Antibacterial effects on *Acinetobacter* species of commonly employed antineoplastic agents used in the treatment of haematological malignancies: an *in vitro* laboratory evaluation

ABSTRACT

Although about 75–80% of neutropenic fevers are thought to be caused by infections, a causal organism can be confirmed microbiologically or suspected clinically in only 30–50%, and even fewer of these cases (16%) have a documented bacteraemia. The cause of neutropenic fever in the remaining cases remains elusive. The reasons for this failure may be due to the difficulty in recovering low numbers of organisms, fastidious organisms which fail to grow using conventional culture media, the presence of non-culturable organisms, or the presence of inhibitory substances in specimens. Previously, the authors showed the presence of *Acinetobacter* in peripheral blood of febrile neutropenic patients with a haematological malignancy, using 16S rDNA polymerase chain reaction (PCR) and sequencing techniques. However, conventional culture was unable to detect these organisms. Hence, it was felt necessary to examine the antibacterial properties of four antineoplastic agents used in the treatment of haematological malignancy, namely bleomycin, cisplatin, doxorubicin and vincristine. A total of 56 wild-type *Acinetobacter* including seven species (*A. calcoaceticus* [n=17], *A. septicus* [n=11], *A. baumannii* [n=10], *A. johnsonii* [n=7], *A. lwoffii* [n=8] *A. haemolyticus* [n=2] and *A. radioresistens* [n=1]) were examined for their susceptibility to the four antineoplastic agents at therapeutic concentration. No inhibition was observed, but inhibition was seen at higher concentrations of both bleomycin and doxorubicin. Time to detection of blood culture bottles containing separate antineoplastic agents (i.e., bleomycin and doxorubicin) was compared to that containing saline using a paired t-test. Samples containing doxorubicin at 1 µg/mL were shown to have a mean time to detection of 21.8 h (range: 15.6–31.4 h). Bottles containing saline had a mean time to detection of 22.9 h (range: 18.2–31.3 h). Statistical analysis showed no significant difference (P=0.3361) between time to detection for blood culture bottles containing doxorubicin at achievable plasma concentration and corresponding negative controls. With regard to bleomycin (300 miu/mL), the mean time to detection was 27.29 h (range: 20.2–38.4 h) in the test bottles, with mean time to detection in the saline negative controls of 22.56 h (range: 17.0–30.1 h). Paired t-test gave P=0.000451, hence a significant difference in time to detection for blood cultures containing therapeutic levels of bleomycin. Overall, the antineoplastic agents vincristine, cisplatin or doxorubicin did not have any inhibitory effects on the *Acinetobacter* organisms examined. At worst, therapeutic concentrations of bleomycin may delay automated detection of an *Acinetobacter* bacteraemia by a mean time of 5.9 h.

KEY WORDS: Antineoplastic agents.
Acinetobacter.
Blood culture.
Hematologic neoplasms.

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Introduction

Cancers, including the haematological malignancies, are often treated with agents that lead to severe immune suppression. Pyrexia of unknown origin (PUO) is a common complication in haemato-oncology patients and encompasses a broad spectrum of diagnostic possibilities. The first line of investigation in such patients presenting with fever is blood cultures drawn through either a central catheter or a peripheral vein. Negative blood cultures are frequently generated on incubation

Previously, in a study involving the molecular detection of

Table 1. Concentrations of antineoplastic agents employed in the study.

Antineoplastic agent	Concentration			
	Supplied (x greater than therapeutic level)	10-fold higher than therapeutic level (adult)	Adult therapeutic level	10-fold lower than therapeutic level (adult)
Bleomycin	3000 iu/mL (x10,000)	3 iu/mL	300 miu/mL	30 miu/mL
Cisplatin	1 mg/mL (x1000)	10 µg/mL	1 µg/mL	0.1 µg/mL
Doxorubicin	2 mg/mL (x2000)	10 µg/mL	1 µg/mL	0.1 µg/mL
Vincristine	1 mg/mL (x100)	100 µg/mL	10 µg/mL	1.0 µg/mL

bacterial and fungal aetiological agents of infection in patients with some form of haematological malignancy, the authors were unable to culture any of the *Acinetobacter* polymerase chain reaction (PCR)-positive blood cultures.[1] In the study, PCR was able to detect five febrile episodes (5/98, 5.1%), where *Acinetobacter* spp. were detected in the blood culture bottle, but which were culture-negative. These findings suggest that *Acinetobacter* may be difficult to detect conventionally from blood culture in haematology patients undergoing cytotoxic chemotherapeutic drug regimens, especially as they have previously been described as causal agents of bacteraemia in patients with malignancy, particularly in those with line-related sepsis.[2,3]

