Use of thermonuclease testing to identify *Staphylococcus aureus* by direct examination of blood cultures

N.M. Kaplan [1]

اختبار الثيرمونوكلياز لكشف العنقوديات الذهبية بالفحص المباشر للمزارع الدموية

ناصر كابلان

الخلاصة: تم فحص المزارع الدموية المُرسَلة إلى مختبرات المكروبيولوجيا السريرية، في مستشفى الملكة علياء العسكري، بعمّان في الفترة من 1999 إلى 2001، وذلك بغية تقييم اختبار إنزيم الثيرمونوكلياز المباشر لكشف العنقوديات الذهبية في مرق المزارع الدموية الذي نمت فيه مكورات إيجابية الغرام. ومن بين 170 مزرعة نمت دراستها، أعطت 129 مزرعة عنقوديات ذهبية إيجابية الغرام بينما أعطت 41 مزرعة مكورات إيجابية الغرام أخرى. وقد تم استخدام أطباق الأغار بزرقة التوليدين والدنا DNA في اختبار نشاط الثيرمونوكلياز. وتم إجراء الاختبارات المعيارية لإنزيم الكواغولاز في الأنابيب على المستفردات. إن الاكتشاف المباشر لفعالية الثيرمونوكلياز في مرق 76 مزرعة دموية تحتوي على العنقودية الذهبية الإيجابية الغرام قد أظهرت ترابطاً تاماً مع اختبارات إنزيم الكواغولاز في الأنابيب التي أُجريت بعد ذلك. فاختبارات النيرمونوكلياز توفر تأكيداً سريعاً ونوعياً وموثوقاً به لتجرثم الدم بالعنقوديات الذهبية من خلال الاختبار المباشر لمرق المزرعة الدموية التي تضم مكورات إيجابية الغرام. وهذا من شأنه أن يوفر العلاج بالمضادات الحيوية بالصورة المثلى وفي الوقت المناسب.

ABSTRACT Blood cultures submitted to the Clinical Microbiology Laboratory, Queen Alia Military Hospital, Amman during 1999–2001 were examined to evaluate thermonuclease testing for identifying *Staphylococcus aureus* in blood culture broths growing Gram-positive cocci. Of 170 cultures studied, 129 yielded Gram positive staphylococci and 41 yielded other Gram-positive cocci. Toluidine blue-deoxynucleic acid agar plates were used to test for thermonuclease activity. Standard tube coagulase tests were performed on the isolates. Direct detection of thermonuclease activity in 76 blood culture broths containing Gram-positive staphylococci showed 100% correlation with subsequent tube coagulase tests. The thermonuclease test provides a fast, specific and reliable confirmation of *S. aureus* bacteraemia by direct examination of blood culture broths that contain Gram-positive cocci. This allows for timely, optimal antibiotic therapy.

Test de la thermonucléase pour l'identification de *Staphylococcus aureus* par l'examen direct des hémocultures

RESUME Les hémocultures soumises de 1999 à 2001 au Laboratoire de microbiologie clinique de l'Hôpital militaire Reine Alia à Amman ont été examinées afin d'évaluer le test de la thermonucléase pour l'identification de *Staphylococcus aureus* dans les cocci à Gram positif qui se développent dans des bouillons d'hémoculture. Sur les 170 cultures étudiées, 129 ont révélé des staphylocoques à Gram positif et 41 d'autres cocci à Gram positif. Des boîtes de gélose au bleu de toluidine et à l'acide désoxyribonucléique ont été utilisées pour tester l'activité de la thermonucléase. Des tests standard de recherche de la coagulase en tube ont été réalisés sur les isolats. La détection directe de l'activité de la thermonucléase dans 76 bouillons d'hémocultures contenant des staphylocoques à Gram positif a montré une corrélation à 100 % avec les tests ultérieurs de recherche de la coagulase en tube. Le test de la thermonucléase permet une confirmation fiable, spécifique et rapide de la bactériémie à *S. aureus* par l'examen direct des bouillons d'hémoculture contenant des cocci à Gram positif, ce qui permet d'instaurer à temps une antibiothérapie optimale.

[1] *Department of Pathology, Queen Alia Military Hospital, Amman, Jordan.*
Received: 10/03/02; accepted: 02/07/02

Introduction

The process by which *Staphylococcus aureus* causes disease is very complex and probably involves a large numbers of factors, both cell-associated and related to the secreted exotoxins [*1*]. Nearly all strains secrete a group of enzymes and cytotoxins, which includes 4 haemolysins (alpha, beta, gamma, and delta), nucleases, proteases, lipases, hyaluronidase and collagenase.

