

False penicillin resistance in *Neisseria meningitidis* following direct susceptibility tests from blood cultures

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Abstract

Blood cultures drawn from a patient with clinically diagnosed invasive meningococcal disease, who had been previously administered benzylpenicillin, had β -lactamases added to increase the probability of recovery of the causative organism. The blood cultures subsequently yielded *Neisseria meningitidis* but direct susceptibility tests by the comparative disk diffusion method demonstrated greatly reduced zones of inhibition to penicillin (1 unit disk). Repeat testing from subcultures showed full penicillin sensitivity. Inoculation of blood culture bottles with a variety of penicillin sensitive bacteria with the addition of β -lactamases showed the same effect of false penicillin resistance, owing to carry over of sufficient β -lactamase from blood culture bottles during inoculation of direct susceptibility plates to inactivate the penicillin in the disks. Direct susceptibility tests to β -lactam agents should not be carried out on positive blood cultures to which β -lactamases have been added.

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Keywords: *Neisseria meningitidis*; β -lactamases; penicillin resistance; blood cultures

Case report

A 48 year old woman presented to her general practitioner with a 24 hour history of sore throat, headache, pyrexia, and myalgia. On examination she had no meningism but had a widespread petechial rash. The general practitioner gave 600 mg benzylpenicillin intramuscularly at 09.15 hours and arranged admission to hospital, where she was found to be hypotensive (75/60 mm Hg), tachycardic (100 beats/min), and had a temperature of 37.3°C. The petechial rash was most prominent on her thighs and abdomen. Aerobic and anaerobic blood cultures (BacTAlert 59268 and 59269; Organon Teknika, Durham, USA) were drawn at 10.45 hours and she was transferred to the intensive care unit where further benzylpenicillin was administered intravenously. The blood cultures were received in the microbiology laboratory at 11.30 hours and broad spectrum β -lactamase mixture (Oxoid, Basingstoke, UK) was added to each of the two blood culture bottles, giving 110 IU of β -lactamase (1 IU = 600 Levy units of activity)/bottle. The bottles commenced incubation at 37°C at 11.45 hours, and both bottles registered as yielding growth 18 hours later. Gram stain from the blood culture bottles

showed Gram negative cocci and a presumptive diagnosis of meningococcaemia was made.

Direct (primary) susceptibility tests were performed using the contents of the blood culture bottles by a modified Stoke's comparative method,¹ using a known penicillin sensitive control strain (ATCC 13102) and a 1 unit penicillin disk. Figure 1 illustrates the appearance of the zone of inhibition of growth for both the blood culture isolate (inner) and control strain (outer) after overnight incubation at 37°C in 6% CO₂. The tests were initially interpreted as showing reduced susceptibility to penicillin and the patient's antibiotic treatment altered to intravenous ceftriaxone 4 g daily.

The susceptibility tests were repeated using the growth from a chocolate agar plate inoculated at the same time as the direct susceptibility tests, and the following day this showed the isolate to be fully sensitive to penicillin. An E-test (AB Biodisk, Solna, Sweden) showed a penicillin minimum inhibitory concentration (MIC) of 0.032 mg/litre.

The isolate was sent to the Meningococcal Reference Unit, where it was confirmed as *Neisseria meningitidis* group C, type 4, subtype NT, with a MIC to benzylpenicillin of 0.04 mg/litre.

The patient developed disseminated intravascular coagulation shortly after admission; she also developed fingertip ischaemia, and needed ventilatory and cardiac inotropic support on the intensive care unit, where she spent the next eight days. She completed a 10 day course of ceftriaxone and received rifampicin for two days before discharge, by which time she had made an otherwise uneventful recovery.

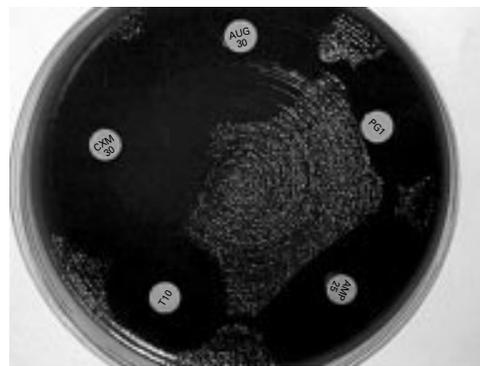


Figure 1 *Neisseria meningitidis* on chocolate agar showing reduction of zones of inhibition around disks containing benzylpenicillin (PG1) and ampicillin (AMP25), but not around tetracycline (T10), cefuroxime (CXM30), or coamoxiclav (AUG30) for isolates inoculated directly from blood cultures to which β -lactamases have (inner) and have not (outer) been added.

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Materials and methods

To study the effect of the addition of broad spectrum β -lactamases to blood cultures on subsequent direct disk susceptibility testing against β -lactam agents, experiments were performed using known penicillin sensitive organisms. These were *N meningitidis* (ATCC 13102), *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus* (NCTC 6571), and *Neisseria gonorrhoeae* (WHO strain A).