A recent audit of *Acinetobacter* positivity in blood culture demonstrated that it constitutes approximately 1–2% of total blood cultures at the authors' centre. Hence, the reason for failing to detect these *Acinetobacter* spp. conventionally remains unanswered.

It has been documented previously that antineoplastic agents could affect microbial growth.[4] Current laboratory practice entails incubation for up to five days for blood cultures before being discarded as negative; thus, the potential exists for the delay or non-isolation of the organism and subsequent belated or inappropriate antimicrobial therapy allocated to the patient.

Therefore, the aim of this study is to establish whether or not certain common antineoplastic agents used in the treatment of haematological malignancies may impede the laboratory detection of *Acinetobacter* from blood cultures.

Materials and methods

Bacterial isolates

Historical blood culture data from the period January 2005 to May 2008 from haemato-oncology patients at Belfast City Hospital were analysed. A total of 56 wild-type *Acinetobacter* blood culture isolates were identified from storage at –80˚C and included seven species (*A. calcoaceticus* [*n*=17], *A. septicus* [*n*=11], *A. baumannii* [*n*=10], *A. johnsonii* [*n*=7], *A. lwoffii* [*n*=8], *A. haemolyticus* [*n*=2] and *A. radioresistens* [*n*=1]). All *Acinetobacter* isolates were cultured on Columbia blood agar (Oxoid CM331, Oxoid, Basingstoke, UK) containing 5% (v/v) defibrinated horse blood (CBA+DHB). The identity of all isolates was confirmed using the API 20NE biochemical kit (bioMérieux.), as well as by 16S ribosomal RNA (rDNA) PCR and gene sequencing techniques, as previously described.[1] In addition, eight control organisms, including four Gram-positive organisms (*Clostridium perfringens* [NCTC8237], *Enterococcus faecalis* [NCTC775], *Staphylococcus aureus* [NCTC6571] and *S. epidermidis* [NCTC11047]), three Gram-negative organisms (*Escherichia coli* [NCTC9001], *Pseudomonas aeruginosa* [NCTC10662] and *Raoultella planticola* [formerly *Klebsiella aerogenes*, NCTC9528]), as well as a yeast (*Candida albicans* [NCTC3089]), were employed in this study.

Antineoplastic agents

The following four antineoplastic agents commonly employed in the clinical treatment of haematological malignancy were used in this study (supplied concentration): vincristine (1 mg/mL, Mayne Pharma), bleomycin (3000 iu/mL, Kyowa Hakko), cisplatin (1 mg/mL, Mayne Pharma) and doxorubicin (2 mg/mL, Pharma Chemie). All agents were obtained from the Belfast City Hospital Haematology Pharmacy Department, with dilutions carried out using sterile distilled water, under stringent health and safety controls.

Examination of gross inhibition

Gross inhibition of antineoplastic agents was performed by placing 10 µL each antineoplastic agent separately onto Mueller-Hinton agar (Oxoid CM337), which had been freshly inoculated with the bacterial suspension and incubated aerobically overnight at 37˚C. Each drug was tested at four concentrations (i.e., [i] supplied concentration, [ii] recommended therapeutic concentration in adults, [iii] 10-fold higher concentration than the recommended therapeutic dosage, and [iv] a 10-fold lower concentration than the recommend therapeutic dosage value) (Table 1). Sterile saline was used as a negative control.

