J Clin Pathol 1984;**37**:1392–1394

Rapid method for detecting β-lactamase producing bacteria in clinical specimens

ITZHAK BROOK, ALAN E GOBER

From the Naval Medical Research Institute, Bethesda, Maryland, and George Washington University, Washington DC

SUMMARY The use of liquid media to detect the production of β-lactamase by β-lactamase producing organisms has been compared with the conventional method of inoculation on to agar media. Pharyngeal cultures were obtained from 162 children treated with penicillin for acute tonsillitis. β-lactamase producing organisms were detected within 72 h in 80 (49%) of the specimens inoculated on to agar media, while β-lactamase production was found in 76 (47%) of the specimens after their incubation in liquid media for 24 h. Twenty one of the cultures were positive only after anaerobic incubation while in liquid media while nine were positive only after aerobic incubation. Incubation in liquid media enabled detection of β-lactamase activity in 53 of the 76 (70%) specimens within 12 h.

Recent work has emphasised the role of β-lactamase producing aerobic and anaerobic bacteria in the failure of penicillin treatment to eradicate upper respiratory tract infections in children.[1–4] β-lactamase producing organisms may reduce the efficacy of treatment by enzymatically inactivating penicillin, allowing infection to persist. These organisms not only protect themselves from penicillin but can also protect penicillin susceptible organisms that are mixed with them at the site of the infection.[5–7] The most prevalent of these organisms are *Staphylococcus aureus, Haemophilus influenzae, H parainfluenzae, Branhamella catarrhalis,* and *Bacteroides* spp *(Bact melaninogenicus* and *fragilis* groups and *Bact oralis).*

Because penicillin treatment is ineffective in many of these mixed infections a simple, inexpensive, and rapid method for identifying β-lactamase producing organisms would be useful. This report describes a method that gives results within 8–12 h and which directs the clinician to the correct choice of antimicrobial treatment.

Patients and methods

PATIENTS

One hundred and sixty two children seen sequentially in the acute care clinic were included in the study; the patients' mean age was 7 years and 8 months (range 4–12 years) and 92 were boys. The patients had symptoms of acute uncomplicated tonsillitis of five to seven day's duration, and all had received oral penicillin V or ampicillin for at least five days.

MICROBIOLOGY

Pharyngeal cultures were obtained from all the children using two sterile cotton swabs, which were introduced into an anaerobic transport media (Port-A-Cul, BBL, Becton Dickson Co, Cockeysville, Maryland). The specimens were taken to the bacteriology laboratory and inoculated within 24 h of collection.

Two methods were used to identify the presence of β-lactamase producing organisms: the conventional method, using solid agar media, and a rapid method, using broth culture, In the conventional method suspected aerobic colonies were tested for β-lactamase production. In the rapid method the broth suspension was used for detecting the enzyme.

CONVENTIONAL METHOD

Sheep blood (5%) and chocolate agar plates were inoculated for the isolation of aerobic organisms. The plates were incubated at 37°C in 5% CO_2, and examined at 24 and 48 h. To isolate the anaerobes the specimens were plated on to prereduced vitamin K_1 enriched Brucella blood agar; anaerobic blood agar plates containing kanamycin (75 mg/l) and vancomycin (7·5 mg/l); an anaerobic blood plate containing phenylethyl alcohol; and into enriched thioglycolate broth. These media were incubated in jars

Accepted for publication 5 September 1984

Effect of incubation environment (aerobic or anaerobic) on the identification of β-lactamase production in clinical specimens using the routine or rapid method

	No of specimens showing β-lactamase activity			
	Total no of β-lactamase producing strains	*Detected in aerobic environment only*	*Detected in anaerobic environment only*	*Detected in both aerobic and anaerobic environment*
Routine method at 24 and 72 h*	80	22	20	38
Rapid method				
after 4 h	8	5	—	3
after 8 h	25	6	5	14
after 12 h	53	8	13	32
after 24 h	76	9	21	46

*Aerobic bacteria were examined after 24 h, and anaerobic bacteria after 72 h.

at 37°C and examined at 72 h. The thioglycolate broth was incubated for 14 days. Aerobic and anaerobic bacteria were identified using previously described methods.[8 9] For detection of β-lactamase producing organisms five colonies of each morphological feature were picked from each plate. Representative aerobic colonies were tested for β-lactamase production after 24 h incubation and suspected anaerobic colonies were tested after 72 hours.

