SINGLE DOSE FLUCLOXACILLIN IN THE TREATMENT OF WOUNDS

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THE UNIVERSITY OF ASTON IN BIRMINGHAM

Single Dose Flucloxacillin In The Treatment Of Wounds

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SUMMARY

Systemic antibiotics are frequently prescribed to patients presenting with contaminated soft tissue wounds in an attempt to prevent development of wound infection. The efficacy of antibiotics employed in this way has not been established.

A double blind, randomised clinical trial to investigate prophylaxis against wound infection in the initial treatment of contaminated wounds was carried out in a hospital accident department. A single oral dose of flucloxacillin 1g was compared with a five-day oral course of flucloxacillin 250mg six hourly. All patients also received long-acting intramuscular penicillin ('Triplopen').

Wounds were assessed seven days after initial treatment using a scored telephone questionnaire. Patients were asked about swelling, redness, pain and exudate associated with their wounds. In cases where the wound assessment suggested the presence of infection, wound swabs were taken if at all possible.

238 patients with contaminated soft tissue wounds were entered into the trial and 202 (85%) were successfully followed up. 100 patients received the single dose of flucloxacillin of whom five were judged to have developed wound infections. 102 patients received the five-day course of whom six developed wound infections. Chi-square analysis of the infection rate in the two treatment groups gave a calculated value, with Yates' correction, of $\chi^2 = 0.18$, ($P > 0.67$), a clear indication of no association between the dosage regime of flucloxacillin and the wound infection rate.

In the clinical situation, pathogenic micro-organisms may often be exposed to levels of antibiotics below the minimum inhibitory concentration and such exposure may alter their susceptibility to phagocytosis. Brief investigations showed that for two clinical isolates of Staphylococcus aureus, a common wound pathogen, low concentrations of flucloxacillin affected bacterial surface hydrophobicity but had little effect on whole blood killing.

Keywords: Wound, Flucloxacillin, Prophylaxis, Single Dose, Clinical Trial

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1. Introduction

1.1 Introduction

It is a common practice in hospital accident departments to prescribe systemic antimicrobial agents to patients who present with wounds which show no clinical signs of infection at the time of treatment. The practice may be described as prophylaxis although it follows a different sequence of events from the antibiotic prophylaxis employed in surgery.

The prophylaxis involved in wounds in accident departments differs from surgical prophylaxis as tissue damage occurs before administration of antimicrobials can be considered and potentially pathogenic micro-organisms are likely to enter the patient's wound at the time of injury. The overall treatment of a wound is directed at cleaning or removing the damaged tissue, reducing the level of contamination, preventing micro-organisms in the wound from developing into an infection, encouraging the healing process and preventing further contamination of the wound. One of the measures often used in an attempt to prevent wound infection is the prescription of antimicrobial agents. There have been relatively few clinical trials investigating the efficacy of antimicrobial agents in this area of medical practice, and those which have been performed have led to varied conclusions. This is a subject appropriate for investigation, where medical practice has made many assumptions and where treatment is continued due to habit rather than scientific data.

1.2 The treatment of wounds in accident departments

The treatment of wounds in hospital accident departments generally consists of cleansing the wound using sterile saline solutions, sterile antiseptic solutions or hydrogen peroxide solution which may or may not be applied under pressure from a syringe. It is notable that the use of sterile irrigating solutions and antiseptics is a relatively new procedure in many hospitals where unsterilised solutions were in use until the mid 1970's. Solutions currently used for irrigation of wounds are generally available in single-use disposable containers whose contents are sterile. Damaged tissue in wounds such as dog bites may be surgically excised and once cleansed, wounds may be sutured, left unsutured or alternatively pulled together with adhesive dressings such as 'Steristrips'.

In addition to the use of surgical wound treatment and dressings, antibiotics may be prescribed in an attempt to prevent wound infection. The decision of whether to prescribe or not may be governed by a number of factors including the wound type and location, the type and degree of contamination, the age of the wound, the age of the patient, the individual views of the prescriber and the policy of the accident department.

A combination of antibiotics commonly prescribed in Stafford in the initial treatment of contaminated wounds is a single dose of long-acting intramuscular penicillin ('Triplopen') and an oral course of flucloxacillin 250mg six hourly for five days. Altogether, the effect of 'Triplopen' (benzylpenicillin 300mg, procaine penicillin 250mg, benethamine penicillin 475mg) lasts for two or three days.

The rationale for the use of 'Triplopen' put forward by Hutton et al. (1978), is the destruction of bacteria, notably clostridia and sensitive pyogenic organisms, thereby reducing the risk of tetanus, gas gangrene and wound infection. The rationale for the use of flucloxacillin is that a large number of local wound infections are caused by Staphylococcus aureus, the majority of which are resistant to penicillin but sensitive to flucloxacillin.

Enquiries at five accident departments in neighbouring health districts revealed considerable variation in the use of prophylactic antibiotics in the treatment of wounds. The response from one department was that antibiotics were never prescribed for soft tissue injuries, that a great deal of reliance was placed upon surgical toilet, and that wound infections were not a problem. At the other end of the scale, medical staff in another department were treating all dirty wounds with antibiotics, notably ampicillin and flucloxacillin or 'Triplopen'. It would seem then that the use of antibiotics for prevention of wound infection varies from district to district, but that the level of prescribing in Stafford is not at great variance with some other local accident departments.

Many wounds which are presented in accident departments are described as dirty or contaminated. The terms dirty and contaminated are generally used interchangeably and are often applied in an inexact way.

A dirty or contaminated wound is usually considered to be one which has been affected by soil, grease, saliva, meat etc., whilst a clean wound is one where the object causing tissue damage was clean e.g. a knife, and where the wound has not been soiled with material likely to harbour micro-organisms.

1.3 Prevention of tetanus in the wounded

Tetanus prophylaxis is an important aspect of wound management and consideration should be given to the risk of tetanus in the treatment of all wounds however trivial.

In patients presenting with dirty wounds who are not adequately immunized against tetanus there is a small risk of the development of clinical tetanus. Two criteria must be fulfilled before tetanus can declare itself. Firstly, virulent organisms must gain access to the tissues, and secondly, the oxidation-reduction potential of these tissues must be low enough to support microbial growth. The second of these two criteria is probably the most important in the development of anaerobic infection and is brought about by impairment of the blood supply to the damaged tissue. A major objective of surgical wound toilet is to ensure the removal of non-viable tissue and foreign bodies, and maintain an environment unsuitable for the growth of anaerobic bacteria (Rubbo, 1966).

Smith et al. (1975) published a set of guidelines for the prevention of tetanus in the wounded, and these are widely used. The guidelines were written in the light of the development of adequate supplies of human tetanus immunoglobulin which became generally available in the United Kingdom in 1974.

The guidelines superseded these authors' previous publication on the subject (Laurence et al., 1966) and will be discussed later.

Active immunisation with adsorbed tetanus vaccine provides the best and safest protection against tetanus and if all wounded patients were known to be actively immune there would be no need for passive protection with antitoxins or antibiotics. In the actively immunised patient a booster dose of adsorbed tetanus vaccine at the time of injury provides simple, safe and effective protection. Careful wound toilet is the only other treatment that should usually be required. The prevention of tetanus in patients who have not been actively immunised has, in the past, caused some therapeutic problems.

In the early 1960's when the use of equine tetanus antitoxin was widespread, there was considerable discussion of the best approach to the passive prevention of tetanus. Filler and Ellerbeck (1960) questioned the large-scale use of equine tetanus antitoxin and noted its tendency to cause adverse reactions and its rapid rate of destruction in patients sensitive to it and in those receiving it on more than one occasion. In patients who are sensitive to equine tetanus antitoxin, the rate that it is destroyed may be increased to such a level that its prophylactic value is almost nil. Furthermore, in the event of clinical tetanus, the effectiveness of antitoxin therapy is reduced in patients who have previously been exposed to it.

Circumstantial evidence has been used to support the use of equine tetanus antitoxin, but its efficacy has not been proved by a controlled clinical trial. An example of the type of information quoted to support the use of antitoxin is an observation that deaths from tetanus after firework injuries on Independance Day in the U.S.A., fell from 102 per 1,000 injuries in 1903, to 3 per 1,000 injuries in 1913, this being ascribed to the use of prophylactic tetanus antitoxin (Cox et al. 1963). It is, however, possible that other factors may have affected the mortality rate, such as an increased awareness of the risk of tetanus and improvements in wound toilet. The antitoxin may have had a beneficial effect on the mortality rate, but the evidence is far from conclusive.

Filler and Ellerbeck (1960) proposed a change in emphasis in passive tetanus prophylaxis from the use of equine tetanus antitoxin to the administration of antibiotics. Their proposals gave consideration to in vitro data regarding the susceptibility of Clostridium tetani to penicillin, tetracycline and chloramphenicol, in vivo data in mice suggesting a decrease in mortality and an increase in survival time of animals exposed to tetanus spores but pre-treated with antibiotics, and the known disadvantages of equine tetanus antitoxin. Filler and Ellerbeck's suggested treatment scheme recommended the use of antibiotics for extensive wounds seen over 48 hours after injury in immunised patients, and all extensive wounds in non-immunised patients, whilst tetanus antitoxin was reserved for use in extensive wounds seen over 48 hours after injury in non-immunised patients. The suggested antibiotic was benzathine penicillin intramuscularly.

Cox et al. (1963) reported the apparently successful withdrawal of tetanus antitoxin from the Accident Department, Sheffield Royal Infirmary, with no apparent increase in the incidence of tetanus. The authors recommended that adequate wound treatment and antibiotics should be substituted for tetanus antitoxin as prophylaxis against tetanus, together with active immunisation by vaccine against future injury. No clinical data was presented to support the notion that antibiotics are helpful when used for tetanus prophylaxis.

In 1964, the Ministry of Health issued a statement concerning protection against tetanus. It was noted that tetanus antitoxin did not need to be used in patients with established basic immunity to tetanus, and that adequate reinforcement was provided by a single dose of tetanus vaccine. It was suggested that in patients with no established basic immunity against tetanus, the use of antitoxin should be considered where surgical toilet was incomplete or delayed. For non-immunised patients whose wounds could be thoroughly treated without delay, wound toilet and the use of antibiotics were recommended, along with initiation of active immunisation. One injection of a combined short- and long-acting penicillin was recommended as an appropriate antibiotic for most cases.

At a time when other clinicians were advocating the use of prophylactic antibiotics as a substitute for tetanus antitoxin, Lucas and Willis (1965), reported an increase in the incidence of casualty associated tetanus in Ibadan, Nigeria, during a period when the use of equine tetanus antitoxin had been discontinued in favour of prophylactic penicillin.

The authors discussed a number of reasons why the substitution of penicillin for antitoxin had been unsuccessful in Ibadan.

A high proportion of the population in Ibadan was not immunised and wounds were often treated after a considerable delay. Furthermore, due to local conditions there was a high risk of wound contamination both before and after hospital treatment. It was suggested that antibiotic prophylaxis may have been considered a satisfactory procedure in Britain and North America where the incidence of tetanus is low, but that it appeared to be unsatisfactory in Ibadan, Nigeria where different local conditions applied.

Recommendations published by Laurence et al. (1966) stressed the virtue of active immunisation against tetanus and the importance of surgical wound toilet. In order to simplify treatment, a scheme of treatment was devised on the basis of immune category (Fig. 1).

Additional prophylaxis consisted of an antibiotic with or without antitoxin. Antibiotic cover was to be provided for at least four days. If the wound was healing well after four days it was suggested that antibiotic cover might be stopped, otherwise antibiotics were to be continued until the wound healed or for at least 10 days in immune category C and four weeks in immune category D.

Recommended antibiotics were oral penicillin V 250mg six hourly or intramuscular fortified procaine penicillin injection daily, or a single dose of intramuscular long-acting penicillin.

Fig.1 Prevention of tetanus in the wounded

All wounds receive surgical toilet

Wounds that are clean, less than six hours old and with negligible tissue damage

Immune category	Treatment
A	Nothing more required
B	Toxoid 1 dose
C	Toxoid 1 dose
D	Toxoid complete course

Other wounds

Immune category	Treatment
A	Nothing more required
B	Toxoid 1 dose
C	Toxoid 1 dose + additional prophylaxis
D	Toxoid complete course + additional prophylaxis

A - Has had a complete course of toxoid or a booster dose within the past year
B - Has had a complete course of toxoid or a booster dose more than 1 and less than 10 years ago
C - Has had a complete course of toxoid or a booster dose more than 10 years ago
D - Has not had a complete course of toxoid or immune status is unknown

Adrenaline injection (B.P.) should be available during prophylactic procedures for anaphylactic shock. In adults 0.5 to 1ml by intramuscular injection.

Laurence et al. (1966)

Tetracycline 250mg six hourly was recommended for patients sensitive to penicillin. The authors suggested that equine tetanus antitoxin was to be used in addition to an antibiotic only if the wound carried a specially high risk of tetanus. The factors thought to be important in governing the use of antitoxin were the extent of wound contamination, the practicability of full surgical toilet, the possibility of a retained foreign body and the age of the wound.

It should be noted that additional prophylaxis was only recommended for patients falling into the category of 'other wounds' and immune category C or D.

The authors' revised guidelines, (Smith et al., 1975) continued to stress the importance of surgical wound toilet and in the light of the introduction of human tetanus immunoglobulin recommended its use in place of antibiotics and equine antitoxin. The authors stated: "equine, bovine and other animal antitoxins may now be regarded as obsolete for tetanus prevention in this country and antimicrobials may no longer be considered to have a primary role".

The current recommendations for the prevention of tetanus are clear. Nevertheless, when the various previous recommendations are taken into account, it is not surprising that there is some confusion over the role of antibiotics as far as tetanus prophylaxis is concerned.

1.4 Antibiotics in the treatment of soft tissue injuries

Each time the protective barriers of the skin are broken through traumatic injury there is a risk of wound infection and antibiotics are frequently prescribed in an attempt to prevent such infection.

There is little clinical data to support the use of antibiotics in the treatment of lacerations. The use of long-acting intramuscular penicillin ('Triplopen') was recommended by Wood (1971) for the initial treatment of hand wounds, following an investigation into the efficacy of various dressings. 265 patients completed a study in which three types of dressing were investigated for their ability to prevent wound infection. Penicillin ('Triplopen') was given at the discretion of the attending doctor, mainly to patients with dirty wounds or to those whose injury had occurred several hours prior to treatment. It was found that 5 out of 60 patients (8%) given 'Triplopen' developed wound infections compared with 57 out of 205 patients (28%) not given 'Triplopen'. The difference in infection rates was said to be statistically significant and on the basis of this result the author recommended the use of prophylactic 'Triplopen' for hand lacerations. It seems undesirable to make strong recommendations based upon observations made following the uncontrolled prescription of 'Triplopen' as factors other than penicillin may have influenced the infection rates in the two groups of patients. For instance, it is not inconceivable that one casualty officer may have had a particularly low threshold for prescription of 'Triplopen' along with a vigorous and successful surgical technique.

As a result, it is possible that a group of patients may have emerged from this investigation with a low incidence of wound infection associated with the prescription of 'Triplopen', but due in fact to good wound toilet.

Edlich et al. (1986), reviewed the use of antibiotics in soft tissue lacerations and described the potential benefits and hazards of antibiotic usage. Antibiotic administration aims to allow optimum healing of wounds without infection, with the possibility of decreased morbidity and a more aesthetically pleasing scar as anticipated additional benefits. Furthermore, successful prophylaxis might reduce the total amount of antibiotic required for the treatment of any one potential infection. On the other hand, it is possible that surgical technique may be relaxed if antibiotics are relied upon. In addition, there is always an incidence of adverse reactions which cause a degree of morbidity and the cost of prophylactic antibiotics should also be considered.

Edlich et al. studied data presented by other authors on this topic and proposed a number of indications for antibiotics in the treatment of wounds. The prescription of antibiotics was recommended for lacerations with a high probability of infection e.g. wounds occurring in high risk anatomical sites such as the feet, wounds presenting with contamination and cases where wound toilet is delayed for longer than six hours.

Two groups of patients in whom it was suggested antibiotics should be mandatory are patients prone to develop infective endocarditis and patients with hip prostheses, on the basis of the significant consequences which might follow the development of endocarditis or an infection of the hip.

Whilst it is clear that the authors thoroughly reviewed the literature on this subject, the basis of the recommendations does not obviously arise from the literature reviewed.