Preparation and inoculation of blood culture bottles

Four isolates each of *A. calcoaceticus*, *A. septicus*, *A. baumannii*, *A. johnsonii* and *A. lwoffii*, which showed sensitivity to both bleomycin and doxorubicin, were selected at random. Isolates were cultured overnight on Columbia blood agar (Oxoid CM331, Oxoid, Basingstoke, UK) containing 5% (v/v) defibrinated horse blood (CBA+DHB) at 37˚C. Bacterial suspensions of each *Acinetobacter* isolate were prepared equal to a 0.5 McFarland standard (approximately 1.5 x 10^8 colony-forming units [cfu]/mL) in 0.9% (w/v) saline, using a Densimat densitometer (bioMérieux). Bacterial suspension (100 µL) containing 20–90 cfu of each isolate was inoculated separately into BacT/ALERT FA Aerobic medium (Organon Teknika, USA), with sterile horse blood (9 mL) and antineoplastic agent (1 mL) to equate to the recommended

therapeutic concentration. Sterile distilled water was used as a negative control in each case.

For each *Acinetobacter* species, four different isolates were tested in duplicate against the antineoplastic agent. All bottles were inserted into the BacT/ALERT 3D (bioMérieux) and incubated at 37°C for up to six days, following the laboratory protocol. Once bottles became positive, time to detection was recorded and aliquots were removed for Gram staining and subsequent culture. The identity of all isolates was confirmed using the API 20NE biochemical kit (bioMérieux) to confirm that *Acinetobacter* organisms were present in each positive bottle.

Statistical analysis

Statistical analysis was performed using Excel 2007 (Microsoft). Determination of significance was by Student's *t*-test for matched-paired samples, where the effect of antineoplastic agent was matched to a particular microorganism. Calculation of the *P* value was used to measure the effect of the antineoplastic agent on the mean time to detection, compared to the negative control. $P<0.05$ was deemed significant, allowing rejection of the null hypothesis.

Results

All antineoplastic agents were not inhibitory to any of the 56 *Acinetobacter* isolates at the therapeutic concentration tested. This was also the case for all control organisms, even at a concentration 10-fold higher than the therapeutic concentration. However, the supplied concentration of all antineoplastic agents, with the exception of vincristine demonstrated activity against control organisms, with varying sizes of zones of inhibition and with no relationship to Gram status (Table 2). All 56 *Acinetobacter* isolates were not inhibited by vincristine and cisplatin at the therapeutic concentration or a 10-fold higher concentration; hence, these agents were not examined further.

However, doxorubicin and bleomycin produced inhibition to the majority of *Acinetobacter* isolates ($n=39$ and $n=40$, respectively), including *A. calcoaceticus*, *A. septicus*, *A. baumannii*, *A. johnsonii* and *A. lwoffii*, only at the highest concentration tested (i.e., that supplied by the manufacturer). Time to detection of blood culture bottles containing separate antineoplastic agents (bleomycin and doxorubicin) was compared to that containing saline using the paired *t*-test (Table 3). Samples containing doxorubicin at 1 µg/mL showed a mean time to detection of 21.8 h (range: 15.6–31.4 h). Bottles containing saline showed a mean time to detection of 22.9 h (range: 18.2–31.3 h). Statistical analysis showed no significant difference ($P=0.3361$) between time to detection for blood culture bottles containing doxorubicin at achievable plasma concentration and the corresponding negative controls.

With bleomycin (300 miu/mL), the mean time to detection was 27.29 h (range: 20.2–38.4 h) in the test bottles, with mean time to detection in the saline negative controls of 22.56 h (range: 17.0–30.1 h). Paired *t*-test gave $P=0.000451$, hence a significant difference in time to detection for blood cultures containing therapeutic levels of bleomycin.

Table 2. Antibacterial properties of four antineoplastic agents (bleomycin, cisplatin, doxorubicin, vincristine) tested against control organisms.

