Fontana R. Genotypic identification of methicillin-resistant coagulase-negative staphylococci by polymerase chain reaction. *Antimicrob Agents Chemother* 1991;**35**:2568–73.

- 5 **Petersson AC**, Miorner H, Kamme C. Identification of *mecA*-related oxacillin resistance in staphylococci by the Etest and the broth microdilution method. *J Antimicrob Chemother* 1996;**37**:445–56.
- 6 **York MK**, Gibbs L, Chehab F, *et al*. Comparison of PCR detection of *mecA* with standard susceptibility testing methods to determine methicillin resistance in coagulase-negative staphylococci. *J Clin Microbiol* 1996;**34**:249–53.
- 7 **Cimolai N**, Trombley C, Zaher A. Oxacillin susceptibility of coagulase-negative staphylococci: role for *mecA* genotyping and Etest susceptibility testing. *Int J Antimicrob Agents* 1997;**8**:121–5.
- 8 **Hussain Z**, Stoakes L, Lannigan R, *et al*. Evaluation of screening and commercial methods for detection of methicillin resistance in coagulase-negative staphylococci. *J Clin Microbiol* 1998;**36**:273–4.
- 9 **Graham JC**, Murphy OM, Stewart D, *et al*. Comparison of PCR detection of *mecA* with methicillin and oxacillin disc susceptibility testing in coagulase-negative staphylococci. *J Antimicrob Chemother* 2000;**45**:111–13.
- 10 **NCCLS**. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*. Approved standard, 5th edn, M7-A5. Wayne, PA: NCCLS, 2000.
- 11 **Brakstad OG**, Maeland JA. Mechanisms of methicillin resistance in staphylococci. *APMIS* 1997;**105**:264–76.
- 12 **Suzuki E**, Miramatsu K, Yokata T. Survey of methicillin-resistant clinical strains of coagulase-negative staphylococci for *mecA* gene distribution. *Antimicrob Agents Chemother* 1992;**36**:429–34.
- 13 **Ryffel C**, Tesch W, Berger-Bachi I, *et al*. Sequence comparison of *mecA* genes isolated from methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Gene* 1990;**94**:137–8.
- 14 **Cimolai N**, Espersen F. Staphylococcal infections. Chapter 11. In: Cimolai N, ed. *Laboratory diagnosis of bacterial infections*. New York: Marcel Dekker, Inc. In press.
- 15 **Hussain Z**, Stoakes L, Massey V, *et al*. Correlation of oxacillin MIC with *mecA* gene carriage in coagulase-negative staphylococci. *J Clin Microbiol* 2000;**38**:752–4.
- 16 **Cimolai N**. Ciprofloxacin and multiresistant staphylococci. *Lancet* 1997;**349**:1030.

## ARCHIVIST .....

### Child protection registers and the accident and emergency department

Several national bodies and government departments have stated that it is important that accident and emergency (A&E) department should have ready access to child protection registers. A survey of UK A&E departments (G Quin and R Evans. *Emergency Medicine Journal* 2002;**19**:136–7) has shown considerable variation in the method of access, criteria for checking, and satisfaction with access to the register.

A postal questionnaire was sent to consultants in 254 major A&E departments and 190 questionnaires (75%) were returned. The most common way of accessing the child protection register (48% of departments) was via the duty social worker but this is time consuming and restricts the number of children who can be checked. One third of departments had access to a copy of the register, (computerised in half of them). Eighteen per cent of departments used combined means of access (social worker plus copy or social worker plus police) or other means. Satisfaction with the means of access was expressed by most consultants using computerised or hard copies of the register (82% and 66% respectively) but by only half of those using only the duty social worker or combined methods. Access to a copy of the register provided only local, and often out of date, information.

Thirty per cent of departments checked all children against the register and the rest either checked children with specified risk factors or relied on staff suspicion of child abuse. Departments with their own copy of the register tend to check all children but distributing copies could put confidentiality at risk. Registration is neither sensitive nor specific for current abuse (many children with nonaccidental injury will not be on the register and children on the register may have accidental injuries). On the other hand about 7% of children put on the register in 1982 were reinjured within a year.

Which policy is the best for the protection of children is not known. It is suggested that outcomes should be compared between departments using different methods.