

Rate of growth of *Pseudomonas fluorescens* in donated blood

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Abstract

Aims—To examine how delayed refrigeration of blood affects the growth of *Pseudomonas fluorescens*, one of the two most important causes of sepsis resulting from transfusion of contaminated blood.

Methods—Two donations of whole blood were each divided into three aliquots and inoculated with 5–10 cfu/ml of a *P fluorescens* strain from a case of transfusion associated sepsis. From each donation, one aliquot was placed at 4°C, one was held at 20°C for six hours prior to refrigeration and the third was held at 20°C for 24 hours prior to refrigeration. Samples were aseptically withdrawn over 17 days and bacterial counts were determined using a pour plate technique.

Results—The rate of growth of *P fluorescens* in blood at 20°C was increased compared with blood at 4°C. At 24 hours the aliquots held at 20°C for six and 24 hours had, respectively, 174 and 29 000 cfu/ml compared with 15 cfu/ml in aliquots held at 4°C. There was no evidence of increased killing of *P fluorescens* at the higher temperature.

Conclusions—These results suggest that blood for transfusion should be refrigerated as soon as possible after collection.

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Keywords: *Pseudomonas fluorescens*, blood, contamination, refrigeration.

Transfusion of blood in which bacteria have multiplied is rare in modern practice, but has a high mortality rate.^{1–4} Two organisms, *Yersinia enterocolitica* and *Pseudomonas fluorescens*, which share the ability to multiply at 4°C, account for most cases. Established methods for prevention of this problem include the use of good aseptic technique, closed collection systems and refrigeration. Standards of practice in many countries require refrigeration of blood within eight hours of collection.^{5,6}

Contamination by *Y enterocolitica* has increased in frequency over the past decade.^{1,2} It appears that *Y enterocolitica* in donated blood derives from an asymptomatic bacteraemia in the donor. Suggested methods to deal with this organism have included delaying refrigeration of blood,^{7,8} in the hope of allowing host defence mechanisms to kill the organisms or allowing the organisms to be engulfed by phagocytes which can subsequently be removed by filtration.⁹ Studies with *Y enterocolitica* appear to support this concept. Interpretation of these experiments, however, is difficult because of

the complex nature of this organism and its response to environmental conditions.¹⁰

We set out to determine how a delay in refrigeration of donated blood might affect the growth of *P fluorescens*, which probably derives from the donor's skin.¹¹ Simple experiments involving inoculation of small numbers of organisms into freshly donated blood are therefore likely to accurately reflect real contamination events.

Methods

P fluorescens strain 079 was isolated from a fatal transfusion reaction involving contaminated platelet depleted blood.¹² The organism was cultured in nutrient broth at 30°C overnight. Aliquots (0.2 ml) of the broth culture were added to 1.8 ml volumes of nutrient broth containing 10% glycerol and stored at –20°C. One vial was thawed, and a viable count performed to calculate the dilution required to inoculate blood samples.

Donations of whole blood were split into aliquots of approximately 100 ml and weighed to give individual volumes. Each aliquot was fitted with a sampling site coupler. A vial of *P fluorescens* culture was thawed and diluted in saline, and inoculated into each aliquot of blood to achieve a concentration of 5–10 cfu/ml. In the first series of experiments two aliquots from each of six donors were incubated at 4°C for 17 days; another two aliquots from each donation were first stored at 20°C for 24 hours and then placed at 4°C for the next 16 days. In second series of experiments separate aliquots from each of two donors were stored at 4°C after initial incubation at 20°C for zero, six and 24 hours, respectively.

Samples were withdrawn for bacterial culture immediately after inoculation to determine the actual size of inoculum, and subsequently at intervals up to 17 days. On each occasion a 3 ml sample was collected from each bag and mixed with 150 µl of 10% (w/v) saponin (Sigma, Poole, Dorset, UK) to lyse red cells. Triplicate pour plates were prepared by mixing 1 ml samples of lysed blood with 19 ml Isosensitest agar (Oxoid Ltd, Basingstoke, UK). Plates were allowed to set and the number of colonies were counted after 48 hours at 30°C. Samples collected after 24 hours were serially diluted in sterile saline and 1 ml aliquots of appropriate dilutions cultured as described above.