Day (1975) investigated the use of antibiotics in the treatment of 160 patients with non-contaminated wounds requiring suture, presenting within four hours of injury. Wound toilet was performed using chlorhexidine 0.015% & cetrimide 0.15% solution and formal debridement was undertaken as necessary followed by closure under aseptic conditions in an operating theatre. Patients were randomly allocated to receive either intramuscular penicillin ('Triplopen') at the time of wound closure, a wound irrigation of tetracycline 100mg just prior to closure or no antibiotics. Assessment after five to seven days showed that the frequency of infection in the two antibiotic treated groups (23%) was significantly greater than that for the control group (7%), with no real difference in infection frequency between the two antibiotic groups. The author was unable to explain satisfactorily the increase in the incidence of infection associated with the use of antibiotics, although it was suggested that resistant strains which survive antibiotic treatment might be intrinsically highly virulent. The author concluded that minor wounds in fit patients not associated with compound fracture should be treated surgically, without prophylactic antibiotics.

Roberts and Teddy (1977) studied the incidence of wound infection amongst 368 patients with hand lacerations which required suturing, who were randomly allocated to one of three treatment groups. Following wound toilet and suturing, patients with uncomplicated hand lacerations were randomly allocated to receive either an intramuscular penicillin ('Triplopen'), oral flucloxacillin 250mg four times a day for seven days or no antibiotic. 305 patients were successfully followed up seven days after initial treatment amongst whom there were 30 infections (9.8%). There was no significant difference in infection rate between the three groups and the authors concluded that the use of prophylactic antibiotics is not justified, with the possible exception of patients who have never received tetanus prophylaxis. Emphasis was placed on the importance of the instruction of casualty officers in the basic techniques of wound toilet.

Hutton et al. (1978) were aware of the widespread use of long-acting intramuscular penicillin in casualty departments as a prophylactic measure against infection when suturing accidental wounds and noted that little work had been done to justify this treatment. They conducted a trial to determine whether or not the incidence of infection was influenced by giving a single dose of 'Triplopen'. 301 patients presenting with accidental wounds less than four hours old and requiring primary suture were admitted to the trial. All wounds were cleaned thoroughly with 1% cetrimide solution and devitalised tissue was excised. Alternate patients received a single intramuscular dose of 'Triplopen'. Of the 285 patients who returned for suture removal, 10 of the 142 (7.0%) who received 'Triplopen' and 9 of the 143 (6.3%) who received no antibiotic developed wound infections.

The difference in incidence of wound infection between the two groups was not significant. Interesting observations were that penicillin-resistant Staphylococcus aureus was cultured from 17 of the 19 infection sites and macroscopically dirty wounds became infected more often (10.1%) than those which were apparently clean (4.8%). The authors concluded that there was no indication for the administration of a single dose of 'Triplopen' as a prophylactic measure against infection when suturing accidental wounds and suggested that its use was more as a result of tradition than for any logical reason. It was stressed that meticulous toilet and surgical excision in all wounds were the most important factors in the prevention of infection.

Samson and Altman (1977) reached a similar conclusion following a clinical trial of oral dicloxacillin for prophylaxis of infection in minor lacerations. Dicloxacillin was chosen due to its 95% efficacy rate against Gram-positive organisms in the locality of the trial. All wounds were irrigated with saline and soaked for five minutes in an equal mixture of povidone-iodine and saline. All non-viable, questionably viable, or grossly contaminated tissue was aggressively debrided. 1334 patients were entered into the study and on the basis of odd or even hospital number each was assigned to either dicloxacillin 250mg four times a day for seven days or no antibiotic. The progress of only 271 patients was fully analysed. Wound infection developed in 17 of these 271 patients (6.3%) and Staphylococcus aureus sensitive to dicloxacillin was isolated from all of them. Infection developed in 3 out of 67 patients (4.7%) who received dicloxacillin and 14 out of 204 patients (6.9%) who received no antibiotic.

The authors stated that the use of dicloxacillin showed no significant effect upon wound infection rates and suggested that its use is contra-indicated in the prophylaxis of wound infection. Control of infection, it was postulated, was completely dependent on thorough cleansing and careful debridement prior to suturing.

Haughey et al. (1981) reported on the results of a randomized clinical trial which set out to determine the efficacy of prophylactic oral cephalexin in the treatment of soft tissue wounds of the hand. All wounds were scrubbed, rinsed, irrigated with high pressure saline and then debrided if necessary. Patients were randomly assigned to receive cephalexin 250mg four times a day for five days or no antibiotic. At return visits wounds were assessed using various criteria for wound infection. The presence of at least two out of four criteria (warmth, redness extending more than 5mm from the wound margin, tenderness, induration extending more than 5mm from the wound margin) was considered to indicate the presence of infection. Alternatively, the presence of purulence, lymphangitis or lymphadenopathy was taken to indicate infection. Patients who did not return for scheduled appointments were telephoned and questioned about signs of wound infection. Patients initially assigned to the antibiotic group who took none of their treatment were re-allocated to the control group and hence the number in each group was uneven. Out of 394 patients, 160 received cephalexin of whom 17 (10.6%) developed wound infections whilst 234 received no antibiotics of whom 18 (7.7%) developed wound infections. There was no statistically significant difference in infection rates. The authors suggested that antibiotic therapy has no place in the initial treatment of uncomplicated soft tissue hand wounds.

The wisdom of re-allocating non-compliant patients to the control group must be questioned as non-compliance is an intrinsic problem associated with all self-administered medicines.

In a clinical trial of prophylactic antibiotics in simple sutured hand wounds, Grossman et al. (1981) reported a remarkably low incidence of wound infection amongst both antibiotic-treated and untreated patients. The progress of 265 patients with hand wounds was followed until suture removal or satisfactory wound healing. Initial treatment involved irrigation with a minimum of 1 litre of saline, use of a povidone-iodine preparation and a sterile surgical technique including mask and gloves for wound closure. Patients were randomly allocated to receive either oral cephalexin 250mg six hourly for six days or intramuscular cephazolin 1g or a placebo injection at the completion of suturing. The overall infection rate was 1.1% representing 3 infections. There were no infections in the cephazolin group, 2 (2.5%) in the cephalexin group and 1 (1.1%) in the placebo group. These results led the authors to conclude that antibiotics are an unnecessary adjunct in the treatment of simple hand lacerations and no replacement for meticulous wound management. Rutherford and Spence (1980) reported on the progress of 485 patients who had had wounds sutured in an emergency department. Severe infection was recorded in 2.5% of patients and possible infection in a further 4.5%. It was noted that antibiotics were used in 7.4% of patients, sometimes in anticipation of infection, and sometimes on signs of inflammation. The authors considered this to be probably an overuse of antibiotics and contended that a firm policy of giving no prophylactic antibiotics was more likely to lead to good suturing.

Removal of sutures and a short course of regularly changed saline soak dressings was the recommended treatment in the event of an infection.

The position of antibiotics in the initial treatment of soft tissue injuries remains controversial. The weight of evidence from clinical trials suggests that prophylactic antibiotics provide no improvement in wound infection rates compared with placebo, and that the quality of wound toilet is the most important determinant of wound infection.

1.5 Antibiotics in the treatment of mammalian bite wounds

Animal bite wounds are a common problem and are said to account for almost 1% of accident department visits in the United States. Dogs account for 80-90% of all animal bites requiring medical attention (Callaham[1], 1980). A number of authors have expressed views on the optimal initial treatment of bite wounds, but few have been able to support their views with strong clinical evidence.

Lee and Buhr (1960) conducted a retrospective study of 69 patients with dog bite injuries which had been investigated bacteriologically. Wound swabs had been taken from 59 patients on the first day of attendance at hospital, whilst the remaining 10 patients had swabs taken some days later when signs of wound infection were present. 20 of the 69 patients developed a wound infection and in 10 of these cases Pasteurella multocida sensitive to penicillin was associated with the infection, either solely or as one of a number of isolates. The authors stressed the importance of Pasteurella multocida as a wound pathogen following dog bite injury.

Nine of the patients whose treatment was reviewed were prescribed prophylactic penicillin and in only one case did a wound infection develop. The authors contended that the study provided evidence to suggest that prophylactic penicillin should be given to all patients with serious dog-bite wounds and particularly sutured wounds which had shown a high rate of infection (47%). The justification for recommending antibiotic prophylaxis on the basis of nine patients studied retrospectively must surely be questionable.

Callaham[2] (1980), in a widely quoted investigation, reported the results of a clinical trial in which 62 patients with dog bites were followed-up after receiving penicillin V or placebo four times a day for five days. Infection developed in 3 out of 30 (10%) patients treated with penicillin V compared with 8 out of 32 (25%) patients given placebo. The author noted that the difference in infection rates between the two groups was not significant but went on to propose that prophylactic antibiotics may be helpful in dog bite wounds, particularly in wounds with a high incidence of infection such as punctures and wounds of the hand. Callaham also noted that in one patient the infecting organism was Staphylococcus aureus resistant to penicillin, and on this basis he suggested that use of a penicillinase-resistant penicillin might be more logical. No clinical data was provided to support this suggestion.

Jones and Stanbridge (1985) investigated the prophylactic use of a five day course of co-trimoxazole in reducing wound infection rates following dog bites in 113 adults and children aged over three years of age.

78 of the patients enrolled in the trial returned for the one week follow-up and the remainder were assumed to have had uninfected wounds. Amongst 55 patients treated with co-trimoxazole 3 (5.5%) developed wound infections, compared with 8 (13.8%) out of 58 patients given placebo. This difference in infection rates between the two groups failed to reach statistical significance. In a sub-group of patients with hand wounds, there were no infections amongst 23 patients given co-trimoxazole and 4 infections out of 24 patients given placebo. The difference in the rate of infection in hand wounds reached marginal significance (P=0.06). The authors came to the conclusion that routine use of antibiotics for dog bite wounds could not be justified but that wounds to the hand should be considered for antibiotic prophylaxis.

Elenbaas et al. (1984) advocated the prophylactic use of antibiotics in the initial treatment of cat bite wounds following a small clinical trial involving 11 patients. Adults with uninfected wounds presenting within 24 hours of injury were considered for entry in a trial in which patients were randomly assigned to receive oxacillin 500mg or placebo four times a day for five days. Four of six patients receiving placebo, but none of the five receiving oxacillin developed a wound infection and this difference was statistically significant (P=0.045). Interestingly, Pasteurella multocida was found to be the responsible pathogen in all three cases of wound infection which were subsequently drained and successfully treated with oxacillin.

Goldstein et al. (1984) stressed the importance of anaerobic bacteria as part of the oral flora of animals and humans. It was noted that anaerobes are to be found in a large proportion of animal bite wounds (39%), human bite wounds (50%) and clenched fist injuries (56%). Anaerobic bacteria in wounds, according to the authors, are always present in mixed culture with aerobic oral flora, and organisms commonly isolated include Bacteroides species, Fusobacterium species, Peptococcus species, Peptostreptococcus species and Veillonella species. It was recommended that initial empiric antimicrobial therapy for bite wounds should be directed against potential anaerobic as well as aerobic pathogens. No clinical information was presented to support the notion of antimicrobial prophylaxis against anaerobic bacteria in bite wounds. Unsubstantiated recommendations such as this can be used by pharmaceutical manufacturers to promote agents with anaerobic and aerobic activity for use as prophylactic agents without supporting clinical evidence. Furthermore, the isolation of bacteria from bite wounds at the time of initial presentation has been found by other authorities to be a poor marker for subsequent wound infection.

Boenning et al. (1983) studied the effect of penicillin prophylaxis on the infection rate amongst 55 children with non-facial dog bites. Their investigation included wound culture at the time of presentation. Forty per cent of cultures yielded potential pathogens, but none of these results predicted subsequent wound infection. In this study, after the wound cultures were performed, non-viable tissue and foreign matter was removed from all wounds followed by immersion or swabbing with povidone-iodine solution and rinsing with saline.

The experimental group was given penicillin V 250mg four times a day for five days. 1 of 25 patients treated with penicillin V developed a local wound infection and 1 of 30 patients in the control group developed an infection. The recommendations of the authors included suggestions that prophylactic antibiotics should not be given to children with superficial non-facial dog bites and that wound cultures are only indicated in the presence of signs of infection, as initial cultures are of no predictive value.

A particularly low rate of wound infection associated with dog bites was obtained by Zook et al. (1980) working in a plastic surgery department and using a vigorous treatment regime. Due to the nature of the department the patient population seen involved mainly children with lacerations and perforations of the head and neck. 215 injuries, including 188 of the head and neck, in 61 patients were treated using a consistent protocol. The average patient age was 5 years. Pressurised irrigation of wounds was undertaken using a minimum of 500ml 0.9% saline for each wound, delivered from a plastic infusion bag with a pressure cuff arount it, or from a sterilised hand operated pressure pump. Irrigation was followed by debridement and then further pressure irrigation. All patients were prescribed antibiotics at the beginning of the surgical procedure and approximately 55% of patients were admitted to hospital and given intravenous antibiotics for 48 hours, followed by oral antibiotics for a further five days. Patients not admitted to hospital were prescribed oral antibiotics for a period of five days. Only 1 wound infection developed, an infection rate of 0.47%.

It is not possible to be sure of the reason for the very low infection rate, and whilst the use of an aggressive and lengthy wound toilet procedure provides a possible explanation, the authors seemed obliged to commend the whole of their method.

Rosen (1985) reported on a trial involving the random allocation of 33 patients with recent dog bite wounds to receive either penicillinase-resistant antibiotics or placebo, following wound toilet involving pressure irrigation with at least 100ml of normal saline both before and after debridement. Patients were asked to return to the accident department 48-72 hours after treatment for wound assessment. Telephone follow-up was attempted for all patients not returning for examination within 72 hours. 9% of patients developed infected wounds and it was noted that hand wounds became infected significantly more often than other wounds.

Rosen concluded that prophylactic antibiotics were not beneficial in the initial treatment of dog bite wounds, suggested that emphasis should be placed on both the importance and techniques of wound debridement and used the work of Zook et al. (1980) to support the idea that particular attention to wound toilet can almost eliminate wound infection completely.

Skurka et al. (1986), published the results of a clinical trial in which the dog bite wounds of 39 children were cultured and then irrigated with povidone iodine and pressurised saline prior to prescription of oral penicillin V or placebo for two days.

Initial wound cultures were of no predictive value for the development of infection, prophylactic penicillin failed to prevent wound infection and the authors concluded that good local care on presentation seemed to be the most important factor in determining future infection. It was suggested that due to the increasing resistance of common pathogens to penicillin, further studies might involve agents such as clavulanate-potentiated amoxycillin ('Augmentin').

In view of the controversy regarding the value of prophylactic antibiotics for bite wounds, it is probably not surprising to find authors of review articles recommending their continued use for wounds in patients with identifiable risk factors (Callaham[1], 1980; Snook, 1982; Anon, 1983), however, there appears to be little supporting evidence based upon clinical trials.

1.6 Povidone-iodine in the treatment of minor wounds

The antiseptic agent povidone-iodine, though not routinely used in Stafford for the treatment of wounds, may be useful in the reduction of wound infection rates. A number of workers have reported on its use, and their results may be of some interest.

Povidone-iodine spray has been reported to reduce infection rates in wounds presented in accident departments. Morgan (1978) reported on an investigation in which 600 patients with superficial wounds considered suitable for primary suture were allocated to two groups of 300 each. In one group the wound was sprayed with povidone-iodine before suture whilst in the other, no spray was used.

The cleansing and treatment of wounds was said to be the same in all other respects and no prophylactic antibiotics were used. Patients were asked to return for assessment six days later. Wound infection was defined as a definite discharge from which a swab could be taken.

The results of the investigation showed that the use of povidone-iodine spray was associated with a statistically significant reduction in the wound infection rate from 14% in the control group to 6% in the group whose wounds were sprayed ($P < 0.025$).

Naunton Morgan et al. (1981), reported on the use of povidone-iodine dry powder spray as prophylaxis against wound infection in patients with injuries to the head or neck or to the hand or forearm. 628 patients were entered into a study in which a standard system of wound toilet, suture, dressing and tetanus prophylaxis was adhered to and the use of povidone-iodine was randomised.

In accordance with the policy of the Accident Department, St. Bartholomew's Hospital, London, on tetanus prophylaxis, intramuscular penicillin ('Triplopen') was administered to patients who were inadequately immunised at the time of injury and whose wounds were defined as dirty i.e. sustained out of doors or indoors with animal contamination.

The use of povidone-iodine was associated with a reduction in the infection rate in wounds of the head and neck, but this did not reach statistical significance. In the wounds of the forearm and hand there was a statistically significant reduction in the infection rate ($P < 0.02$).

The authors recommended the use of povidone-iodine dry powder spray as a simple, safe and efffective prophylactic agent for the prevention of wound infection.

Povidone-iodine may well be a useful agent for the prevention of local wound infections. The effect of other commonly used agents, e.g. chlorhexidine/cetrimide solution and hydrogen peroxide solution, on the incidence of wound infection in accidental wounds appears to be considerably less well documented. It is likely that commercial interests have favoured investigations into the efficacy of modern proprietary preparations containing povidone-iodine rather than traditional generic products.