The rapid differentiation of *S. aureus* from coagulase-negative staphylococci in blood cultures is important because of the high association of *S. aureus* isolation with clinically significant bacteraemia [*2*]. Several methods for the rapid identification of *S. aureus* in samples of growing blood cultures have been described. These include coagulase [*3*], commercial latex agglutination [*4*] and lysostaphin susceptibility tests [*5,6*]. The potential use of nucleic acid probes as an alternative method for rapid identification has been assessed [*7*] and used for rapid identification of *S. aureus* directly from positive blood cultures containing Gram-positive cocci in clusters [*8*].

Thermonuclease (TNase) is a heat-stable nuclease that has both endo- and exonucleolytic properties and can cleave DNA or RNA. The detection of TNase activity is another specific diagnostic test which can be used to identify *S. aureus* isolates [*9–12*], and has been used for the rapid, accurate and direct detection of *S. aureus* in foods [*13–16*]. In this study we evaluate the use of direct TNase testing of blood culture broths growing Gram-positive cocci as a method for rapid identification of *S. aureus*.

Methods

From May 1999 to September 2001, we examined 170 blood cultures submitted to the Clinical Microbiology Laboratory of Queen Alia Military Hospital in Columbia and thioglycollate broths (Becton, Dickinson and Company, Sparks, United States of America) by the recommended procedures [*17*].

On arrival at the laboratory, all samples were incubated at 35 °C for up to 7 days or until growth was detected. Bottles were observed macroscopically for visible evidence of bacterial growth (e.g. turbidity, haemolysis, gas production, chocolatization of the blood, and the presence of visible colonies or a layer of growth on the fluid meniscus), twice for the first 2 days, and then once daily thereafter. Gram stain was performed on macroscopically positive blood culture bottles immediately, and also on macroscopically negative bottles after 9–24 hours, 2–4 days and 5–7 days of incubation.

Standard tube coagulase tests were performed [*18*] on all staphylococcal subculture isolates. Subcultures were taken and direct antimicrobial susceptibility tests performed on microscopically positive bottles. Where the presence of Gram-positive cocci was demonstrated, 3 mL of broth were removed from the sample and centrifuged to remove erythrocytes. The supernatant was placed in a sterile glass tube, heated at 100 °C for 15 minutes and then cooled to room temperature. Toluidine blue-deoxynucleic acid agar was prepared [*19*] and poured into plastic Petri dishes. The plates were stored at 4 °C, wrapped in a plastic bag, and warmed to 37 °C for 1 hour before inoculation.

The large end of a sterile Pasteur pipette was used to punch out a maximum of 6 wells per plate. The wells were filled with the heat-treated samples. Positive (*S. aureus*) and negative (*Escherichia coli*) controls prepared from brain heart infusion broth cultures were included with each series of samples. The inoculated plates were

incubated in an upright position under aerobic conditions at 35 °C and inspected after 1 hour, 2 hours and 24 hours.

The presence of a pink zone around the well indicated a positive result. The pink halo was evidence of the breakdown of DNA [toluidine blue dye is blue when complexed with intact DNA but becomes metachromatic (pink) when complexed with free nucleotides]. A negative result was indicated by the absence of the pink zone. A small clear zone around a well was not indicative of a positive test as some coagulase-negative staphylococci could destroy the dye without denaturing the DNA.

Results

The type and number of the isolated organisms and the approximate timing of their microscopic detection and TNase testing are shown in Table 1. A total of 76 blood culture samples from 63 patients yielded *S. aureus*. There were no methicillin-resistant *S. aureus* isolates.

Within a period of 9–24 hours after incubation of the blood culture bottles, 89.5% of *S. aureus* and 86.8% of coagulase-negative staphylococci were detected microscopically. By TNase testing, *S. aureus* was identified as early as 11–12 hours after receipt in both aerobic and anaerobic blood culture bottles.

The results for TNase tests of 170 blood culture samples growing Gram-positive cocci and the results for tube coagulase tests of 129 staphylococcal subculture isolates are shown in Table 2. For the 76 blood culture samples that yielded *S. aureus*, there were no discrepancies between the direct TNase testing of the broths and the tube coagulase testing of the subculture isolates.

No positive thermonuclease results were found in the 53 samples containing coagulase-negative staphylococci and the 41 samples containing other Gram-positive cocci, including 14 *Micrococcus* spp., 11 *Streptococcus viridans*, 6 *Enterococcus*

Table 1 Type and number of isolated organisms and approximate timing of their microscopic detection and thermonuclease testing

Organism isolated	No.of organisms isolated			
	Time elapsed after incubation			Total
	9–24 hours	2–4 days	5–7 days	
Staphylococcus aureus	68	3	5	76
Coagulase negative staphylococci	46	1	6	53
Micrococcus	13	–	1	14
Streptococcus viridans	9	1	1	11
Enterococcus sp.	4	1	1	6
Streptococcus pneumonia	3	1	–	4
Streptococcus pyogenes	1	–	–	1
Streptococcus agalactiae	1	–	–	1
Anaerobic Gram-positive cocci	2	1	1	4
Total	147	8	15	170

Table 2 **Results of thermonuclease and tube coagulase tests**

Organism isolated	No. of blood culture samples	% of direct TNase-positive broths	% of tube coagulase-positive isolates
Staphylococcus aureus	76	100	100
Coagulase-negative staphylococci	53	0	0
Other Gram-positive cocci	41	0	Not done

spp., 4 *Streptococcus pneumoniae*, 4 anaerobic Gram-positive cocci, 1 *Streptococcus pyogenes*, and 1 *Streptococcus agalactiae*.