An inoculum of approximately 100 colony forming units/ml was prepared in normal saline from colonies on plates incubated overnight, and 1 ml was injected aseptically into duplicate sets of blood culture bottles containing 40 ml of broth. For each organism, one set had 110 IU broad spectrum β -lactamases added (1 ml volume) and one set had 1 ml sterile normal saline added as control. To encourage growth, 1 ml of sterile horse blood was also added to each blood culture bottle. The final concentration of β -lactamases in each bottle was 2.6 IU/ml.

After overnight incubation, three drops of blood culture were spread on appropriate susceptibility test media by rotary plater using a cotton wool swab, on to the inner part of the plate for cultures to which β -lactamases had been added, and on to the outer part of the plate where they had not. Antibiotic impregnated disks were then applied to the uninoculated area between the isolates, and the plate incubated at 37°C for 18 hours in an appropriate atmosphere. Disks used were benzylpenicillin (1 unit), cefuroxime (30 μ g), ampicillin (25 μ g), coamoxiclav (30 μ g), and tetracycline (10 μ g).

Results

In all cases, the zone around the penicillin disk was much reduced where the isolate was from blood culture bottles inoculated with β -lactamases, whereas a large zone was seen where isolates were from blood culture bottles that had not been inoculated. The effect was less pronounced with ampicillin 25 μ g and not seen at all with cefuroxime, tetracycline, and coamoxiclav. Similar results were found from both aerobic and anaerobic blood culture bottles.

Discussion

For presumptive cases of invasive meningococcal disease immediate treatment with benzylpenicillin in the community is recommended, before admission to hospital.² Where penicillin has been administered to the patient, it has long been known that the subsequent chances of isolating an organism sensitive to the administered agent from blood cultures can be increased by promptly adding β -lactamases to the blood culture bottles.³ It is not known how common this practice is in the UK, but it is not widespread in the USA.⁴ Little has recently been published on the use of β -lactamases in blood culture media, particularly in automated blood culture systems, but one study⁵ demonstrated that the addition of β -lactamases increased the isolation rates of organisms from blood significantly in clinical

practice, although *N meningitidis* did not feature in that series. It is perhaps surprising that no clinical study has investigated the effect of adding β -lactamases to blood cultures from patients with invasive meningococcal disease treated with benzylpenicillin before admission to hospital. Recent guidelines on the diagnosis and investigation of acute bacterial meningitis fail to mention the technique.⁶ Other agents used to neutralise the action of previously administered antibiotics have included sodium polyanethol sulphate, which inactivates aminoglycosides and polymyxins, and thiol broth, which slowly inactivates penicillins. Currently, resins may be included in modern automated blood culture systems, although the benefits of using bottles containing such inhibitors of antibiotics are still debated.⁷ In the case described here, we were able to add β -lactamases to the blood cultures within 45 minutes of them being taken. The recovery of penicillin sensitive *N meningitidis* from the cultures of a patient who had received a large dose of penicillin two and a quarter hours previously suggests that this was valuable.

The use of direct (primary) susceptibility tests where the inoculum is the specimen itself, in this instance blood cultures, has both advantages and disadvantages.⁸ The main advantage for blood cultures is the speed of reporting susceptibility results (being available the next day), and the main disadvantages are that the inoculum cannot be controlled and that, unless care is taken, there is a likelihood of introducing contaminants, which can be misleading.⁹⁻¹⁰ If the inoculum density is correct on the susceptibility plates, and there is only one organism present, the results of direct susceptibility tests can be reliable,¹¹⁻¹² although some authors recommend confirmation by testing isolated colonies from subcultures in a standardised manner.¹³

The effect of the β -lactamases added to blood culture bottles on subsequent direct susceptibility tests has, to my knowledge, not been described previously. These studies show that this can greatly affect the zone sizes around disks containing β -lactam agents, and this applies to a range of β -lactam sensitive bacteria. The reason for this would seem to be the effect of β -lactamases in the inoculum from the blood culture bottle on the β -lactam in the impregnated disks, leading to inactivation of the latter. That this is seen with penicillin and ampicillin, yet not with tetracycline or coamoxiclav, would therefore be expected. Tetracycline is unaffected by β -lactamase, and coamoxiclav contains the β -lactamase inhibitor clavulanic acid. The lack of effect around the cefuroxime disk may result from reduced activity of the β -lactamase mixture against cephalosporins compared with penicillins.¹⁴⁻¹⁵

Further studies would be useful to ascertain the value of adding β -lactamases to blood cultures from patients with meningococcal disease, particularly in view of the frequent use of pre-admission benzylpenicillin in this condition, and also to determine whether there is an optimum final concentration of β -lactamases in blood cultures to give adequate inactivation of

penicillin with minimal effect on direct sensitivity testing results.

Conclusion

Direct susceptibility tests to β -lactam agents should not be carried out on positive blood cultures to which β -lactamases have been added because there is a risk of interpreting the results as showing false β -lactam resistance.

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