1.7 Antimicrobial prophylaxis in medicine and surgery

Antimicrobial prophylaxis has a wide variety of applications in both medicine and surgery and may employ multiple dose or single dose regimes. Recommendations for specific applications of antimicrobial prophylaxis are given in the British National Formulary (1987).

Prophylaxis is recommended for prevention of secondary cases of meningitis, diphtheria and tuberculosis in appropriate contacts of the infected patient. This type of prophylaxis is likely to be prescribed to contacts after exposure to the infection and therefore bears similarities to prophylaxis in contaminated wounds.

The most common application of prophylaxis is in surgery where it is well established for a number of procedures and was reviewed in the Drug and Therapeutics Bulletin (1981). Surgical prophylaxis generally involves the administration of one or more antimicrobial agents to the patient immediately prior to a procedure, allowing the operation to be conducted under antimicrobial cover. The aim of the technique is to produce high levels of antimicrobial agent in the tissues at the time of greatest risk of microbiological contamination i.e. during the surgical procedure. Subsequent doses of antimicrobials may be administered during or after the surgical procedure. Prophylaxis should generally last for no more than forty eight hours as resistant bacteria may develop and extended therapy is of no further benefit and is wasteful.

In addition to the procedures for which recommendations are given in the British National Formulary, many other areas have been investigated where surgical prophylaxis may reduce morbidity.

1.8 The use of antimicrobial agents in single doses

Antimicrobial agents have been successfully administered in single doses in a number of therapeutic fields such as the treatment of gonorrhoea, syphilis, trichomonal vaginitis, vaginal candida infections and urinary-tract infections, and prophylactically in surgery.

1.8.1 Single dose treatment of urinary-tract infections

A number of studies have investigated single-dose treatment of urinary-tract infections, particularly in uncomplicated infections in women.

The first study, conducted by Grüneberg and Brumfitt (1967), compared the efficacy of a single dose of the long-acting sulphonamide sulphormethoxine (Fanasil) with a seven day course of ampicillin 500mg eight hourly. Results showed that either treatment cured 22 out of 25 patients (88%) as indicated by follow-up after two and six weeks. The incidence of side-effects, mainly rashes or diarrhoea, was significantly lower amongst patients treated with the single dose of sulphormethoxine (4%) compared with those treated with ampicillin (16%).

Bailey and Abbott (1978) compared a single dose of co-trimoxazole (2.88g) with a five day course of 0.96g twice a day in 40 women with urinary-tract infections. In both groups of patients 17 out of 20 (85%) were considered cured one week after completion of treatment. It was concluded that single-dose therapy for uncomplicated urinary tract infections is simple, effective, economical and well tolerated by patients.

The treatment of urinary tract infections using single doses of amoxycillin 3g, co-trimoxazole 1.92g or trimethoprim 400mg was investigated by Harbord and Grüneberg (1981). 64 women with symptoms suggestive of urinary-tract infection and two mid-stream urine specimens showing a pure growth of more than 100,000 organisms per ml were randomly assigned to receive one of the three treatments. On the basis of a urine specimen seven days after treatment the cure rates were 18 out of 20 (90%) for amoxycillin, 21 out of 24 (87.5%) for co-trimoxazole and 19 out of 20 (95%) for trimethoprim. The authors suggested that single-dose treatment of urinary tract infection should be tried more widely.

Fang et al.(1978) used the antibody-coated bacteria technique in order to distinguish lower urinary-tract infections (negative) from upper urinary tract infections (positive). The antibody-coated bacteria technique may allow the site of a urinary-tract infection i.e. kidney or bladder to be elucidated. It is suggested that infection of the kidney causes coating of the infecting bacteria with antibodies through the immune response, whilst this does not occur in infections of the bladder. On the basis of the results of this test, 43 female patients with urinary-tract infection without antibody-coated bacteria in the urine were identified and assumed to have lower urinary-tract infections. 22 patients were given a 3g single dose of amoxycillin whilst the remaining 21 patients were treated with a ten day course of amoxycillin and all patients were cured of their infection.

A small number of investigators have found single-dose treatment of urinary-tract infections to be less effective than conventional therapy. Greenberg et al. (1981) compared a single 2g oral dose of cefaclor with a course of cefaclor 250mg three times a day for ten days in the treatment of uncomplicated urinary-tract infection in 52 women. Four weeks after the completion of therapy only 10 out of 30 (33%) patients given the single dose had culture-negative urine compared with 18 out of 22 (81%) given the ten day course. From retrospective analysis of results of the antibody-coated bacteria test in this investigation it was found that the cure rate was similar using single-dose or multiple-dose therapy in those patients for whom the test was negative.

The authors suggested that the test might be used to identify patients with urinary-tract infections who would be likely to respond to single-dose therapy.

Eriksson et al. (1981) reported on 59 women presenting with uncomplicated urinary-tract infection who were treated with either a single dose of 3g amoxycillin or a seven day course of sulphamoxole 500mg twice daily. In the single dose group treatment was successful in 18 out of 28 patients (64%), whereas 28 out of 31 (90%) patients were successfully treated in the group who received the seven day course. Differences in therapeutic outcome in this trial may have been due to differences in sensitivity of the infecting organisms to the two antibiotics used rather than differences in the dosing schedule.

In a review of single dose antimicrobial treatment of uncomplicated urinary-tract infections, Philbrick and Bracikowski (1985) pooled data from six trials employing single-dose amoxycillin and four trials involving single-dose co-trimoxazole. Single-dose amoxycillin (3g) provided a significantly lower cure rate (69%) than multiple-dose treatment (84%), but single-dose co-trimoxazole was indistinguishable from multiple-dose therapy. However, the number of patients treated with co-trimoxazole was not enough to exclude type II error, i.e. an erroneous conclusion that there is no difference between two treatments when in fact there is a difference which is too small to be detected with the amount of data available. However, Freiman et al. (1978) had already noted that the majority of clinical trials which labelled therapies as "no different from control" had not given the new therapies a fair test through the use of inadequate samples.

It has been suggested by Ronald (1986) that single-dose therapy with four tablets of co-trimoxazole should be the preferred treatment for acute cystitis and asymptomatic bacteriuria in adult women, with a follow-up urine culture after two weeks to confirm cure. The author noted that only relatively small numbers of patients have been included in published studies comparing single-dose with multiple-dose therapy, but that forty-six out of fifty studies found the two therapies to be equally effective for the treatment of acute cystitis in adult women. It was also noted that advertising has not identified single-dose therapy as an effective treatment. There is clearly likely to be little commercial interest in promoting single-dose therapy instead of more prolonged courses.

In this area of therapeutics, much has been written on the subject of the best way to treat an infection but a final conclusion has not been firmly established due in part to the relatively small numbers of patients included in the published trials allowing the frequent possibility of Type II error.

1.8.2 Treatment Of Other Infections With Single-Dose Therapy

In the treatment of gonorrhoea, a single intramuscular dose of procaine penicillin is recommended along with a single dose of oral probenecid, BNF (1987). Alternatively a single dose of ampicillin or amoxycillin may be given orally along with a single dose of probenecid (Garrod et al. , 1981). It is particularly beneficial to use single-dose therapy in the treatment of sexually transmitted diseases such as gonorrhoea since the success of the treatment may be jeopardised by poor attendance at hospital clinics or poor compliance with self-medication.

Vaginal trichomoniasis may be treated successfully with a single oral dose of metronidazole 2g as an effective and convenient alternative to a seven day course (Garrod et al., 1981).

1.8.3 Single Dose Antimicrobial Prophylaxis

The use of a single prophylactic dose of an antimicrobial agent has been investigated for a number of procedures. Guidance is given in the BNF (1987) on the use of single-dose prophylaxis in a number of types of abdominal surgery. It is suggested that operations on the stomach or oesophagus for carcinoma, or cholecystectomy in patients with possibly infected bile should be covered with a single pre-operative dose of gentamicin or a cephalosporin. Resections of the colon and rectum for carcinoma, and resections in inflammatory bowel disease should be covered with a single pre-operative dose of gentamicin and metronidazole, and hysterectomy should be covered by a single dose of metronidazole.

Strachan et al. (1977) reported on the efficacy of single-dose prophylaxis against wound sepsis after cholecystectomy using cephazolin 1g intramuscularly pre-operatively. Wound sepsis was recorded in 11 out of 65 control patients (16.9%) who were not given the antibiotic compared with 2 out of 63 patients (3.2%) given the single pre-operative dose. The difference in infection rates was statistically significant ($P < 0.025$). Cholecystectomy is associated with a high risk of wound infection (11-20%) which is in part due to the presence of bacteria in the bile at operation which account for 20 to 40% of post-operative wound infections. The high risk of bacterial contamination in this procedure may account for the success of prophylaxis.

Greenall et al. (1979), found a single intravenous dose of metronidazole to provide convenient and effective prophylaxis against wound infection following appendicectomy, a procedure which is commonly followed by wound infection involving Bacteroides spp. 100 patients undergoing appendicectomy were randomised to receive either an infusion of metronidazole 500mg in 100ml or physiological saline 100ml over 10 minutes following induction. 51 patients received saline and 49 metronidazole. Pus producing wound infection was seen in 1 wound (2%) from the metronidazole-treated group and 12 wounds (24%) from the control group. The authors noted that the majority of organisms isolated from the infected pus of the group given saline were not susceptible to metronidazole. It is possible that obligate anaerobes such as Bacteroides fragilis may inhibit phagocytosis and thereby allow other organisms to multiply (Ingham et al., 1977). Such a mechanism might explain the effectiveness of metronidazole in reducing the incidence of infections involving a wide spectrum of organisms.

The use of a single pre-operative dose of intravenous metronidazole has also been advocated by Arnbjörnsson and Mikaelsson (1984) as prophylaxis against infectious complications following appendicectomy in children. When no pre-operative antibiotic prophylaxis was employed, 32 infectious complications developed out of 301 patients (10.6%), however, when the use of metronidazole 7.5mg per kg intravenously as a single pre-operative dose was instituted the rate of infection fell significantly to 1 out of 215 patients ($< 0.5\%$). This was claimed to be the lowest reported rate of infectious complications following appendicectomy.

A single dose of antibiotic has been shown to be effective in reducing infective complications following cesarean section in patients at high risk of infectious morbidity. Saltzman et al. (1985) reported on 100 women who underwent cesarean section and who were considered at high risk for development of post-operative infections because they had been in active labour or had ruptured membranes for longer than four hours. Patients were randomly assigned to receive either placebo or a single 2g dose of ceftizoxime at the end of cord clamping. The incidence of endometritis was significantly reduced from 12 out of 49 (24.5%) in the placebo group to 3 out of 50 (6.0%) in the antibiotic treated group. Febrile morbidity was also significantly reduced from 16 out of 49 (32.7%) in the placebo group to 7 out of 50 (14.0%) in the treated group.

The British Society for Antimicrobial Chemotherapy (1982) has recommended the use of prophylactic single-dose antibiotics for the prevention of endocarditis in patients with heart-valve lesions, septal defects, patent ductus or prosthetic valves undergoing dental procedures. There is no firm evidence in man for the protective effects of antibiotics for dental procedures and fewer than 15% of patients with infective endocarditis give a history of dental treatment in the three months preceding their illness, however it is suggested that circumstantial evidence is strong enough to support the use of prophylactic antibiotics in this way. A 3g dose of oral amoxycillin is recommended for dental procedures in patients who are at risk. This recommendation has been widely adopted and it has been noted in the Drug and Therapeutics Bulletin (1985) that it is unlikely for logistic and ethical reasons that the efficacy of the practice will ever be determined.

A use of antibiotics which may be regarded as partly prophylaxis and partly treatment of infection is the antibiotic cover which may be given to drainage of abscesses. Blick et al. (1980) reported on the use of clindamycin in the surgical treatment of acute abscesses. Patients with an abscess, defined as at least 3ml of pus, were randomly allocated to one of four treatment groups and received either clindamycin 300mg intramuscularly pre-operatively and 150mg orally six hourly for four days post-operatively, or the pre-operative dose only, or the post-operative course only, or no antibiotic at all. Blood filling the abscess cavity after intramuscular clindamycin was found to have the same drug concentration as venous blood. The results of the trial are shown in table 1.

The authors noted that amongst forty patients who did not receive pre-operative intramuscular clindamycin there were five cases of bacteraemia, one of which developed into septicaemia. Amongst the forty patients who received pre-operative intramuscular clindamycin there were no cases of septicaemia and one case of bacteraemia which was due to an organism resistant to clindamycin. It was concluded that a single injection of an effective antibiotic is sufficient to protect the patient against bacteraemia and permit optimum healing.

1.9 Advantages of single-dose antibiotic therapy

Where single-dose prophylaxis or treatment of infection can be shown to be as effective as multiple-dose therapy the benefits may be severalfold:

(i) In terms of convenience to the patient, the treatment becomes shorter and may mean a reduction in time spent in hospital.

Table 1 **Clindamycin in the surgical treatment of acute abscesses**

Antibiotic Treatment	Number of patients in group	Number of patients with bacteraemia	Number of patients with septicaemia	Mean number of days healing time
Clindamycin pre-op Clindamycin post-op	19	0	0	9.63
Clindamycin pre-op Placebo post-op	21	1	0	9.66
Placebo pre-op Clindamycin post-op	19	4	1	12.95
Placebo pre-op Placebo post-op	21	0	0	16.05

Blick et al. (1980)

(ii) A reduction in in-patient stay may reduce hospital costs.

(iii) The frequency of adverse drug reactions may be reduced.

(iv) Single-dose therapy is generally less expensive than multiple-dose therapy in terms of the amount of antibiotic required.

(v) Single-dose therapy reduces the exposure of bacteria to antibiotics and may extend the period of usefulness of antibiotic agents.

(vi) Supervised administration of a single dose of an antibiotic guarantees patient compliance.

1.9.1 Potential advantages of a single oral dose of flucloxacillin in an accident department

If a single oral dose of flucloxacillin successfully replaced a five day course in patients attending the accident department with contaminated wounds at least 3 benefits might be expected to be seen immediately.

(i) Patients leave the department having received all their prescribed medicine and with no instructions to follow regarding any course of antibiotics.

(ii) Compliance is guaranteed.

(iii) A decrease in pharmacy expenditure. A five-day course of prophylactic flucloxacillin 250mg six hourly costs around £1 compared with 20p for a single dose of 1g.

Approximately 1,000 courses of prophylactic flucloxacillin are used by the accident department in Stafford annually, representing a potential saving of £800 per year in Mid-Staffordshire Health Authority. It should be noted that the cost of flucloxacillin has fallen by around 75% over the past three to four years due to the availability of generic products.

2. Methods

A randomised, double-blind clinical trial was conducted in the Accident and Emergency Department, Stafford District General Hospital between March 1986 and April 1987. Approval was obtained from the Mid-Staffordshire Health Authority ethical committee.

Full details of the trial are given in the Flucloxacillin Trial Protocol (Appendix 1) and the reader may find it useful to refer to that section at this point.

2.1 Patient numbers

It was estimated that the number of patients likely to be recruited to the trial over a period of one year might be about 400. This was based on a count of prescriptions issued from the Accident and Emergency Department over a period of one month in 1985. 140 prescriptions for flucloxacillin had been issued with approximately half of the usage aimed at preventing the development of wound infection and the remainder for the treatment of established infections. Taking into account the selection and exclusion criteria of the trial, around 35 patients would have been eligible for entry to the study during that month.

2.2 Patient selection

Patients presenting with contaminated soft tissue wounds who fulfilled all of the selection criteria and none of the exclusion criteria were invited by the attending doctor to participate in the trial. The doctor explained the nature of the investigation and asked the patient to read and sign the consent form (Appendix 1b).

The consent form was designed to inform patients about the purpose of the trial and the commitment required, without the use of an excessive number of medical or technical terms.

2.2.1 Selection criteria - contaminated wounds

The term 'contaminated' was applied in a broad sense. Wounds were considered to be contaminated if they had been in contact with soil, gravel, greasy machinery, agricultural or gardening equipment, raw meat, saliva or any other potential source of microbiological contamination.

2.2.2 Exclusion criteria

1. Patients taking antibiotics prior to presentation, due to the strong possibility of concurrent therapy affecting the bacterial flora at the wound site.
2. Patients describing previous intolerance to penicillins such as hypersensitivity reactions were prescribed alternative antibiotics e.g. erythromycin.
3. Patients who did not receive 'Triplopen' e.g. due to refusal on the grounds of pain, in order that the only difference in antibiotic treatment given to patients entering the trial should lie in the dosage of flucloxacillin.
4. Patients with wounds already showing signs of infection such as erythema or pus at presentation.
5. Patients presenting a considerable time after injury i.e. more than 24 hours, as the early treatment of contaminated wounds may be an important factor in the reduction of the microbiological challenge at the wound site and the risk of infection.