Discussion

The presence of living microorganisms in the blood of a patient carries the potential for considerable morbidity and mortality. In some large studies, a mortality rate from bacteraemia of 20%–50% has been reported [*20*]. *S. aureus* remains one of the most common causes of community-acquired and nosocomial localized and systemic infections. Large epidemiological studies consistently show that the incidence of *S. aureus* infections has been increasing steadily over the past few decades in all age groups [*21,22*], including neonates [*23*]. Despite the recent advances in antibiotic development, *S. aureus* infections still carry a significantly high morbidity and mortality in children and adults [*23–25*]. Thus, prompt and accurate identification of the etiological agents of bacteraemia remains one of the most important functions of the microbiology laboratory.

In our study, agreement between direct TNase tests on blood culture samples and subsequent tube coagulase tests on the isolates was 100%, a finding which parallels the excellent correlation between TNase tests and coagulase tests on clinical staphylococcal isolates in previous studies [*9–12,26–28*].

S. epidermidis, the most frequently encountered coagulase-negative species in blood cultures [*29,30*], has not been identified as a species that gives a positive TNase result [*31*]. After discussion with the clinicians, 85% of *S. epidermidis* isolated from blood cultures were judged to be contaminant [*32*]. Guidelines for proper blood culture collection and reduction of contamination have recently been published [*33*].

All positive TNase reactions were detected 2 hours after inoculation, although they generally intensified over a 24-hour period. This is a shorter incubation time than the 4-hour time previously noted [*12*], and strengthens the clinical usefulness of the test as a rapid method.

The positive samples were taken from the Columbia aerobic and the thioglycollate anaerobic bottles (48 from aerobic bottles and 28 from anaerobic bottles), although a previous study has noted an inhibitory effect of anaerobiosis on TNase production [*13*].

Although staphylococci and streptococci are generally easily presumptively identified by cellular morphology in blood cultures [*34*], the morphology may occasionally be indistinct. Because some streptococci, particularly group D, may produce thermonuclease activity [*35*], these sam-

ples were tested to detect possible false-positive reactions.

We conclude that direct detection of TNase activity in blood culture broths growing Gram-positive cocci provides a specific and reliable method for the rapid identification or exclusion of *S. aureus*. These rapid results are clinically relevant and enable physicians to make more timely and cost-effective decisions about antibiotic therapy. The procedure is technically easy to perform and to interpret.

References

1. Dinges MM, Orwin PM, Schlievert PM. Exotoxins of *Staphylococcus aureus*. *Clinical microbiology reviews*, 2000, 13:16–34.
2. MacGregor RR, Beaty HN. Evaluation of positive blood cultures. *Archives of internal medicine*, 1972, 130:84–7.
3. Wasilauskas BL, Ellner PD. Presumptive identification of bacteria from blood cultures in four hours. *Journal of infectious diseases*, 1971, 124:499–504.
4. Doern GV, Robbie LI. Direct identification of *Staphylococcus aureus* in blood culture fluid with a commercial latex agglutination test. *Journal of clinical microbiology*, 1982, 16:1048–51.
5. Knight RG, Shlaes DM. Rapid identification of *Staphylococcus aureus* and *Streptococcus pneumoniae* from blood cultures. *Journal of clinical microbiology*, 1983, 17:97 9.
6. Severance PJ, Kauffman CA, Sheagren JN. Rapid identification of *Staphylococcus aureus* by using lysostaphin sensitivity. *Journal of clinical microbiology*, 1980, 11: 724–7.
7. Wolcott MJ. Advances in nucleic acid-based detection methods. *Clinical microbiology reviews*, 1992, 5:370–86.
8. Oliveira K et al. Rapid identification of *Staphylococcus aureus* directly from blood cultures by fluorescence *in situ* hybridization with peptide nucleic acid probes. *Journal of clinical microbiology*, 2002, 40:247–51.
9. Barry AL, Lachica RVF, Atchison FW. Identification of *Staphylococcus aureus* by simultaneous use of tube coagulase and thermonuclease tests. *Applied microbiology*, 1973, 25:496–7.
10. Menzies RE. Comparison of coagulase, deoxyribonuclease and heat-stable nuclease for identification of *Staphylococcus aureus*. *Journal of clinical pathology*, 1977, 30:60–8.
11. Rayman MK et al. Reassessment of the coagulase and thermostable nuclease tests as means of identifying *Staphylococcus aureus*. *Applied microbiology*, 1975, 29:451–4.
12. Shanholtzer BL, Peterson LR. Clinical laboratory evaluation of the thermonuclease test. *American journal of clinical pathology*, 1982, 77:587–91.
13. Erickson A, Deibel RH. Production and heat stability of staphylococcal nuclease. *Applied microbiology*, 1973, 25: 332–6.
14. Koupal A, Deibel RH. Rapid qualitative method for detecting staphylococcal nuclease in food. *Applied and environmental microbiology*, 1978, 35:1193–7.
15. Lachica RVF, Hoeprich PD, Genigeorgis C. Metachromatic agar-diffusion microslide technique for detecting staphylococcal nuclease in foods. *Applied miorobiology*, 1972, 23:168–9.
16. Park CE, Derea HBE, Rayman MK. Evaluation of staphylococcal thermonuclease (TNase) assay as a means of screening foods for growth of staphylococci and possible enterotoxin production. *Canadian journal of microbiology*, 1978, 24:1135–9.

