Aztreonam selective agar for Gram positive bacteria

W Wood, G Harvey, E S Olson, T M S Reid

Abstract

Aztreonam blood agar, a new selective medium for Gram positive aerobic bacteria, was evaluated in comparison with conventional media for skin swabs. Aztreonam agar increased the number of isolates of *Staphylococcus aureus* by 17%. By producing purer growths on primary isolation, it significantly speeded up the identification and sensitivity testing of staphylococci and streptococci. All major Gram positive aerobic pathogens grow on this medium.

Aztreonam agar is now an established addition to our culture media. It is used for swabs which are likely to have a mixed Gram positive and Gram negative flora, such as ears, burns, ulcers, and for the sputa of patients with cystic fibrosis.

(J Clin Pathol 1993;46:769-771)

Staphylococcus aureus and *Streptococcus pyogenes* are the major pathogens in skin sepsis. Unfortunately many sites, such as ulcers, wounds, burns and ears often have a mixed flora with *Pseudomonas* spp or coliforms such as *Proteus* spp. The presence of large, sometimes swarming, colonies of Gram negative bacilli may conceal staphylococci and streptococci or delay their identification and antibiotic sensitivity testing by necessitating subculture for purity.

Several selective media have been developed to overcome this problem. Most of them are genera or species specific—for example, 10% sodium chloride nutrient agar, salt milk agar,¹ and phenolphthalein phosphate agar,² for staphylococci, and crystal violet blood agar,³ and polymyxin B, neomycin, fusidic acid medium⁴ for streptococci.

Aztreonam is a monobactam antibiotic with a broad spectrum of activity against Gram negative aerobic bacilli including *Pseudomonas aeruginosa*. It has virtually no activity against Gram positive aerobic bacteria.⁵ A pilot study had shown that adding 8 mg/l of aztreonam to blood agar gave the optimal concentration for the suppression of Gram negative bacteria while still allowing Gram positive bacteria to grow, and was comparable with 10% sodium chloride blood agar for the isolation of staphylococci.

Methods

A prospective study of swabs submitted for routine bacteriological examination was undertaken. Swabs were selected for inclu-

sion if they came from sites likely to yield a mixed flora or if the Gram stain indicated a mixed flora.

All swabs were received in Amies transport medium (Difco). Most were from general practitioners. Transport time to the laboratory ranged from a few hours to two days. All specimens were inoculated within three hours of receipt.

The following media were used:

blood agar: Columbia agar base with 5% defibrinated horse blood (Lab M);

gentamicin agar: blood agar with 5 mg/l of gentamicin (Roussel);

aztreonam agar: blood agar with 8 mg/l of aztreonam (Squibb);

MacConkey agar: (Lab M).

These were prepared according to the manufacturers' instructions. For aztreonam blood agar, aztreonam 8 mg/l was added to molten blood agar immediately before the plates were poured.

The media were poured in split plates in the following combinations: blood/MacConkey, blood/gentamicin. Aztreonam agar was poured in whole plates, half a plate being used for each specimen. Quality control was performed on all batches of aztreonam agar before use. Each batch was deemed satisfactory if it supported the growth of *S aureus* (NTCC 6571) and *S pyogenes* (local strain) and prevented the growth of *Escherichia coli* (NTCC 10418) and *Ps aeruginosa* (NTCC 10662). Plates were stored at 4°C and used within five days of pouring.

Swabs were plated for single colonies. Media were inoculated in the following order: blood/MacConkey, blood/gentamicin, aztreonam agar.

The blood/gentamicin plates were incubated overnight at 37°C in an atmosphere of 90% nitrogen, 5% hydrogen, 5% carbon dioxide. The other plates were incubated overnight at 37°C in air plus 5% carbon dioxide. Identification of isolates was by standard procedures.

Growth of clinically important Gram positive bacteria and all Gram negative bacteria was recorded by a semiquantitative method for each medium: 1-10 colonies +; 10-20 colonies ++; >20 colonies +++. Gram positive isolates requiring subculture for purity from the conventional media were recorded.

Results

Two hundred and thirty three swabs were entered into the study: 112 grew Gram negative bacteria; 95 of these contained clinically

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Accepted for publication
9 February 1993

Results for swabs containing mixed growths of Gram positive and Gram negative isolates

Swab type (numbers received)	No of mixed isolates on conventional media	No of mixed isolates with Gram positive only on aztreonam	Number of mixed isolates requiring subculture in absence of aztreonam
Abscess (6)	4	1	3
Wound (25)	7	1	3
Skin (26)	11	4	5
Ulcers (89)	38	4	19
Ear (78)	16	4	4
Umbilical cord (3)	2	0	1
Total (233)	81	14	35

Photograph of two blood/MacConkey agar plates (plate 1 and 3) and one aztreonam agar plate (plates 2).

Wound swab inoculated onto plate 1 and top half of plate 2 shows growth of *P mirabilis* and *E coli* on conventional media and a profuse growth of *S pyogenes* only on the aztreonam agar.

Sputum sample from a patient with cystic fibrosis inoculated onto plate 3 and bottom half of plate 2; showing growth of *Ps aeruginosa* on the conventional media and a profuse growth of *S aureus* on the aztreonam agar.

important Gram positive organisms. Of these 95 swabs, 14 (12.5%) grew pathogenic Gram positive bacteria (principally *S aureus*) only on aztreonam agar (table). The remaining 121 swabs were either sterile, grew pure Gram positive organisms, or grew normal skin flora.