6. Patients unable to take capsules, as placebo medication was only available in that form.
7. Patients taking oral contraceptives, as many antibiotics particularly broad-spectrum antibiotics such as ampicillin and tetracycline may impair the activity of oral contraceptives (Stockley, 1981; Hansten, 1985). Although flucloxacillin is not particularly noted for its interference with oral contraceptives, the possibility of problems could not be ruled out as a 1g dosage is not commonly used.
8. Patients unavailable for wound assessment by telephone at home or at work.

2.3 Treatment packs

The trial medication was packed as described in Appendix 1a in plastic 'Snap-safe' containers within a small cardboard dispensing carton. Inside the cardboard carton, in addition to the medication, was a package insert (Appendix 2) reinforcing the instructions given on the medicine containers and by the hospital staff.

Flucloxacillin 250mg capsules and matching placebo capsules were obtained from H.N. Norton & Co. Limited, South Woodford, London.

2.4 Assessment

Patients' wounds were assessed by structured telephone questionnaire (Appendix 1c) seven days after initial treatment.
If wound assessment was not possible at that time, questions were asked with reference to the state of the wound after seven days.

During the assessment, the condition and progress of the wound was discussed in terms of swelling, redness, pain and exudate and a numerical score was allocated to each patient on the basis of these findings. In addition, patients were asked to comment on any observed symptoms during treatment. These were noted and an initial attempt was made to judge the probability of relationship to flucloxacillin intake.

Patients were asked to describe their compliance with their in-instructions and each individual's compliance was defined as either 'good', 'uncertain' or 'poor'. 'Good' compliance was defined as a claim to have taken one capsule either four times a day for five days or three times a day for six or seven days. 'Uncertain' compliance was recorded if the claimed adherence to the instructions was inconsistent with the number of capsules supplied or if patients could not describe their own perceived compliance. 'Poor' compliance was recorded if patients claimed to have taken capsules less frequently than eight-hourly.

2.5 Pilot study

In order to ensure that patients would be likely to understand and give meaningful answers to the telephone wound assessment, a brief pilot study was undertaken. Details were noted of 72 patients aged 16 to 64 prescribed prophylactic flucloxacillin in the initial treatment of wounds. Of these, 30 patients would have been suitable for entry to the trial. One week later, 12 were contacted by telephone and had their wounds assessed using the scored questionnaire (Appendix 1c). All of these patients seemed to respond adequately to the structured questions.

One patient achieved a wound score of 7 points strongly suggestive of infection. The presence of infection was confirmed during a clinical examination by the consultant surgeon.

2.6 Infected wounds

If any wound assessment suggested the presence of infection, patients were encouraged to visit either the Accident and Emergency Department or their General Practitioner for additional treatment and a wound swab.

Culture and sensitivities of wound isolates was performed by the Microbiology Department, St. George's Hospital, Stafford. Swabs were incubated at 37^{o}C both anaerobically and aerobically for 24 hours on Columbia agar plates containing 7% defibrinated horse blood (Difco Laboratories, East Molesey, Surrey). Any bacterial colonies found on examination after incubation were identified by standard methods.

3. Results

3.1 Matching of patients

There are a number of variables which might have had the potential to influence the outcome of wound treatment. Details of the distribution of variables between the two treatment groups are shown in tables 2, 3, 4 and 5. Chi-square analysis suggested the randomised allocation of patients to the two treatment groups provided well matched patients with respect to age, sex, wound type and wound location.

3.2 Rates of wound infection amongst the two treatment groups

In all, 238 patients were entered into the trial. 36 patients who could not be contacted for wound assessment or who had been entered into the trial in contravention of exclusion criteria have been excluded from the analyses. 202 patients (85%) were successfully interviewed using the structured telephone questionnaire. The distribution of patients between the two treatment groups and the frequency of wound infection is shown in Fig 2. Chi-square analysis of the infection rate in the two treatment groups gave a calculated value with Yates' correction of $\chi^2 = 0.18$, $(P > 0.67)$, so there is a clear indication of no association between the dosage regime of flucloxacillin and the wound infection rate. The P value describes the probability of obtaining a χ^2 value as large or larger than the calculated value when there is no association between two variables. If the P value is large it is very likely that the result is a random one.

Table 2 Dosage Of Flucloxacillin And Patients' Age

Age/years	Number of Patients (%)		
	Single dose of flucloxacillin	Five-day course of flucloxacillin	Total
16→24	38 (38.0)	43 (42.2)	81 (40.1)
25→34	23 (23.0)	20 (19.6)	43 (21.3)
35→44	13 (13.0)	14 (13.7)	27 (13.4)
45→54	14 (14.0)	16 (15.7)	30 (14.8)
55→64	12 (12.0)	9 (8.8)	21 (10.4)
Total	100	102	202

$\chi^2 = 1.09$, $P > 0.90$ with 4 degrees of freedom

Table 3 Dosage Of Flucloxacillin And Patients' Sex

Sex	Number of Patients (%)		
	Single dose of flucloxacillin	Five-day course of flucloxacillin	Total
Male	82 (82.0)	88 (86.3)	170 (84.2)
Female	18 (18.0)	14 (13.7)	32 (15.8)
Total	100	102	202

$\chi^2 = 0.69$, $P > 0.41$ with 1 degree of freedom

Table 4 Dosage Of Flucloxacillin and Wound Type

Wound Type	Number of Patients (%)		
	Single dose of flucloxacillin	Five-day course of flucloxacillin	Total
Laceration	44 (44.0)	46 (45.1)	90 (44.5)
Bite	32 (32.0)	34 (33.3)	66 (32.7)
Puncture Wound	13 (13.0)	13 (12.7)	26 (12.9)
Crush Injury	11 (11.0)	8 (7.9)	19 (9.4)
Graze	0 (0.0)	1 (1.0)	1 (0.5)
Total	100	102	202

$\chi^2 = 0.61$, $P > 0.89$ with 3 degrees of freedom*.

*Wound types 'laceration' and 'graze' were combined for the purpose of chi-square analysis, leaving 4 possible wound types.

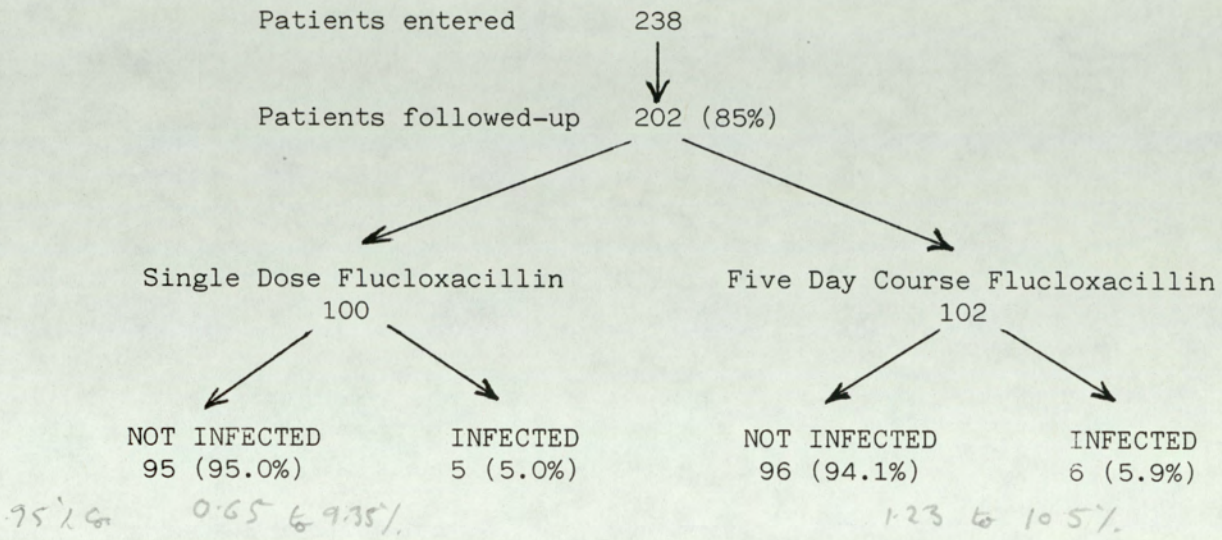
Table 5 Dosage Of Flucloxacillin And Wound Location

Wound Location	Number of Patients (%)		
	Single dose of flucloxacillin	Five-day course of flucloxacillin	Total
Hand	61 (61.0)	65 (63.7)	126 (62.4)
Arm	6 (6.0)	7 (6.9)	13 (6.4)
Foot	9 (9.0)	7 (6.9)	16 (7.9)
Leg	16 (16.0)	13 (12.7)	29 (14.4)
Head	6 (6.0)	7 (6.9)	13 (6.4)
Body	2 (2.0)	3 (2.9)	5 (2.5)
Total	100	102	202

$\chi^2 = 0.97$, $P > 0.91$ with 4 degrees of freedom*.

*Wound locations 'head' and 'body' were combined for the purpose of chi-square analysis, leaving 5 possible wound locations.

Figure 2 **Distribution of wound infections between patients treated with a single dose of flucloxacillin or a five-day course**



Overall infection rate = 5.4%

χ^2 = 0.18, P > 0.67 with 1 degree of freedom.

3.3 Wound scores

The wound scores associated with the two flucloxacillin treatment groups are shown in table 6. Chi-square analysis gave a value of $\chi^2 = 5.1$, $(P > 0.27)$, suggesting no association between the wound score distribution and the flucloxacillin dosage regime.

3.4 Wound Infections

11 patients' wounds were judged to have become infected, 5 of whom had received the single dose of flucloxacillin and six of whom had received the five day course. Details of these wounds and any micro-organisms isolated from them are shown in tables 7 and 8.

3.5 Type and location of infected wounds

The distributions of wound type and wound location amongst the patients with wounds considered to be infected are shown in tables 9 and 10. Due to the low incidence of wound infection overall, the number of patients with infections associated with any specific wound type or location is small. There are no particularly notable trends towards high or low wound infection rates amongst the various wound types and locations.

3.6 Adverse Reactions

Of the 202 patients, 59 reported adverse effects which may have been related to antibiotic treatment. The problems reported and their frequency are shown in table 11. Chi-square analysis of the proportion of patients in each group reporting possible adverse reactions showed no statistically significant difference between the two dosage regimes, $\chi^2 = 2.60$ $(P > 0.10)$.

Table 6 Wound score and dosage of flucloxacillin

Wound score: points	Number of patients		
	Single dose of flucloxacillin	Five-day course of flucloxacillin	Total
0	50	59	109
1	24	17	41
2	12	6	18
3 or 4	9	14	23
5 or more	5	6	11
Total	100	102	202

$X^2 = 5.1$, $P > 0.27$ with 4 degrees of freedom

Table 7 **Five Cases Of Wound Infection Amongst Patients Initially Treated With A Single Dose Of Flucloxacillin**

Case	Age	Sex	Occupation	Wound Description	Compliance	Wound Score	Wound Swab Results	Antibiotic Sensitivities of Wound Isolates
1	21	M	Miner	Laceration to left knee whilst playing rugby. Contaminated with mud.	Poor	5	Heavy growth of beta-haemolytic Streptococcus group A	Sensitive to: Penicillin, Flucloxacillin, Erythromycin Resistant to: Tetracycline
2	34	M	Engineer	Laceration to left index finger on grass cutting equipment.	Good	5	No wound swab taken due to prescription of 'Cicatrin' powder	-
3	42	F	Press Operator	Dog bite on right forearm. V-shaped laceration and one puncture wound.	Good	8	'No Bacterial Growth'	-
4	25	M	Miner	Laceration to right forearm. Hit by rockfall from roof of mine	Good	5	Scanty growth of Staphylococcus aureus	Sensitive to: Flucloxacillin, Erythromycin, Co-trimoxazole, Cephradine etc. Resistant to: penicillin
5	45	F	Assembler	V-shaped laceration to right index finger on machinery at work.	Good	8	No wound swab sought due to prescription of 'Cicatrin' powder by G.P.	-

Table 8 Six Cases Of Wound Infection Amongst Patients Initially Treated With A Five-Day Course Of Flucloxacillin

Case	Age	Sex	Occupation	Wound Description	Compliance	Wound Score	Wound Swab Results	Antibiotic Sensitivities of Wound Isolates
6	18	M	Factory	Crushed right hand between two metal plates.	Good	8	Moderate growth of Staphylococcus aureus	Sensitive to: Flucloxacillin, Erythromycin, Co-trimoxazole, Cephradine, etc. Resistant to: Penicillin
7	21	M	Unemployed	Laceration to face whilst cutting a bicycle wheel on machinery.	Uncertain	5	No wound swab obtained. Patient did not seek medical attention	-
8	33	M	Engineer	Dog bite to left little finger.	Good	7	No wound swab obtained. Patient did not seek medical attention	-
9	47	M	Publican	Caught right hand under a barrel. Deep laceration of ring finger and superficial laceration of little finger.	Good	4	NO BACTERIAL GROWTH	
10	51	M	Miner	Fell and cut base of thumb at work.	Poor	7	No wound swab obtained due to damage in transit by post	-
11	19	M	Farmworker	Lacerated right hand on a broken beer glass	Good	5	NO BACTERIAL GROWTH. nb. 'Cicatrin' powder applied to wound four days prior to wound swab	-

Table 9 Wound type amongst infected wounds

Wound type	Number of patients with infected wounds	% Infection rate for this wound type
Laceration	7	8
Bite	2	3
Puncture wound	0	0
Crush injury	2	11
Graze	0	0

Table 10 Wound location amongst infected wounds

Wound location	Number of patients with infected wounds	% Infection rate for this wound location
Hand	7	6
Arm	2	15
Foot	0	0
Leg	1	3
Head	1	8
Body	0	0

Table 11 Reported adverse effects and dosage of flucloxacillin

Adverse reaction reported	Number of patients (%)		
	Single dose of flucloxacillin	Five-day course of flucloxacillin	Total
Diarrhoea/Increased frequency of motions	11	14	25
Other gastro-intestinal e.g. nausea, dry mouth	3	9	12
Malaise	6	4	10
Rash	1	2	3
Other	3	6	9
Total	24 (24)	35 (34)	59 (29)

There does, however, appear to be a slight tendency towards a higher incidence of adverse effects amongst the patients treated with the five day course of flucloxacillin, but a larger sample would have been needed to determine whether this was significant.

3.7 Compliance

Compliance of patients with the instruction to take one capsule every six hours for five days was assessed by questioning over the telephone (Appendix 1c) and as such is perhaps better described as claimed compliance. Patients' claimed compliance is shown in table 12. It is noteworthy that the overall rate of claimed compliance is very high (76%). Comparison of the two treatment groups showed no statistically significant difference in claimed compliance, $\chi^2 = 1.34$, $(P > 0.51)$.

Claimed compliance of patients with wound infections is shown in table 13, with no notable differences from the overall pattern of compliance.

Table 12 **Claimed compliance (all patients) and dosage of flucloxacillin**

Claimed Compliance	Number of Patients (%)		
	Single dose of flucloxacillin	Five-day course of flucloxacillin	Total
Good	76 (76)	78 (76)	154 (76)
Uncertain	14 (14)	10 (10)	24 (12)
Poor	10 (10)	14 (14)	24 (12)

$\chi^2 = 1.34$, $P > 0.51$ with 2 degrees of freedom

Table 13 **Claimed compliance in patients with infected wounds and dosage of flucloxacillin**

Claimed Compliance	Number of Patients (%)		
	Single dose of flucloxacillin	Five-day course of flucloxacillin	Total
Good	4	4	8 (73)
Uncertain	0	1	1 (9)
Poor	1	1	2 (18)

4. Discussion

4.1 Overall Wound Infection Rate

The overall wound infection rate for the patients who completed the trial was 5.4%, representing 11 infections amongst 202 patients. This level of infection represents a similar order of magnitude to infection rates reported by others who have investigated the place of antibiotics in the initial treatment of accidental wounds. Grossman et al. (1981) recorded an infection rate for hand lacerations of 1.1%, whilst Roberts and Teddy (1977) obtained a rate of 9.8% for similar wounds. Infection rates for dog bite wounds have been recorded as low as 0.5%, using a vigorous wound toilet protocol (Zook et al., 1980) and as high as 11.2% (Callaham[2], 1980). Rutherford and Spence (1980), used antibiotics in only a small proportion (7.4%) of their sutured wounds, and found an infection rate of 7.0%.

It would appear then, that the infection rate amongst the patients studied in the trial was neither notably high nor low. This result might well be expected to be the case in a modern district general hospital.