17. Freeman R. Bacteriology of normally sterile body fluids. In: Hawkey PM, Lewis DA, eds. *Medical bacteriology; a practical approach*, 1st ed. Oxford, IRL Press, 1989:21–42.

18. Bushell AC. Bacteriology of superficial, and deep tissue infection. In: Hawkey PM, Lewis DA, eds. *Medical bacteriology; a practical approach*, 1st ed. Oxford, IRL Press, 1989:91–137.

19. Hawkey PM, Lewis DA, eds. *Medical bacteriology: a practical approach*, 1st ed. Oxford, IRL Press, 1989:293–8.

20. Reimer LG, Wilson ML, Weinstein MP. Update on detection of bacteremia and fungemia. *Clinical microbiology reviews*, 1997, 10:444–65.

21. Ayliffe GA. The progressive intercontinental spread of methicillin-resistant *Staphylococcus aureus*. *Clinical infectious diseases*, 1997, 24(suppl. 1):S74–9.

22. Pittet D, Wenzel RP. Nosocomial bloodstream infections: secular trends in rates, mortality, and contribution to total hospital deaths. *Archives of internal medicine*, 1995, 155:1177–84.

23. Kallman J et al. Increase in staphylococci in neonatal septicaemia: a fourteen-year study. *Acta paediatrica*, 1997, 86:533–8.

24. Lautenschlager S, Herzog C, Zimmerli W. Course and outcome of bacteraemia due to *Staphylococcus aureus*: evaluation of different clinical case definitions. *Clinical infectious diseases*, 1993, 16: 567–73.

25. Mehtar S. The continuing problem of 'hospital staphylococci': why? *Journal of chemotherapy (Florence, Italy)*, 1994, 6(suppl. 4):25–40.

26. Madison BM, Baselski VS. Rapid identification of *Staphylococcus aureus* in blood cultures by thermonuclease testing. *Journal of clinical microbiology*, 1983, 18:722–4.

27. Ratner HB, Stratton CW. Thermonuclease test for same-day identification of *Staphylococcus aureus* in blood cultures. *Journal of clinical microbiology*, 1985, 21:995–6.

28. Skulnick M et al. Evaluation of three methods for the rapid identification of *Staphylococcus aureus* in blood cultures. *Diagnostic microbiology and infectious disease*, 1994, 19:5–8.

29. Marsik FJ, Brake S. Species identification and susceptibility to 17 antibiotics of coagulase-negative staphylococci isolated from clinical specimens. *Journal of clinical microbiology*, 1982, 15:640–5.

30. Sewell CM et al. Clinical significance of coagulase-negative staphylococci. *Journal of clinical microbiology*, 1982, 16: 236–9.

31. Gramoli JL, Wilkinson BJ. Characterization and identification of coagulase-negative, heat-stable deoxyribonuclease-positive staphylococci. *Journal of general microbiology*, 1978, 105:275–85.

32. Kirchhoff LV, Sheagren JN. Epidemiology and clinical significance of blood cultures positive for coagulase-negative staphylococci. *Infection control*, 1985, 6:479–86.

33. Ernst DJ. The right way to do blood cultures. *RN*, 2001, 64:28–31.

34. Agger WA, Maki DG. Efficacy of direct Gram stain in differentiating staphylococci from streptococci in blood cultures positive for Gram-positive cocci. *Journal of clinical microbiology*, 1978, 7:111–3.

35. Park CE et al. A survey of microorganisms for thermonuclease production. *Canadian journal of miorobiology*, 1980, 26:532–5.