The susceptibility of *P fluorescens* strain 079 to complement was tested by incubating bacteria in citrated plasma and in 10% serum (diluted to 10% in complement fixation test buffer (Oxoid)). Viable counts after one hour at 37°C were compared with saline and buffer controls. *Escherichia coli* R2 was used as a complement sensitive control strain.

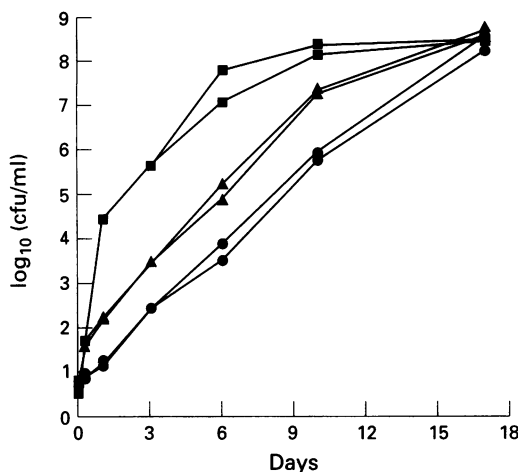
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Growth of *P fluorescens* in experimentally contaminated blood from two donors. Each point represents the mean of triplicate viable counts. ●, Aliquots placed at 4°C immediately after collection; ▲, aliquots held at 20°C for six hours; ■, aliquots held at 20°C for 24 hours prior to refrigeration.

Results

In the initial experiment duplicate aliquots from each of six donors were refrigerated at 4°C immediately after inoculation and a second set of duplicate aliquots were held at 20°C for 24 hours prior to refrigeration. *P fluorescens* grew in all of the units at a temperature dependent rate. There was no apparent lag phase and no evidence of bacterial killing.

To relate these findings more clearly to current blood transfusion practice, we decided to repeat the experiment and to include a holding time of six hours at 20°C prior to refrigeration. Similar results were obtained, with a doubling time of 14.4 hours at 4°C compared with two hours at 20°C (figure). Holding the blood at 20°C for 24 hours resulted in an increase of approximately 100-fold in the number of bacteria present in the blood between 24 hours and six days. The difference in numbers of bacteria then reduced as growth approached a plateau of approximately 3×10^8 cfu/ml.

P fluorescens strain 079 was found to be completely resistant to killing by 10% serum and 100% plasma, which is compatible with the observation of unimpeded growth of the organism in whole blood.

Discussion

The results of our investigation suggest that prolonged storage of blood at 20°C, even for six hours, exacerbates the growth of *P fluorescens*. As contamination by *P fluorescens* is likely to occur from an exogenous source, probably the donor's skin, good aseptic technique with an emphasis on skin cleaning remains central to the prevention of contamination by this organism.¹¹ There is no extant evidence to suggest that filtration would effectively remove *P fluorescens* from contaminated blood.

It is reasonable to question whether the differences in growth rate reported here represent real differences in hazard. We have not demonstrated any difference in the ability of *P fluorescens* to survive at the different temperatures, merely a difference in rate of growth. However, many units are transfused in the first

few days after collection at which time the differences in numbers of organisms could be sufficient to increase the mortality rate. Data on the effect of transfusing large numbers of bacteria into humans are not available, and animal data yield variable results which cannot be directly applied to humans.^{13,14}

It is often assumed that endotoxin is the cause of illness in these circumstances, but it is likely that other bacterial factors also contribute to the outcome.^{15,16} Endotoxin assays are a less sensitive measure of bacterial contamination than are bacterial counts in experimentally contaminated blood¹⁷ and may be influenced by binding of the endotoxin to plasma or red cells. Bacterial endotoxins also vary in their toxicity and the relative potency of *P fluorescens* endotoxin is not known. We believe, therefore, that endotoxin assay results would not have contributed in a major way to the interpretation of our results.

From the evidence presented here, we are concerned about the suggestion to hold blood at 20°C for between six and 24 hours before refrigeration at 4°C. The incidence of colonisation of skin of normal individuals with *P fluorescens* is approximately 1 in 400¹¹ so that the potential for increased morbidity caused by favouring the growth of this organism is high. In view of the lack of clear evidence of benefit from prolonged holding of blood at 20°C we conclude that the practice of rapid refrigeration of blood should not be changed.

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