4.2 Dosage regime of flucloxacillin and development of wound infection

The similarity of the wound infection rates in the two treatment groups, 5.0% amongst the patients given single-dose flucloxacillin and 5.9% amongst those given the five day course, was remarkable. Not only was there no statistically significant difference in infection rate between the two groups, but also, the P value ($P > 0.67$) gives a clear indication of no association between the dosage regime of flucloxacillin and the wound infection rate.

It seems reasonable that a single 1g dose of flucloxacillin might be used instead of a five day course. Unfortunately, due to the nature of the trial, it is not possible to use these results to comment upon the merit of antibiotic therapy following wound toilet compared to wound toilet alone in the prevention of infections.

A number of investigators have employed penicillinase-resistant penicillins as prophylactic agents against wound infection in controlled clinical trials and have concluded that their routine use cannot be justified (Roberts and Teddy, 1977; Samson and Altman, 1977; Elenbaas et al., 1982). Nevertheless, the use of these antibiotics in this way is still widespread. It should be noted that neither the conclusions of Roberts and Teddy nor Samson and Altman were based on double-blind trials. In Roberts and Teddy's trial, when wounds were assessed both doctors and patients knew whether antibiotics had been given or not. Such a situation may have allowed doctors involved in wound assessment to influence their results either intentionally or unintentionally. In Samson and Altman's trial, patients were aware of their treatment, but the doctors performing the wound assessments were not. Under these circumstances it is possible that the patients' treatments may have been divulged during questioning, and again this may have influenced the results of the investigation. Furthermore, Samson and Altman's work is notable for the difference between the number of patients entered into the study (1334) and the number successfully followed-up (271). Indeed, only 67 of these patients received antibiotics.

Such discrepancies, due in part to the elimination of non-compliant patients from the trial analysis, may have had a considerable effect upon the outcome of the trial and hence the validity of this work should be questioned.

Elenbaas et al. (1982) reported the results of a double-blind clinical trial in which patients presenting with dog-bite wounds within 24 hours of injury received either oral oxacillin 500mg four times a day for five days or placebo. 46 patients completed the study, with no significant difference in infection rate between the two groups ($P > 0.05$). Due to the small number of patients involved in this trial there is a considerable risk that the results may be subject to a type II error.

It would appear that although three groups of workers have investigated and advised against the use of penicillinase-resistant penicillins for the prevention of infection in accidental wounds, each of their clinical trials suffered from poor design, which may explain their apparent lack of impact upon prescribing habits.

The use of a penicillinase-resistant penicillin for prophylaxis in cat bite wounds has been recommended by Elenbaas et al. (1984). That particular investigation has already been criticised on account of the small number of patients involved. Perhaps more importantly, cat bite wounds, which may have particular complicating factors associated with them, were not encountered in the trial in Stafford.

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4.3 Bacterial wound isolates

It was only possible to obtain wound swabs from 6 of the patients with wounds considered to be infected. 3 swabs yielded Gram-positive cocci susceptible to flucloxacillin and 3 showed no bacterial growth. Assuming that the organisms isolated were also those responsible for the wound infections, it is of some interest that in all three cases they were susceptible to flucloxacillin. A number of possible explanations of this observation might be proposed:

(i) It is possible that there may have been insufficient penetration of flucloxacillin to the site of infection in spite of apparently adequate dosage. Such a situation might arise if wound toilet were inadequate, leaving devitalised tissue or foreign material within which bacteria might be protected from the effects of antibiotics.

(ii) Invading bacteria may have entered wounds and set up infections after the elimination of the single dose or five day course of flucloxacillin or during a prolonged period of non-compliance. This may have occurred by penetration of bacteria through dressings, during changing of dressings, through the wearing of wet dressings or through the removal and non-replacement of dressings.

(iii) It may be that in the first instance a number of different species of bacteria entered some or all of the wounds which developed infections. Some obligate anaerobes interfere with the phagocytosis and killing of aerobic bacteria in vitro (Ingham et al., 1977).

Although anaerobes probably have a limited viability in a well cleaned healthy wound, their effects on phagocytosis may have lasted long enough to allow aerobic bacteria to set up an infection. Short term viability of anaerobic bacteria might also explain why they are not detected with wound swabs. If this is indeed a valid explanation of the basis of infections caused by organisms susceptible to the prophylactic agent, perhaps an anti-microbial active against anaerobes, such as metronidazole, should be investigated with respect to prophylaxis of wound infections.

One further patient was judged to have developed a wound infection but was excluded from the trial analysis as his wound was two days old at the time of entry to the trial. The patient, a 34 year old male service engineer, presented with a two day old dog bite of the right calf and received a single dose of flucloxacillin. Telephone wound assessment after seven days gave a wound score of 7 points, suggestive of infection. On attendance at the accident department the wound was described as red and oozing, and the edges of the wound were separated. An area of inflammation of 4cm diameter was noted. A wound swab showed a moderate growth of Pasteurella multocida sensitive to penicillin, erythromycin, co-trimoxazole, gentamicin etc. The wound was cleaned and povidone iodine ointment was applied. Without knowledge of the microbiological results from the wound swab the patient was prescribed oral erythromycin 250mg four times a day, and the infection resolved after a week.

4.4 Type and location of infected wounds

It is interesting that there appeared to be no particularly strong trends in terms of wound infection rates amongst the various wound types and locations, although with such a small number of infected wounds it would be difficult to reach meaningful conclusions. It has been suggested that puncture wounds are more likely than other wounds to become infected (Callaham[1], 1980), whilst injuries of the hand or arm have been noted by a number of authors to carry a high risk of infection (Callaham, 1978 & 1980[2]; Hutton et al., 1978; Rosen, 1985).

4.5 Wound scores

The comparison of the distribution of wound scores between the two treatment groups indicating no association between wound score and flucloxacillin dosage ($P > 0.27$) reinforces the overall conclusion of the trial. Clearly it is difficult, if not impossible, to specify a definitive wound score which in every case divides infection from non-infection. In deciding upon the presence or absence of infection in a wound, the clinician must look for signs of infection and assess the extent of these signs. The wound assessment questionnaire aims to provide a similar decision making process. Since many wounds may show mild erythema or serous exudate without clinical wound infection, the numerical scoring system may provide an indication of the tendency towards infection in wounds considered uninfected. It is possible that there may have been a greater tendency towards infection in one of the treatment groups which was reflected in terms of higher wound scores overall but which was not shown as a difference in scores greater than 4 points.

However, the statistical analysis of all the wound scores suggesting that they were distributed similarly between the two treatment groups supports the notion that even if the score required for classification of wounds as infected had been set at a figure other than five points, the overall conclusion of the trial, that the two treatments were associated with a similar rate of wound infection, would remain the same.

4.6 Adverse Reactions

A high proportion of patients (29%) reported adverse effects. This might be explained in a number of ways:

(i) All reported problems were recorded, with the exception of complaints of pain at injection sites. It was difficult, and possibly unfair, to make a value judgement concerning the relationship of reported problems to treatment during a brief telephone conversation and without knowledge of patients' previous medical histories, and therefore such a judgement has not been attempted.

(ii) The phrasing of the question, "Have you had any illnesses or symptoms during the last week?" might be considered to be conducive to a positive reply. If this is the case, patients may have felt encouraged, or even obliged, to try to recall any period during the week when they may have felt unwell. Patients who reported rather non-specific symptoms such as "feeling unwell" have been included under the heading 'malaise'.

The slight trend towards a higher incidence of adverse effects amongst the patients treated with the five day course of flucloxacillin would tend to favour use of the single dose, all things being equal.

It also lends a degree of support to the suggestion that one of the advantages of single-dose prophylaxis might be a reduction in the incidence of adverse effects.

4.7 Compliance

76% of all patients assessed claimed 'good' compliance as defined in the method. For comparison, based upon the results of a number of studies of compliance with short courses of oral therapy for acute illness, Lima et al. (1976) noted that on average approximately one third of patients will comply adequately, one third will not and the remainder will do so variably. It seems unlikely, therefore, that the true level of 'good' compliance in this study would have been as high as 76%.

Compliance with drug regimes is a particularly difficult parameter to measure. Tablet counts are frequently employed, but are susceptible to manipulation if patients dispose of tablets without consuming them. Blood levels of drugs may be measured in an attempt to check for compliance, however such measurements are rather invasive, only indicate the serum concentration at the time of sampling and again may be open to manipulation by the patient. Direct questioning of patients is clearly susceptible to the possibility of deliberate deceit in addition to variations between patients' perceived level of compliance and their actual compliance.

In this study in which patients were interviewed by the trial organiser over the telephone, interviewees may have felt a need to please the interviewer by claiming to have taken their capsules as requested. Patients who may have felt guilty about their non-compliance may also have made false claims.

Furthermore, in a situation in which questioning occurs via the telephone rather than face to face, it is perhaps relatively easy to deceive the interviewer.

4.8 Entry of patients to the trial

The system for entry of patients to the trial was designed to enable participating doctors to enrol patients simply and quickly. It was necessary, therefore, that the protocol did not contain complicated stipulations regarding which wounds were suitable for inclusion in the trial and which were not.

Patients were only considered for entry to the trial if the attending doctor felt that prophylactic antibiotics would normally have been indicated on the grounds of contamination or difficulty in cleansing. As wound contamination is so variable in terms of type and extent, the threshold for prescribing antibiotics is likely to have varied considerably between individual doctors. It should be noted that in all, twelve senior house officers participated in the trial, all of whom were undertaking a six month training period in the Accident and Emergency Department. These doctors were not experienced specialists in accident surgery and each would have had his or her own threshold for prescribing which, due to inexperience, may have varied during their training period. Some or all of these factors may have influenced the variety in the nature of wounds entered into the trial.

4.9 Patient numbers

The number of patients enrolled into the trial fell somewhat short of the predicted figure of 400.

The shortfall in the number of patients seemed to be due to failure to recruit some patients suitable for the trial. Participating doctors commonly claimed that they were sometimes too busy to spend time explaining details of the trial to patients. Perhaps it should be noted that there were no incentives to doctors to put in the extra work necessary to enrol patients in the trial. Thus, although future trial organisers may wish to consider the use of written wound assessments at the time of presentation (4.10), the additional time required on the part of the doctors involved may adversely affect the ease of patient recruitment.

4.10 Initial assessment of wounds

The trial protocol, which was designed with the aim of causing the minimum possible increase in workload for the accident department, did not demand of the prescriber a written assessment of wounds at the time of entry to the trial. In order to highlight which wound features, if any, were indicative of a high risk of infection, it might well have been useful to have recorded a detailed wound description at presentation. Aspects which might be useful to record include the length, area and depth of the wound, the type and degree of contamination, the time delay between injury and treatment and the wound type and location.

4.11 Single dose flucloxacillin 1g

The choice of the size of the single dose of flucloxacillin, in the absence of any specific guidelines, was to some extent based upon the use of single dose co-trimoxazole in the treatment of urinary-tract infections.

The most commonly investigated single-dose of co-trimoxazole is a dose of 1.92g (four tablets) equivalent to one day's therapy at the conventional dosage. It seemed to be reasonable that a dose of flucloxacillin equivalent to one day's therapy should be used, hence the choice of 1g. In fact, 'Floxapen' is licensed for a dosage of up to 8g daily, in divided doses six to eight hourly. Clinical experience of high dose oral flucloxacillin has been documented by Knight (1983), who reported the use of flucloxacillin and amoxycillin together, in the treatment of bronchiectasis, both at a dosage of 1g per 20kg bodyweight twice daily. The author found no gastrointestinal or other side-effects amongst four patients treated.

If antibiotics such as flucloxacillin are indeed beneficial in the prevention of infection in accidental wounds and if adequate treatment can be provided by a single 1g dose, it would be interesting to know the minimum effective dose. Unfortunately, the efficacy of antibiotics in this usage remains controversial and hence the optium dosage cannot be defined until this more pressing question has been resolved.

4.12 Standardisation of wound treatment

In terms of antibiotic therapy, patients received one of two treatments as specified in the trial protocol. However, specific procedures were not laid down in the trial protocol for wound toilet and wound dressings. This approach was in line with a general philosophy of trying to improve patient care by investigating the use of a new treatment with a number of potential benefits whilst leaving unchanged all other practices of the accident department, and causing the mimimum increase in workload.

The details of wound debridement, cleansing and dressing were decided by the individual doctors and nurses treating each patient. Such decisions were made within the framework of a departmental practice to irrigate contaminated wounds with a sterile solution of chlorhexidine gluconate 0.015% and cetrimide 0.15% or in some cases hydrogen peroxide solution 6% in the presence of heavy soiling. Dressings used were either dry dressings or paraffin gauze dressings where adherence to the healing tissues was to be avoided.

If, as has often been suggested, the most important factor in the prevention of wound infection is meticulous and vigorous local wound care, then differences in wound toilet may have been a significant factor in the progress of wounds towards either healing or infection. It may be considered that local wound toilet is of such paramount importance to the successful treatment of contaminated wounds that its details should be carefully specified in future trials of antibiotics so that differences in infection rates between treatment groups, which may be quite small differences, are unlikely to be due to variations in wound toilet. Variations in expertise at performing surgical toilet might be reduced by ensuring that all wounds studied are treated by one doctor. Such a policy might, however, have serious repercussions upon the number of patients enrolled into a clinical trial.

4.13 The validity of wound assessment by telephone questionnaire

The treatment of accidental wounds aims to promote prompt healing without disfigurement or loss of sensory or motor function.

To these ends, wound infection is a complication to be avoided if at all possible.

When a patient receives follow-up treatment following wounding, the clinician must assess the wound for the presence or absence of infection. Usually this assessment is a clinical one. A number of criteria are used in order to reach a clinical judgement regarding presence or absence of wound infection and these include redness, swelling, exudate and pain. As in the case of many clinical judgements, classification of wounds as infected or not infected usually depends upon the presence of more than one of these signs, the extent of the signs and their progression over a period of time.

It may be that in the majority of cases systematic multifactorial wound assessment will provide a reasonable indication of the presence or absence of local wound infection. This concept has recently been pursued by Wilson et al. (1986) who described a scoring method for post-operative wound infections, for use in clinical trials of prophylactic antibiotics, based upon repeated observations of various wound characteristics.

The assessment of patients' wounds by telephone questionnaire in this trial required the patient to make specific observations regarding the condition of his or her wound. The presence or absence of swelling, redness, pain and exudate, and their extent and progression was elucidated in order to identify the wounds with strong evidence of infection.

Whilst each wound assessment was dependent upon the responses of a different observer, these responses were elicited in a regimented fashion with each potential indicator of the presence of infection dealt with individually.

The use of a scored wound assessment in this trial successfully detected a number of wound infections corroborated by clinical and microbiological evidence. The future of this type of assessment may lie in computer-aided diagnosis in which a patient's signs and symptoms are entered and followed by a form of numerical analysis from which a diagnosis or differential diagnosis is proposed.

One criticism of the telephoned wound assessment which is difficult to overcome is the exclusion from the trial of patients who could not be contacted by telephone. Patients excluded from the trial for this reason may well have included the poorest members of society who had no access to a telephone for financial reasons. The exclusion of patients due to difficulties in communication is clearly undesirable and the effect of this problem upon the trial, if any, is unknown.

P A R T 2

The Effect Of Low Concentrations Of Flucloxacillin On Surface Hydrophobicity And Phagocytosis of Staphylococcus Aureus

5. Introduction

5.1 Pharmacokinetics Of Flucloxacillin

Systemic anti-microbial agents are usually administered to patients as multiple-dose courses, sometimes as single doses, and are frequently rapidly eliminated by renal excretion in particular. As a consequence of the short half-life of many antibiotics e.g. amoxycillin and ampicillin around 60 minutes, benzylpenicillin around 30 minutes and flucloxacillin around 45 minutes, (Hyde and Willson, 1985) pathogenic bacteria may be exposed to levels below the minimum inhibitory concentration (MIC) at certain stages of treatment.

The mean free serum concentration of flucloxacillin four hours after a 250mg oral dose was reported to be approximately 0.05$\mu g\ ml^{-1}$ and after a 500mg dose it was 0.12$\mu g\ ml^{-1}$ (Sutherland et al., 1970). The mean total serum concentration for the same groups of patients four hours after oral dosing was 1.0$\mu g\ ml^{-1}$ after 250mg and 2.2$\mu g\ ml^{-1}$ after 500mg. This type of data suggests that when a six hourly dosing regimen is employed, pathogenic bacteria with an MIC of, for example, 0.5$\mu g\ ml^{-1}$ may be exposed to sub-MIC levels for a considerable proportion of the dosing interval, particularly if it is only the free fraction of flucloxacillin which is available for antibacterial activity.

5.2 The Effect Of Protein Binding On The Activity Of Antibiotics

The figure of 94.7% flucloxacillin protein bound given by Sutherland et al. (1970) is comparable with the results reported by Bodey et al. (1972) of 92.6% binding of flucloxacillin to human serum proteins and by Bergeron et al. (1976) of 94.6% protein binding. A ten to twenty-fold drop in the activity of flucloxacillin in 95% serum compared with nutrient broth was reported by Sutherland and attributed to the effects of protein binding, hence the antibacterial action of the compound was considered to be due to the free fraction only.