RAPID METHOD

The two original swabs were each introduced into a tube of peptone yeast glucose broth (Scott Laboratories, Fiskville, RI) and shaken. One tube was incubated aerobically and the other anaerobically. β-lactamase activity was measured after 4, 8, 12, 24, and 48 h.

DETECTION OF β-LACTAMASE PRODUCTION

The Cefinase disc (BBL Microbiology System, Cockeysville, MD 21030, USA), which is impregnated with the chromogenic cephalosporin nitrocefin, was used for detecting the enzyme.[10] In the conventional method colonies were picked from the agar media and placed on the disc, while in the rapid method the tube was shaken and 0·1 ml of the broth was aspirated into a 1·0 ml syringe and placed on the disc.

Results

A total of 91 β-lactamase producing aerobic and anaerobic bacteria were detected in 80 (49%) specimens. The aerobic organisms were *Staph aureus* (24 isolates), *Branhamella catarrhalis* (11), *H influenzae* (7), and *H parainfluenzae*. (5). The anaerobic bacteria were *Bact melaninogenicus* group (21 isolates), *Bact oralis* (13), *Bact ruminicola* ssp *brevis* (8), and *Bact fragilis* (2).

With the conventional method, β-lactamase producing organisms were detected in 38 specimens in both the aerobic and anaerobic plates (Table). In 22 instances β-lactamase producing organisms were detected only in the cultures incubated aerobically, and in 20 instances these organisms were recovered only from specimens incubated anaerobically.

With the rapid method maximal β-lactamase production was detected within 24 h in 76 specimens, all of which were also positive for enzyme production using the conventional method. Forty six specimens were positive in both aerobic and anaerobic tubes, 21 were positive in an anaerobic environment only, and nine were positive in an aerobic environment only. After incubation in an aerobic environment, a difference was noted between the rates of isolation with the routine and the rapid methods (22 *v* 9). This discrepancy is explained by the higher rate of detection of the enzyme in both the aerobic and anaerobic environment in the rapid method.

Although maximal activity was detected within 24 h, 53 of the 76 (70%) tubes in which β-lactamase was eventually detected showed the presence of the enzyme within 12 h; in 25 (33%) the enzyme was detected within 8 h; and in eight (11%) the enzyme was found within 4 h.

Discussion

Detection of β-lactamase production using conventional methods may take up to 72 h, especially if anaerobic strains are sought. Shortening the time needed for detecting β-lactamase producing organisms may facilitate administration of appropriate antimicrobial treatment, especially in cases where penicillin treatment is ineffective. Although incubation of the specimen in liquid media does not give the identity of the organisms, the time for the detection of β-lactamase producing organisms is consid-

erably reduced. We have also shown that since some of the bacteria grow better in either aerobic or anaerobic atmospheres, for maximal detection of β-lactamase producing organisms, the specimens should be incubated in both environments.

The rapid simple test may have wide practical implications in the management of patients receiving penicillin treatment. If applied to all clinical swabs, it may shorten the time needed to detect β-lactamase producing organisms, which may be preventing the eradication of infection. Furthermore, when applied to specimens from patients who have failed to respond to treatment such a test can direct the clinician to the possible emergence of β-lactamase producing organisms.

A recent report described five patients with clinical failure after penicillin treatment associated with isolation of β-lactamase producing anaerobic organisms.[3] We have recently investigated the presence of β-lactamase producing organisms in clinical specimens from 185 children with orofacial or respiratory tract infections who failed to respond to antimicrobial treatment, including penicillins, which were given to 148 (80%) of them.[1] β-lactamase producing aerobic and anaerobic bacteria were detected in 75 (40·5%) of the 185 children. The β-lactamase producing strains included all 11 strains of the *Bact fragilis* group, 30 (45·4%) of the 66 strains of the *Bact melaninogenicus* group, five (41·7%) of the 12 strains for *Bact oralis,* and 41 (97·6%) of 42 strains of *Staph aureus*. These reports show that previous penicillin treatment substantially increases the incidence of β-lactamase producing organisms in infected sites and may account for the clinical failure with penicillin.