Organism	Antineoplastic agent			
	Vincristine (1 mg/mL)	Doxorubicin (2 mg/mL)	Cisplatin (1 mg mL)	Bleomycin (3000 iu/mL)
Staphylococcus aureus	R	I	R	I
Escherichia coli	R	R	I	I
Pseudomonas aeruginosa	R	R	I	R
Enterococcus faecalis	R	I	R	R
Clostridium perfringens	R	I	I	R
Staphylococcus epidermidis	R	I	R	I
Klebsiella aerogenes	R	I	R	I
Candida albicans	R	R	R	I

I: inhibition, R: resistant

Discussion

Although approximately 75–80% of neutropenic fevers are thought to be caused by infections, a causal organism can be confirmed microbiologically or suspected clinically in only 30–50%, and even fewer of these cases (16%) have a documented bacteraemia. The cause of neutropenic fever in the remaining cases remains elusive.[5] The reasons for this failure to isolate a pathogen are often not apparent but may be due to the difficulty in recovering low numbers of organisms, fastidious organisms which fail to grow using conventional culture media, or the presence of inhibitory substances.

Detection of bacteria in blood has an important role in diagnosis for a febrile patient in order to establish the presence of infection (thereby excluding a non-infectious cause of fever), to reassure the clinician about the chosen empirical therapy, and to streamline antibiotic treatment after assessment of the antibiotic sensitivity of an isolate. Blood cultures are considered one of the most significant specimen types that the microbiology laboratory processes, and every laboratory has strict notification policies to ensure that positive blood cultures are reported promptly to the clinician.[6]

Several studies have suggested that antineoplastic agents may affect the recovery of bacteria and fungi using automated blood culture systems. Kinnunen *et al.*[4] investigated the effect that seven antineoplastic agents had on the recovery of Gram-positive cocci, Gram-negative rods and yeasts, using two different automated blood culture systems. This study revealed that two of the seven antineoplastic agents assessed may have a significant effect on growth of bacteria in blood culture systems.

Experiments carried out by Bodet *et al.*[7] investigated the effects of 14 antineoplastic agents

Table 3. Mean time to detection (h) in blood cultures containing 20-90 cfu *Acinetobacter* spp. which had been artificially spiked with bleomycin and doxorubicin compared against controls.

		Mean time to detection (h)			
Organism	Mean cfu/mL	Bleomycin	Saline	Doxorubicin	Saline
Acinetobacter baumannii	60	32.8	22.2	24.2	22.7
Acinetobacter johnsonii	45	21.5	21.8	20.6	25.2
Acinetobacter calcoaceticus	55	24.5	19.5	19.4	19.9
Acinetobacter lwoffii	40	24.8	21.8	21.0	21.1
Acinetobacter septicus	70	34.2	23.3	23.8	25.5

cfu: colony-forming units/mL blood culture medium

against 101 bacterial isolates. Their study demonstrated that three of the agents tested (i.e., 5-fluorouracil, mitomycin and etoposide) had inhibitory effects at achievable plasma concentrations.

The present study was unable to detect any inhibition of the *Acinetobacter* organisms by all antineoplastic agents at therapeutic concentrations. However, when higher concentrations of doxorubicin and bleomycin were employed, there was considerable inhibition of the *Acinetobacter* isolates. Although these concentrations would never be attained *in vivo*, the potential of bactericidal or static effects remain viable at lower concentrations.

Representative isolates of sensitive species were selected at random for blood culture inoculation to establish any increase in time to detection. From the results obtained, a significant increase was obtained for samples that contained bleomycin at therapeutic levels. This delay was 15.8 h in the most extreme case, but addition of bleomycin delayed detection by 5.9 h. Ultimately, such a delay in detection, or total inhibition, would result in postponement in identification and susceptibility testing of the aetiological agent and optimisation of antimicrobial therapy. Recently, a large, retrospective study of patients with severe sepsis showed that delayed administration of appropriate antibiotic was associated with an increase in mortality of 7.6% for each hour of delay.[8]

In conclusion, synergistic or antagonistic interactions might occur when multiple antineoplastic drugs or antineoplastic-antibiotic drug combinations are used in an analogous manner to that observed with some antimicrobial drugs, as demonstrated by Henriksson *et al*.[9] Instigation of altered blood culture incubation times may be necessary, as well as the development of a specialised blood culture bottle or additive tailored to patients undergoing certain chemotherapeutic regimes, in order to minimise or eliminate any residual antimicrobial effects of antineoplastic agents. □

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