In a review, Wise et al. (1980) stated that only the protein-free fraction of an antibiotic can act against bacteria and other factors being equal, a low-bound drug should be superior to one with higher binding.

More recently, however, Lacey (1985) reported that protein binding of a number of antibiotics does not affect their anti-staphylococcal activity. Work involving 50% whole blood with nutrient broth suggested that protein binding had no effect on the antistaphylococcal activity of five antibiotics including the isoxazolyl penicillin dicloxacillin which is 96% protein bound. An explanation put forward was that the antibiotics may bind to penicillin-binding proteins in bacteria more firmly than they do to plasma proteins, hence overcoming the effect of protein binding. If these observations provide a suitable model of in vivo conditions then the same finding would be likely to apply to flucloxacillin as its structure is almost identical to that of dicloxacillin.

Kunin (1986) proposed an explanation of Lacey's results noting that use of 50% whole blood in nutrient broth diluted the serum albumin which is the major serum binding protein for beta-lactam antibiotics. Binding, according to Kunin, is highly dependent on albumin and falls as a logarithmic function of the concentration.

George (1986) repeated Lacey's experiments using slightly different methods and proposed that the latter's results may have been affected by the citrate phosphate dextrose adenine solution (CPD adenine) contained as an anticoagulant in Lacey's expired transfusion blood. No suggestions were put forward to explain any mechanism by which CPD adenine might affect the anti-staphylococcal activity of antibiotics.

Following the criticism from Kunin and George, Lacey (1986) re-affirmed his opinion, claiming that experiments involving diluted whole blood are relevant to the patient and noting that some antibiotics are effective in clinical use even though they are highly protein bound with a free fraction which might be considered sub-inhibitory.

The question of whether the total serum antibiotic concentration or the unbound concentration is of greatest clinical importance remains unresolved.

5.3 The Mode Of Action Of Beta-Lactam Antibiotics

The cytoplasmic membrane surrounding the bacterial protoplast has very little intrinsic strength and if not overlaid by the cell wall it would be ruptured by internal osmotic pressure if the cell were to be placed in a hypotonic solution. The shape and integrity of bacteria is maintained by the strong, rigid and structured cell wall. The cell wall's strength is derived from peptidoglycan a cross-linked aminosugar polymer. Peptidoglycan in Gram-positive cell walls is susceptible to degradation by the enzyme lysozyme and when this occurs it is followed by rapid cell lysis.

Beta-lactam antibiotics such as penicillins and cephalosporins produce their lethal action through the inhibition of enzymes involved in peptidoglycan assembly. This causes weakening of the cell wall until it loses the ability to resist the internal turgor pressure. Bulges often develop at the site of cell division and these may enlarge until the cell bursts.

Around 50% of the weight of the Gram-positive cell wall is peptidoglycan, the remainder being made up of various polymers such as teichoic acids, teichuronic acids and proteins. Peptidoglycan is composed of a network of linear polysaccharide chains, up to 200 disaccharide units long, which are cross-linked by short peptide chains.

In order to grow and divide bacteria must expand their cell wall. This involves extending the peptidoglycan matrix in a controlled manner such that the shape and integrity of cells is retained.

Cell wall precursors are synthesized in the cytoplasm and then transferred to a lipid carrier molecule which transports them across the cytoplasmic membrane. Finally, they are inserted into the cell wall.

The final stage in peptidoglycan synthesis involves a number of enzymes including transpeptidases and carboxypeptidases which in combination refashion the peptidoglycan network during cell growth. When bacteria are exposed to beta-lactam antibiotics a small quantity of antibiotic, of the order of 200-4000 molecules per cell, becomes covalently bound to the cells. This small number of molecules is responsible for damaging and eventually killing the cell. The antibiotic molecules are bound to transpeptidases and carboxypeptidases and inhibit these enzymes. Although the cells continue to grow they are unable to produce cross-linked peptidoglycan, develop abnormal shapes and eventually stop growing. Cells often lyse, although filament-shaped bacteria and other unusual forms may persist without lysis.

The transpeptidases and carboxypeptidases bind covalently to the beta-lactam bond of penicillins thus forming inactive intermediate compounds sufficiently stable to ensure that cross-linking of peptidoglycan cannot occur. Due to a similarity of structure it is likely that the enzymes mistake beta-lactam antibiotic molecules for their genuine substrate peptidoglycan, cleave the beta-lactam bond and become inactivated due to the relative stability of the antibiotic-enzyme intermediates or penicillin binding proteins (PBPs).

A number of PBPs exist and most beta-lactam antibiotics bind to all of them with preference for certain of the PBPs at low concentrations. As inhibition of different PBPs may result in various changes in cell shape, the morphological effects of antibiotics depend upon the binding characteristics of the antibiotic and the concentration employed. Lysis, filamentation or formation of other abnormal cell shapes may occur (Hammond et al., 1984).

In addition to the effect of beta-lactam antibiotics on the synthesis of peptidoglycan, the triggering of autolytic enzymes in the cell wall plays an important part in the destruction of bacteria.

5.4 The Effect Of Sub-MIC Levels Of Antibiotics On Bacteria

The clinical relevance of periods of sub-MIC levels of antibiotics has yet to be established; however there are indications that low levels of antibiotics may indeed influence the therapeutic outcome of treatment either by an effect on bacteria or on the process of phagocytosis or on both.

Sub-MIC levels of antibiotics have been shown to produce abnormal bacterial morphology and ultrastructure and to inhibit the rate of bacterial growth in vitro, and as noted by Rolinson (1977) the activity of an antibiotic is not an all or nothing effect. Variation in the speed and extent of antibacterial effect is usually seen over a wide range of concentrations and within that range the MIC represents one particular degree of antibacterial effect.

Concentrations below the MIC may diminish the growth rate and the final amount of growth, whilst at concentrations above the MIC the speed at which growth is arrested or the rate at which cells are killed may be greatly increased.

Lorian et al. (1979) proposed the term minimal antibiotic concentration (MAC) as a means of describing antibacterial activity at low concentrations and defined it as the lowest concentration of an antibacterial agent that produces a 1 log decrease in the number of organisms per ml as compared with a control culture in a drug-free medium. Lorian (1985) has since described the minimum antibiotic concentration as the lowest concentration of an antibacterial agent that affects either bacterial structure or growth rate or both.

The morphological changes induced in some bacteria may show variability depending on the beta-lactam antibiotic to which the organism is exposed. In the case of Gram-positive cocci all beta-lactam antibiotics produce similar morphological effects (Lorian, 1985). Over about three to four hours staphylococci which are exposed to penicillins or cephalosporins produce cells which are larger than normal and contain one or more thick cross walls.

In addition to effects on morphology, low concentrations of antibiotics may reduce the adhesive properties of bacteria and this may occur in a number of ways. Four possible mechanisms by which bacterial adhesion may be affected have been discussed by Chopra (1986).

Antibiotics may alter the overall shape of the bacterium and hence its ability to approach receptors on the host's cells. The release of adhesins from the bacterial cell surface may be promoted. Formation of functionally deficient adhesins may be induced. Synthesis or secretion of surface adhesins may be suppressed.

5.5 The Role Of Phagocytosis In Infection

Phagocytosis is a cellular phenomenon which serves three major purposes. The relative importance of each function depends upon the type of organism involved and the cells carrying out the activity.

Phagocytosis is the main means of feeding in protozoa, where food particles are engulfed in a food vacuole and digested by the release of enzymes and absorption of the resulting products. In mammals, phagocytic white blood cells i.e. polymorphonuclear leucytes or neutrophils and monocytes, form the first line of defence against invading micro-organisms through their ability to phagocytize and kill them. Phagocytic cells also dispose of dying and damaged cells and cellular debris (Fietta, 1983; Castle, 1984). In relation to infection, phagocytosis of bacteria is the most important of the functions described.

Phagocytosis can only occur when the neutrophil and bacterial cell are in close proximity, hence when foreign bacteria are present phagocytes need to migrate to the site of infection. Low concentrations of natural breakdown products of bacteria act as chemoattractants to neutrophils, indicating the presence and location of invading organisms.

The process of phagocytosis involves adherence of the bacterium to the neutrophil surface and its ingestion followed by killing and digestion.

The nature of the surface of both the neutrophil and the bacterium is important in determing whether phagocytosis will occur. Van Oss (1978) described the phagocytosis of bacteria in terms of the relative hydrophobic surface properties of bacterial cells and phagocytes. Bacteria which are more hydrophobic than phagocytes are relatively easily phagocytized whereas bacteria which are less hydrophobic than phagocytes tend to resist phagocytosis.

The ingestion of particles by phagocytes is accompanied by an increase in oxygen consumption or 'respiratory burst'. This is due to the generation of toxic oxygen derivatives such as superoxide anions, hydrogen peroxide and hydroxyl radicals which are unstable and highly reactive. These oxygen derivatives provide killing mechanisms for the destruction of foreign cells and are probably important in the microbicidal activity of phagocytes.

The ability of phagocytes to kill ingested bacteria is an important one as survival of bacteria following ingestion may lead to persistent or progressive disease. Organisms which can survive ingestion by phagocytes, facultative intracellular bacteria, include Mycobacterium tuberculosis, Legionella pneumophila and some strains of Staphylococcus aureus.

Intraphagocytic organisms are protected from the action of some antimicrobial agents presumably due in part to limited entry of the agents into the phagocytes. The success of antibiotic treatment of infections due to intracellular bacteria depends on the extracellular effects of the antibiotic on the bacteria, penetration of the antibiotic into phagocytic cells and the intracellular effects of the antibiotic on both phagocytes and bacteria (Hand, 1984).

5.6 The Effect Of Sub-MIC Levels Of Antibiotics On Phagocytosis

In vitro findings from a number of investigators have shown that the process of phagocytosis may be potentiated after pre-exposure of a variety of bacterial species to antibiotics. Morphological changes in bacteria observed by microscopy have been correlated with increased susceptibility to phagocytosis in vitro. Gemmell (1984), who reviewed the effects of low concentrations of antibiotics on the potentiation of phagocytosis of pathogenic bacteria, noted that morphologically abnormal bacteria have been recognised following antibiotic therapy in humans and recommended further studies into in vivo potentiation of phagocytosis.

Consideration should be given to the effect of antibiotics, if any, on the hydrophobicity of both bacterial cells and phagocytes, for in the clinical situation it is not only bacteria which are exposed to antibiotics e.g. ampicillin has been reported to make phagocytes less hydrophobic and bacteria much less hydrophobic resulting in overall depression of phagocytosis (van Oss, 1978).

5.7 The Effect Of The Growth Environment On Bacteria

The properties and composition of the bacterial cell surface may be considerably influenced by the environment in which growth occurs. Factors which may be important are the growth rate, whether the organisms are multiplying on a surface or in suspension, and the chemical composition of the growth medium.

Practical and ethical problems dictate that the majority of investigations into infection involve in vitro studies on bacteria isolated from clinical infections which are subsequently subcultured in growth media. Assumptions concerning the in vivo properties of bacteria are often extrapolated from in vitro data and such information may not be entirely valid.

Growth in standard culture media produces bacteria whose characteristics in terms of virulence, immunogenicity and susceptibility may differ considerably from those obtained in vivo. The rate of growth of bacteria can influence their surface properties and it may be of some importance that growth rates in vivo tend to be slower than those in vitro. It is notable that bacteria grow on surfaces in vivo whereas they frequently grow in suspension in vitro. The significance of surface growth has not been fully elucidated although it has been noted that surface growing bacteria form microcolonies enmeshed in a polysaccharide matrix and this pattern of growth may confer protection against antibiotics and immunological defence systems (Brown & Williams, 1985).

The availability of essential nutrients may vary from one growth environment to another and their lack of availability produces dramatic changes in bacterial cell structure and biochemistry. Ionic iron, for example, is relatively unavailable in vivo as it is mainly to be found intracellularly in haemoglobin or the iron storage protein ferritin, whilst the small extracellular portion is bound to transferrin. In all of these forms, iron is strongly bound and invading bacteria are presented with the problem of acquiring enough iron to enable growth to occur. Successful pathogens possess a means of obtaining iron through production of low molecular weight compounds called siderophores which bind iron as strongly as the host's iron binding proteins. Siderophores are usually produced in response to iron deprivation, an ability which may be encoded genetically and which correlates with increased virulence (Brock, 1986). Shand et al. (1985) reported on Gram-negative bacteria isolated directly and without subculturing from the urine of patients with urinary-tract infections and observed that the organisms had grown under iron-restricted conditions resulting in changes in their outer-membrane components. It is not clear, however, whether all urinary-tract infections are iron restricted.

5.8 The Effect Of Sub-MIC Levels Of Antibiotics In Clinical Practice

Rolinson (1977) has suggested that in the treatment of certain infections it is likely that merely a reduction in the rate of growth of the pathogen would be sufficient to result in a therapeutic effect and for this a sub-MIC level of antibiotic might well be adequate.

In more serious infections, particularly when body defence mechanisms are impaired, a partial inhibition of growth may not be sufficient and a bactericidal effect which is rapid and as complete as possible may be called for, requiring antibiotic concentrations considerably higher than the MIC.

There is little clinical experience of antibiotic regimens designed to treat infections using sub-MIC levels of antibiotics. Ben Redjeb et al. (1982) reported on a clinical trial in which 20 patients with symptomatic urinary tract infections (10^5 colony-forming units of E. coli per ml of urine in addition to pyuria) received 10mg ampicillin in three divided doses each day for three days. After 2 days, 16 of the 20 patients had culture-negative urine without pyuria whereas all of the 18 similar control patients showed no change. The urine of the 16 patients in whom the treatment had been successful remained culture-negative for four days after finishing treatment, confirming that 10mg of ampicillin per day produces considerable antibacterial activity in the urine. The concentrations of ampicillin in the urine producing this antibacterial effect ranged from one fifth to three-quarters of the MIC of the infecting organism. It was suggested that the success of the treatment was due to a reduction in the number of bacteria produced by sub-MICs of ampicillin and to an effect on the adherence of E. coli to urinary epithelial cells such that non-adhering bacteria were washed away by the urine.

The exact role of sub-inhibitory concentrations of antibiotics during the treatment of infections remains largely unexplored and unknown. It may be desirable to make observations on bacteria in vivo at the site of infection in order to fully understand the interaction between bacteria and antibiotics at low concentrations. In practice, it is very inconvenient to attempt such observations, particularly in humans, and may be unethical. It is likely that much work will continue to be performed in vitro.

Flucloxacillin is an example of an antibiotic which is commonly prescribed in doses which provide sub-MIC serum levels for considerable portions of the dosing interval and which is used in the treatment of minor wounds where bacteria are present in soft tissues in relatively small numbers. In prophylaxis, the object of prescribing an antibiotic is to prevent any bacteria from developing into a clinical infection and since the bacteria are likely to be exposed to sub-MIC levels of the antibiotic, investigation of the sub-MIC effects of flucloxacillin on S. aureus seems to be warranted.

6. METHODS

6.1 Cultures

Four clinical isolates of Staphylococcus aureus coded 05, 067, 178 and 262 were obtained from the Microbiology Department, St. George's Hospital, Stafford. Stock cultures were stored on nutrient agar (NA) slopes at $4^{o}C$. S. aureus 05 and 262 were supplied as benzylpenicillin-sensitive isolates and S. aureus 067 and 178 as benzylpenicillin-resistant isolates. The penicillin sensitivity and resistance of the isolates was confirmed using penicillin Neosensitabs. S. aureus 262 and 178 were respectively the most sensitive and resistant to penicillin and were selected for further investigation. Phage typing, performed by the Hospital Infection Research Laboratory, Dudley Road Hospital, Birmingham, indicated that both strains are quite commonly isolated from clinical specimens. Phage types were as follows:-

S. aureus 178 (Penicillin resistant) 29/52/52A/80

S. aureus 262 (Penicillin sensitive) 42E

6.2 Minimum Inhibitory Concentration (MIC) Of Flucloxacillin For S. aureus 262 and 178

A tube minimum inhibitory concentration (MIC) for flucloxacillin was obtained for S. aureus 262 and 178. 0.1ml of an overnight culture of each isolate at a dilution of 10^{-2} was inoculated into 2.5ml double strength nutrient broth (NB) containing various concentrations of flucloxacillin and made up to 5ml with distilled water. Concentrations were tested in triplicate and the test tubes were incubated at $37^{o}C$ and inspected for growth after 24, 48 and 72 hours.