This test may be applied in the management of children with recurrent tonsillitis who fail to respond to penicillin treatment. Because of its previously high success in eradicating group A β-haemolytic streptococci and other oral pathogens, penicillin has been the mainstay of treatment of respiratory tract infections. β-lactamase producing organisms can, in addition to causing inflammation themselves, limit the efficacy of pencillin treatment by protecting the penicillin susceptible pathogens present in mixed infections, thus allowing the infection to persist.[7 11 12] In a recent study of the bacteriology of recurrent tonsillitis, we found β-lactamase producing organisms in 39 of our 50 patients.[13] These were isolates of *Bact melaninogenicus* group, *Bact oralis,* and *H influenzae*. Detection of such β-lactamase producing organisms in children with recurrent tonsillitis should suggest to the clinician the use of antimicrobial agents effective against both group A β-haemolytic streptococci and β-lactamase producing organisms.

The opinions and assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

The authors gratefully acknowledge the technical assistance of JE Perry and the editorial assistance of Ms Donna Boyle.

References

[1] Brook I. Beta lactamase-producing bacterja recovered after clinical failure with penicillin. *Arch Otolaryngol* 1984;**110**:228–21.
[2] Brook I, Calhoun L, Yocum P. Beta lactamase-producing isolates of *Bacteroides* species from children. *Antimicrob Agents Chemother* 1980;**18**:164–6.
[3] Heimdahl A, Von Konow L, Nord CE. Isolation of beta lactamase-producing *Bacteroides* strains associated with clinical failure with penicillin treatment of human orofacial infections. *Arch Oral Biol* 1980;**25**:689–92.
[4] Tunér K, Nord CE. Beta lactamase-producing anaerobic bacteria in recurrent tonsillitis. *J Antimcrob Chemother* 1982; **10** (suppl A): 153–6.
[5] Brook I, Pazzaglia G, Coolbaugh JC, Walker RI. In vivo protection of Group A beta hemolytic streptococci from penicillin by beta lactamase-producing *Bacteroides* species. *J Antimicrob Chemother* 1983;**12**:599–606.
[6] Brook I, Yocum P. 1983. In vitro protection of Group A beta-hemolytic streptococci from penicillin and cephalothin by *Bacteroides fragilis. Chemotherapy* 1983;**19**:18–23.
[7] Simon HM, Sukair W. Staphylococcal antagonism to penicillin group therapy of hemolytic streptococcal pharyngeal infection: Effect of oxacillin. *Pediatrics* 1963;**31**:463–9.
[8] Lennette EH, Balows A, Hausler W, Truant JP. *Manual of clinical microbiology.* 3rd ed. Washington DC: American Society for Microbiology, 1980.
[9] Sutter VL, Citron DM, Finegold SM. *Wadsworth anaerobic bacteriology manual.* 3rd ed. St Louis: CV Mosby Co, 1980.
[10] Gabay EL, Sutter VL, Finegold SM. Rapid beta lactamase testing in Bacteroides. *J Antimicrob Chemother* 1981;**4**:413–6.
[11] De Louvois J, Hurley R. Inactivation of penicillin by purulent exudates. *Br Med J* 1977;iii:998–1000.
[12] O'Keefe JP, Tally FP, Barza M, Gorbach SL. Inactivation of pencillin G during experimental infection with *Bacteroides fragilis. J Infect Dis* 1978;**137**:437–42.
[13] Brook I, Yocum P, Friedman EM. Aerobic and anaerobic flora recovered from tonsillar tissue of children with recurrent tonsillitis. *Ann Otol Rhinol Laryngol* 1981;**90**:261–3.

Requests for reprints to: Dr Itzhak Brook, Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20814, USA