Aztreonam agar only increased the isolation rate of Gram positive organisms in the presence of Gram negative organisms: it is a selective medium only and not an enrichment medium. No significant difference was found between aztreonam agar and conventional media for the isolation of streptococci. Although streptococci grow very well aerobically on aztreonam agar, they grow equally well anaerobically on gentamicin blood agar. The selective properties of aztreonam agar substantially reduced the need to subculture mixed growths for purity (table).

Aztreonam agar was noted to support the growth of the following organisms: *S aureus*; *S pyogenes*; β haemolytic streptococci of Lancefield's groups B, C, and G; *S pneumoniae*; *S milleri* and other viridans type streptococci; enterococci; coagulase negative staphylococci; diphtheroids; *Listeria monocytogenes*.

Four plates contained growths of aztreonam resistant Gram negative bacteria of the following species. Each species only occurred once: *Xanthomonas maltophilia*; *Ps aeruginosa*; *Klebsiella* sp; *Moraxella catarrhalis*.

Discussion

In this study the Gram negative bacteria which obscured growths of staphylococci were principally *Proteus mirabilis*, *Ps aeruginosa*, and *E coli*. *Proteus* species are intrinsically resistant to polymyxin B which is sometimes used in selective media for Gram positive organisms. Aztreonam agar was highly effective in suppressing the growth of Gram negative bacteria.

In the absence of aztreonam agar 12.5% of clinically important Gram positive isolates would have been missed due to overgrowth by Gram negative bacteria. Aztreonam agar permits the growth of Gram positive bacteria without contamination by Gram negative bacteria, thus allowing sensitivity and identification tests to be performed on the primary isolate and avoiding the delay of at least 24 hours required to subculture mixed growths for purity. This has obvious advantages for both the patient and hospital costs.

Since this study we have successfully isolated *Corynebacterium jeikeium*, *Arcanobacterium haemolyticum*, *Erysipelothrix rhusiopathiae* and *Bacillus* species on aztreonam agar. We thus consider it capable of supporting the growth of all major Gram positive aerobic pathogens.

Aztreonam blood agar is now part of our routine culture media protocol for skin ulcers, skin grafts, ears, abscesses, and also where the Gram stain indicates a mixed Gram positive and Gram negative flora. It is also used to assist the isolation of Gram positive bacteria when subculturing mixed growths for purity.

Aztreonam agar is especially useful in the primary isolation of *S aureus* from the sputum of patients with cystic fibrosis and is now part of our standard protocol for such patients.

We thank Gillian Aggasild, Dennis Golder, and Sheila Mackay and all the other MLSOs for their help with this study.

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J Clin Pathol 1993;46:771-772

Pseudotumoral amyloidosis of β_2 -microglobulin origin in the buttock of a patient receiving long term haemodialysis

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Abstract

A 52 year old man who had been receiving haemodialysis for 13 years, with a history of renal tuberculosis, right ischial tuberculous osteomyelitis, and dialysis arthropathy, developed a soft tissue tumour in his left buttock. Histological analysis, immunohistological staining, and electron microscopic examination of the surgically removed tumour showed massive deposits of β_2 -microglobulin (β_2 -M) amyloid. This case shows the expanding clinical spectrum of this type of amyloidosis, and it is suggested that amyloid infiltration should be considered in the differential diagnosis of gluteal tumours in these patients.

(*J Clin Pathol* 1993;46:771-772)

Joint and tenosynovial deposits of β_2 -microglobulin (β_2 -M) amyloid are common in patients receiving long term haemodialysis. In some, amyloid deposits give rise to tumours in and around joints.¹⁻³ Deposition of β_2 -M in viscera and other organs is less common; it is much less abundant than in affected joints and seems to be especially common in the vessel walls.⁴⁻⁶ Deposits of β_2 -M amyloid in soft tissue have been described in a few patients receiving haemodialysis.⁷⁻¹⁰ We report a patient who had been receiving haemodialysis for 13 years, with a soft tissue tumour in the gluteal region, which proved to be massive localised deposits of β_2 -M amyloid.

Case report

A 52 year old man had been receiving haemodialysis since 1977 for end stage renal disease, caused by renal tuberculosis. In 1985 he sustained a cerebral vascular accident with

left hemiparesis. In 1986 he developed a right ischial tuberculous osteomyelitis with gluteal abscess, which spontaneously drained through the popliteal space, and was successfully treated with antituberculous drugs for nine months.

In 1989 he was diagnosed as having amyloid arthropathy, which affected his right knee with relapsing joint effusions, and an osteolytic lesion in the left acetabulum. A synovial fluid aspirate from the right knee showed amyloid deposition. A subcutaneous fat biopsy specimen at this time did not show such deposits.

In 1990 he received right sided carpal tunnel decompression for carpal tunnel syndrome. Histological evidence of β_2 -M amyloid was found in the resected tenosynovial tissue. During the same year he complained of pain in the left buttock on sitting. Examination showed a subcutaneous mass measuring about 5 cm in diameter in the left gluteal region. During surgery a firm hourglass-shaped, greyish tumour in the subcutaneous tissue without encapsulation was found. The surgically removed mass contained neither bony nor cartilaginous tissue.

Light microscopic examination of the formaldehyde fixed, paraffin wax embedded mass from this patient showed multiple nodular areas of amorphous eosinophilic material, which stained with Congo red and showed dichroism under polarised light. Amyloid was deposited among collagen bundles and some small nodules were surrounded by multinucleated cells. Calcification foci, necrotic areas, thrombotic lesions, acute and chronic inflammatory cells and granulomatous inflammation were absent. Ziehl-Neelsen stains were negative.

Immunohistochemical studies using the avidin-biotin-complex technique, with monoclonal antibodies anti-A amyloid, and poly-

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Accepted for publication
17 February 1993