6.3 Determination Of A Sub-inhibitory Concentration Of Flucloxacillin

Growth curves, viable counts and microscopy were used in order to find a suitable sub-MIC level of flucloxacillin for further investigation which did not affect the growth rate of cultures, but which affected the bacteria sufficiently to cause morphological changes visible by microscopy.

6.3.1 Growth Curves

All growth curves were performed under similar conditions. 0.1ml of overnight culture (ONC) of bacteria grown in tryptone soy broth (TSB) was inoculated into 25ml of sterile TSB in a 100ml baffled conical Pyrex flask and placed in a shaking water bath (Mickle Laboratory Engineering Co. Limited, Gomshall, Surrey) at 37°C and speed no.7. Optical density of the cultures was measured at regular intervals by spectrophotometer (Cecil CE 292 Digital Ultraviolet Spectrophotometer) at a wavelength of 470nm.

6.3.2 Growth In The Presence Of Flucloxacillin

Cultures of S. aureus 262 and 178 were incubated in the presence of various concentrations of flucloxacillin, 0.0125μg ml^{-1} (⅛ MIC), 0.05μg ml^{-1} (½ MIC) and 0.1μg ml^{-1} (MIC). Using Gram's stain, samples were inspected by microscopy during late log phase for the presence of cells showing morphological changes compatible with cell wall disruption.

6.3.3 Investigation Of The Viability Of Bacteria Incubated In The Presence Of Sub-inhibitory Concentrations Of Flucloxacillin

In order to investigate whether the viability of S. aureus 262 and 178 incubated in the presence of low concentrations of flucloxacillin had been affected, viable counts were performed, as described in section 6.3.4 on cultures grown to late log phase.

6.3.4 Viable Counts

Viable counts were carried out by placing seven 20µl drops of culture diluted serially in 0.85% saline, onto each of two nutrient agar plates. The number of colonies associated with each 20µl drop was counted after 24 hours incubation at 37°C using a Gallenkamp colony counter.

In order to investigate whether viable counts were being affected by cell association or clumping of S. aureus, a series of culture samples were agitated for 120 seconds by vortexing the serially diluted samples on a Whirlimixer (Fisons, Loughborough, England) prior to measurement of optical density and plating.

6.4 Investigation Of Bacterial Surface Hydrophobicity

6.4.1 Contact Angle Measurement

Contact angles for drops of 0.85% saline were measured by using cellulose acetate membrane filters layered with bacteria from 100ml of suspension of OD_{470nm} 5 as described by van Oss (1978).

S. aureus 262 and 178 were grown in TSB at 37°C in the presence and absence of a sub-inhibitory concentration of flucloxacillin (0.05µg ml^{-1}) until late log phase, OD_{470nm} 5.

The cells were harvested by centrifugation at 9000g for 10 minutes, washed twice with 0.85% saline and resuspended in saline. Using suction and sintered glass filter apparatus the cells were layered onto cellulose acetate membrane filters which had previously been soaked three times in boiled distilled water to remove any residual detergent from the filter. The filter was removed from the sintered glass filter apparatus with a pair of tweezers, cut into half with a scalpel and each half filter was stuck to a glass microscope slide using a water-based adhesive (Gloy paste, Henkel Chemicals Limited, London). The pads of bacteria were allowed to dry in air to remove excess moisture. The time required for drying may be judged by a change in appearance of the surface of the pad of bacteria from gloss to matt. The contact angle is the angle between a solid surface i.e. the bacterial layer, and a liquid drop i.e. 0.85% saline. The initial contact angle of a 10µl drop of saline was observed using a goniometer. At least fifteen drops of saline were observed for each bacterial culture and the average value taken.

6.4.2 Bacterial Adherence To Hydrocarbons (BATH)

The method described by Rosenberg et al. (1980) was used with slight modifications. S. aureus 262 and 178 were grown in TSB at 37°C in the presence and absence of a sub-inhibitory concentration of flucloxacillin (0.05µg ml^{-1}) until late log phase OD_{470nm}5. The cells were harvested by centrifugation at 8000 r.p.m. for ten minutes, then washed twice and resuspended to OD_{470nm} 1.0 in phosphate-urea-magnesium (PUM) buffer; pH 7.1: 22.2g $K_2HPO_4.3H_2O$, 7.26g KH_2PO_4, 1.8g urea, 0.2g $MgSO_4.7H_2O$ and distilled water to 1000ml.

1.5ml of washed bacterial cells in PUM buffer was added to 10µl and 20µl n-octane in 6mm diameter glass test tubes previously washed in 0.1M HCl to remove any detergent. The mixture was allowed to equilibrate for 15 minutes at room temperature and then agitated for 120 seconds using a vortex mixer. After allowing the two phases to separate for 30 minutes, the aqueous layer was carefully removed with a Pasteur pipette and transferred to a 1ml disposable cuvette. Absorbance at 470nm was compared with the original bacterial suspension in PUM buffer. Each combination of bacterial suspension with n-octane was tested in five separate tubes.

6.5 Investigation Of The Effect Of A Sub-inhibitory Concentration Of Flucloxacillin On The Killing Of S. aureus 262 and 178 In Whole Blood

The method described by Jones et al. (1979) was used with slight modifications.

S. aureus 262 and 178 were grown in TSB at 37°C in the presence and absence of a sub-inhibitory concentration of flucloxacillin (0.05µg ml^{-1}) until late log phase OD_{470nm} 5. Cells were harvested by centrifugation at 9000g for ten minutes, washed twice in sterile saline and resuspended to OD_{470nm} 0.2 in 0.85% saline.

Fresh human whole blood was obtained in a lithium-heparin tube and used within one hour of collection.

At time zero 0.6ml fresh whole blood was mixed with 0.6ml of bacterial suspension in a small plastic tube at 37°C in a gently shaking water bath. Immediately, and at regular intervals 0.1ml samples were withdrawn from the mixture and plunged into 0.9ml sterile distilled water at 0°C and left for 5 minutes in order to lyse the blood cells. Viable counts were performed on these samples in order to assess the effect, if any, of whole blood on the number of viable bacteria during the course of the experiment.

In an attempt to estimate phagocytosis blood-bacteria films were prepared directly from the experimental mixture at regular intervals. A small drop was placed onto a glass microscope slide and smeared with a cover slip in order to produce fish tail edges where phagocytes may be viewed. The blood-bacteria films were air dried and stained in the following manner:-

1. Films were fixed with methanol (BDH Chemicals) for 4 minutes.
2. Excess methanol was washed off with May and Grünwald's stain (BDH) and the film allowed to stand for 4 minutes.
3. Excess May and Grünwald's stain was washed off with Giemsa's stain (BDH) diluted 1:20 in Sorensen's buffer and the film allowed to stand for 10 minutes.
4. Excess Giemsa's stain was washed off with Sorensen's buffer.

The number of bacteria associated with 30 phagocytes randomly counted under the microscope was recorded.

7. RESULTS

7.1 Minimum Inhibitory Concentration Of Flucloxacillin For S. aureus 262 and 178

After 72 hours incubation, S. aureus 262 had grown in the presence of flucloxacillin 0.05μg ml^{-1}, but not with 0.1μg ml^{-1}. The MIC was taken to be between 0.05 and 0.10μg ml^{-1}. In the case of S. aureus 178, growth occurred in all three tubes at 0.05μg ml^{-1} flucloxacillin, one of three tubes at 0.1μg ml^{-1}, and none of the tubes at 0.25μg ml^{-1}. The MIC of flucloxacillin for S. aureus 178 was taken to be approximately 0.10μg ml^{-1}.

7.2 Determination Of A Sub-inhibitory Concentration Of Flucloxacillin

7.2.1 Growth Of S. aureus 262 and 178 In The presence Of Flucloxacillin

Growth of S. aureus 262 and 178 in the presence of flucloxacillin 0.0125, 0.05 and 0.1μg ml^{-1} produced no detectable effects on growth rates during the log phase. There was a rapid slowing of growth of S. aureus 178 with flucloxacillin 0.1μg ml^{-1} during late log phase and a similar but smaller effect on S. aureus 262, resulting in a reduction in the total amount of growth in both cases (Figs. 3 & 4).

Samples stained by Gram's method after 6¼ hours' incubation, showed that even the smallest concentration of flucloxacillin (0.125μg ml^{-1}) had a noticeable effect upon cell morphology (Table 14).

7.2.2 Viability Of Bacteria Incubated In The Presence Of Flucloxacillin

The viability of the bacteria was not appreciably affected after incubation for five hours with flucloxacillin 0.0125 and 0.05μg ml^{-1} (Tables 15 & 16).

Fig 3 Growth curves for S. aureus 262 control and with flucloxacillin 0.0125, 0.05 and 0.1μg ml^{-1}

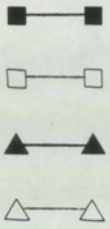
Key

Control

0.0125μg ml^{-1} Flucloxacillin

0.05μg ml^{-1} Flucloxacillin

0.1μg ml^{-1} Flucloxacillin



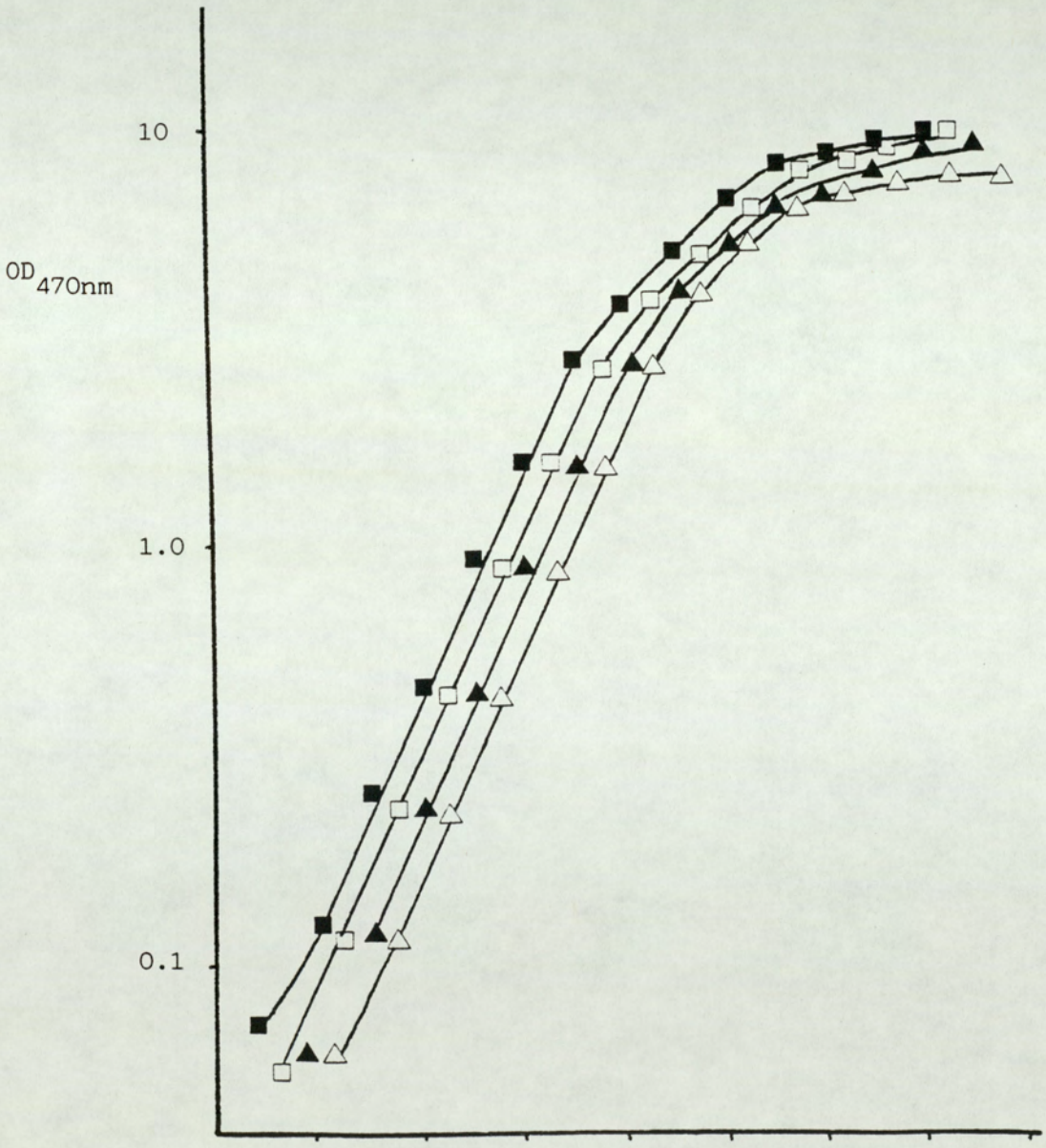

10
1.0
0.1
OD_{470nm}
Time: 1 hour intervals

Fig 4 Growth curves for S. aureus 178 control and with flucloxacillin 0.0125, 0.05 and 0.1μg ml^{-1}

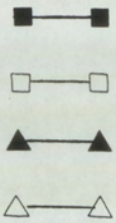
Key

Control

0.0125μg ml^{-1} Flucloxacillin

0.05μg ml^{-1} Flucloxacillin

0.1μg ml^{-1} Flucloxacillin



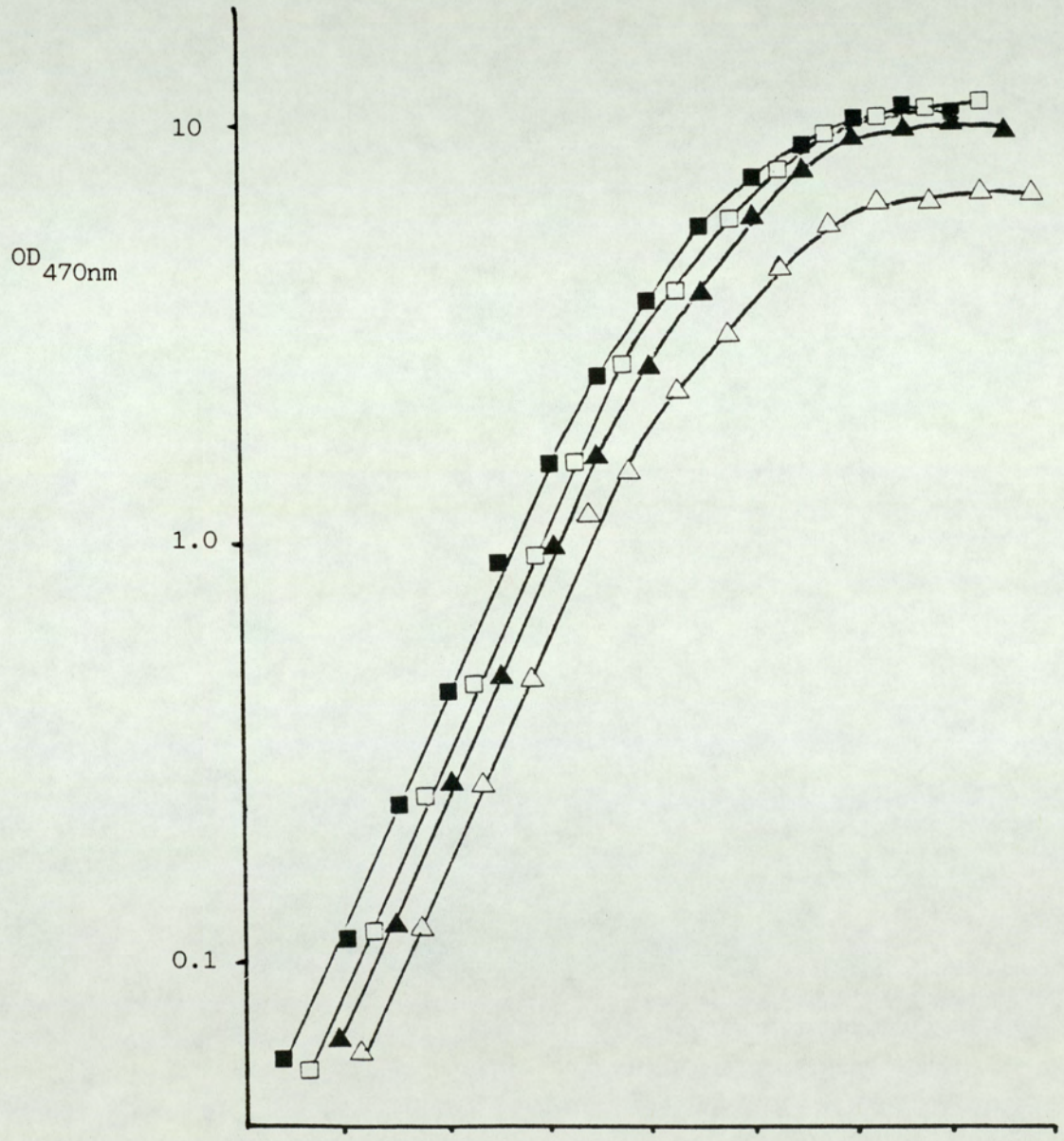

10
1.0
0.1
OD_{470nm}
Time: 1 hour intervals

Table 14 **Morphology Of S. aureus 262 and 178 after 6¼ hours incubation**

Growth medium	S.aureus 262	S. aureus 178
TSB	Many clumps of 30-40 cells. Individual cells of around 1μm diameter	Clumps of about 10-20 cells. Individual cells slightly larger than S. aureus 262, i.e. 1.0 to 1.5μm diameter.
0.0125μg ml^{-1} Flucloxacillin in TSB (⅛ MIC)	Clumps of about 15-20 cells. Some smaller groups of 2, 4 or 6 cells. Some enlarged cells of 1 to 2μm diameter.	Clumps of 10-20 cells and 2, 4, 6, 8 cells. A few enlarged cells of approximately 2μm diameter.
0.05μg ml^{-1} Flucloxacillin in TSB (½ MIC)	Many large cells of around 2μm diameter. Large cells comprise about 30% of sample, mainly in clumps of 6-10 cells. Some larger clumps also.	Some enlarged cells, more than at 0.0125μg ml^{-1}. Cells mainly in clumps of 2, 4, 6, 8 or 10, although some larger clumps. No signs of lysis.
0.1μg ml^{-1} Flucloxacillin in TSB (MIC)	More enlarged cells than normal sized cells. Signs of lysed cells.	Mostly very enlarged cells > 2μm diameter. Large cells are about 4 x diameter of normal cells. Signs of lysed cells.

Table 15 **Viability of S. aureus 262 Cells Grown In The Presence Of Sub-inhibitory Concentrations of Flucloxacillin**

Culture Medium	Optical Density OD 470nm	Viable Count CFU x 10^9 ml^{-1}
Control (TSB)	6.92	2.48
Flucloxacillin 0.0125µg ml^{-1} in TSB (⅛ MIC)	6.96	2.28
Flucloxacillin 0.05µg ml^{-1} in TSB (½ MIC)	6.64	2.37

Table 16 **Viability of S. aureus 178 Cells Grown In The Presence Of Sub-inhibitory Concentrations Of Flucloxacillin**

Culture Medium	Optical Density OD 470nm	Viable Count CFU x 10^9 ml^{-1}
Control (TSB)	7.50	2.64
Flucloxacillin 0.0125µg ml^{-1} in TSB (⅛ MIC)	7.26	3.53
Flucloxacillin 0.05µg ml^{-1} in TSB (½ MIC)	6.80	1.37

7.2.3 The Effect Of Vortex Mixing On Optical Density And Viable Count

Vortex mixing had no effect on the optical density or viable count of S. aureus 262 (Table 17) and hence subsequent investigations did not employ vortexing of bacterial suspensions.

7.2.4 Choice Of A Sub-inhibitory Concentration Of Flucloxacillin

0.05µg ml^{-1} (½ MIC) flucloxacillin was chosen as a suitable sub-inhibitory concentration to use for further investigation of the two clinical isolates of S. aureus as it had a negligible effect on growth curves and viability, but produced morphological changes in the bacterial cells when viewed under a microscope.

7.3 The Effect Of A Sub-inhibitory Concentration Of Flucloxacillin On The Surface Hydrophobicity of S. aureus 262 and 178

7.3.1 Contact Angle Measurement

The contact angle for S. aureus 178 was considerably greater (10^{o}) than that of S. aureus 262 when grown in TSB and in both cases growth in the presence of flucloxacillin produced a marked increase in contact angle, i.e. an increase in the measured surface hydrophobicity (Table 18).

7.3.2 Bacterial Adherence To Hydrocarbons (BATH)

In an aqueous suspension, the interaction of bacteria with hydrocarbons such as n-octane produces partition of the bacteria between the aqueous and non-aqueous layers. An indication of hydrophobicity is provided by the percentage loss of optical density in the aqueous layer after interaction and equilibration of the two layers. The greater the loss of optical density, the greater the bacterial surface hydrophobicity.

Table 17 **The effect of vortex mixing on S. aureus 262**

Time (h)	Optical Density (470nm)		Viable Count (CFU x 10^8 ml^{-1})	
	Non-vortexed	Vortexed	Non-vortexed	Vortexed
2	0.65	0.70	2.1	2.9
4	9.8	9.3	31	21
18	19.8	20.3	150	140

Table 18 **Contact angles of S. aureus 262 and 178 Grown in TSB**

Organism	Flucloxacillin μg ml^{-1}	Contact Angle (^{o})		Mean Contact Angle (^{o})
		Experiment 1	Experiment 2	
262	0.00	20 $\pm$ 4.7	13 $\pm$ 3.5	17
	0.05	32 $\pm$ 3.6	21 $\pm$ 4.3	27
178	0.00	32 $\pm$ 7.1	21 $\pm$ 4.6	27
	0.05	45 $\pm$ 8.8	38 $\pm$ 4.5	42

Results shown are the means $\pm$ standard deviation values for 18 to 44 10μl drops of 0.85% saline

Table 19 shows that S. aureus 178 was the more hydrophobic of the two staphylococcal isolates under the conditions employed. Flucloxacillin markedly decreased the measured surface hydrophobicity of S. aureus 178 whilst the effect on S. aureus 262 was similar but considerably smaller.

7.4 Whole Blood Killing

S. aureus 262 appeared to be susceptible to whole blood killing, with a kill in excess of 90% produced within 60 minutes on two occasions (Figs. 5 & 6). Growth in the presence of flucloxacillin 0.05μg ml^{-1} did not affect the rate or extent of kill of S. aureus 262, indeed the similarity of the results on both occasions was remarkable.

S. aureus 178 appeared to be killed more rapidly than S. aureus 262 during the first 15 minutes of the experiments. Growth of S. aureus 178 in the presence of flucloxacillin 0.05μg ml^{-1} produced an apparent rise in viable count initially, followed by rapid killing (Figs. 5 & 6).

The number of bacteria associated with neutrophils at timed intervals after mixing whole blood with the bacterial suspension did not appear to provide information which might usefully be used to follow the blood-bacteria interaction (Tables 20 & 21).

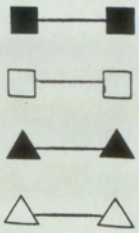
Table 19 **Surface hydrophobicity of S. aureus 262 and 178 as determined by BATH**

Organism	Fluclox. ($\mu g\ ml^{-1}$)	% Decrease* in OD 470nm					
		n-octane (10μl)			n-octane (20μl)		
		Expt.1	Expt.2	Mean	Expt.1	Expt.2	Mean
262	0.00	80	68	74	89	67	78
	0.05	86	52	69	89	54	72
178	0.00	94	93	93	93	97	95
	0.05	47	52	49	40	64	52

*Mean of 5 replicates

Fig 5 **Whole blood killing of S. aureus 262 and 178 grown with and without flucloxacillin 0.05μg ml^{-1}, Expt. 1**

Key



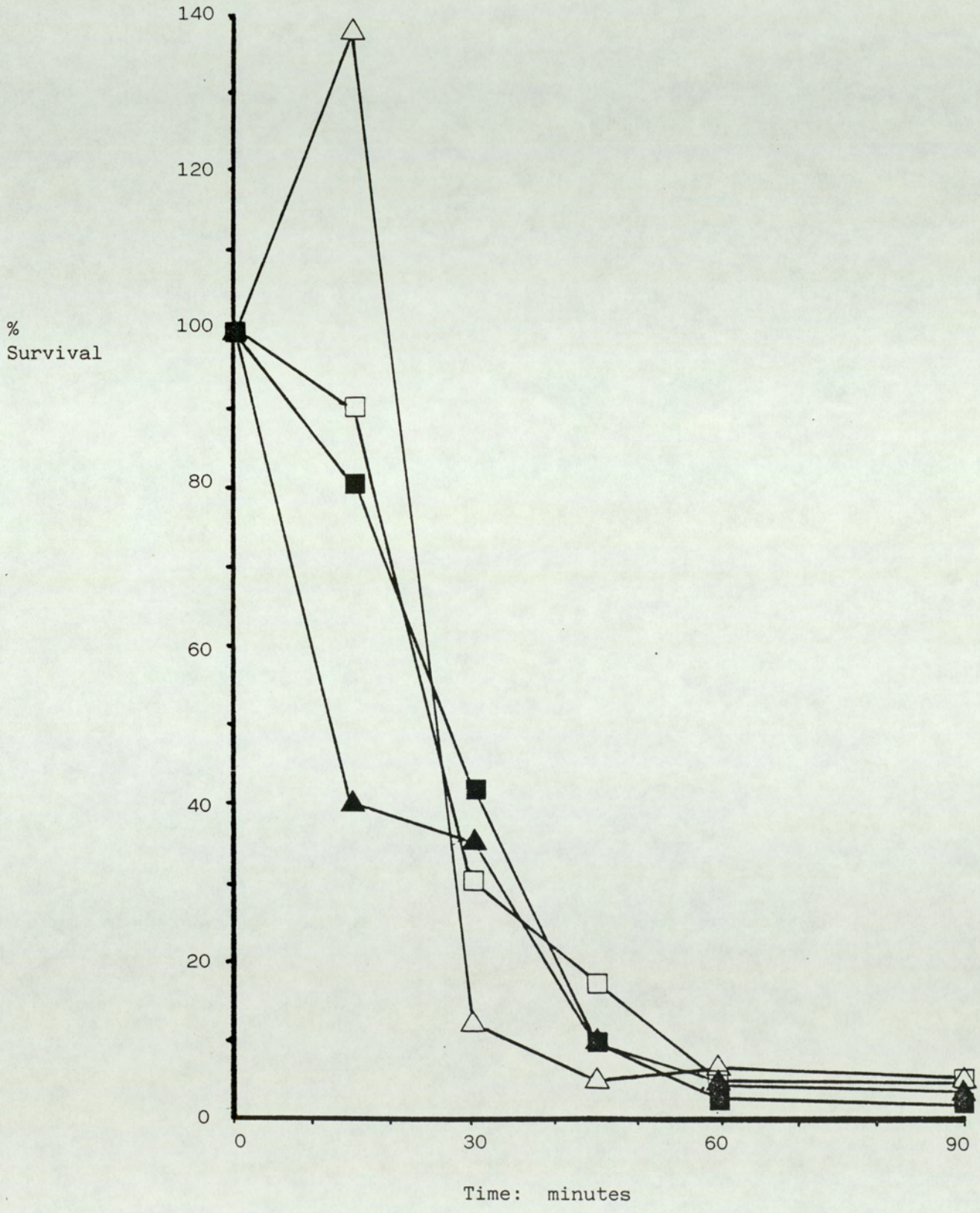
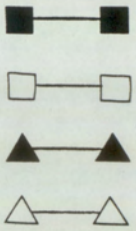

140
120
100
80
60
40
20
0
%
Survival
0
30
60
90
Time: minutes

Fig 6 **Whole blood killing of S. aureus 262 and 178 grown with and without flucloxacillin 0.05μg ml^{-1}, Expt. 2**

Key



S. aureus 262 control

S. aureus 262 + flucloxacillin 0.05μg ml^{-1}

S. aureus 178 control

S. aureus 178 + flucloxacillin 0.05μg ml^{-1}

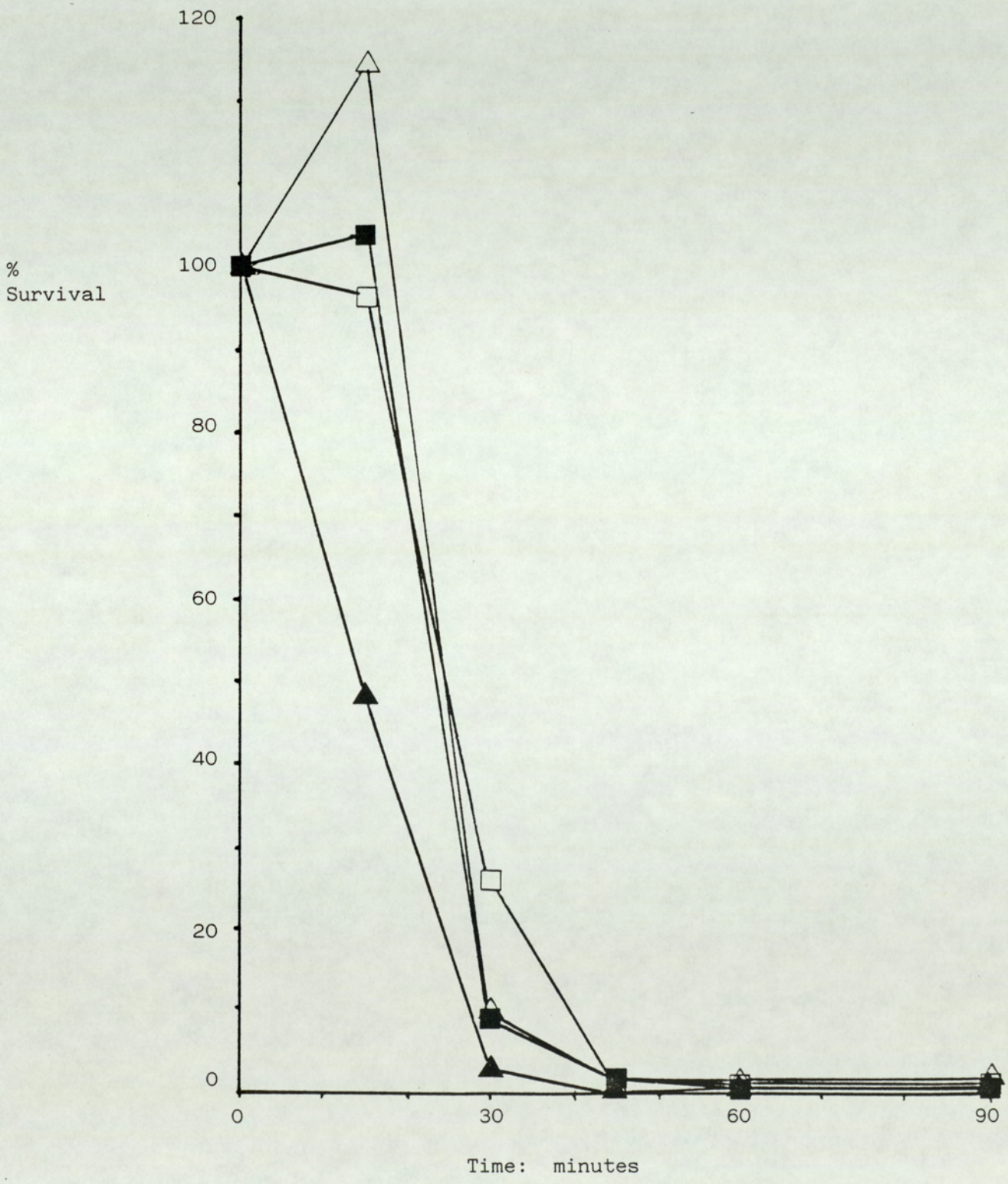

120
100
80
60
40
20
0
% Survival
0
30
60
90
Time: minutes

Table 20 **Association of S. aureus 262 and 178 with neutrophils during whole blood killing (Expt.1)**

Time (min)	Number of bacteria per neutrophil*			
	S. aureus 262		S. aureus 178	
	Flucloxacillin concentration		Flucloxacillin concentration	
	0.00μg ml^{-1}	0.05μg ml^{-1}	0.00μg ml^{-1}	0.05μg ml^{-1}
30	12	8	3	0.6
60	12	9	3	0.7
90	8	5	8	3

*Mean of 30 consecutive neutrophils

Table 21 **Association of S. aureus 262 and 178 with neutrophils during whole blood killing (Expt.2)**

Time (min)	Number of bacteria per neutrophil*			
	S. aureus 262		S. aureus 178	
	Flucloxacillin concentration		Flucloxacillin concentration	
	0.00μg ml^{-1}	0.05μg ml^{-1}	0.00μg ml^{-1}	0.05μg ml^{-1}
15	5	3	1	1
30	5	4	3	3
60	7	5	3	2

*Mean of 30 consecutive neutrophils

8. DISCUSSION

8.1 Bacterial surface hydrophobicity

Following growth under the conditions described in 6.3.1 S. aureus 178 was observed to have a larger contact angle than S. aureus 262 and showed a greater loss of optical density with n-octane using the BATH technique, thus both methods of assessment indicated S. aureus 178 to have a greater surface hydrophobicity than S. aureus 262.

Growth of the isolates in the presence of the sub-inhibitory concentration of flucloxacillin (0.05$\mu g\ ml^{-1}$) produced a considerable increase in surface hydrophobicity of both S. aureus 262 and 178 as measured by contact angle. A decrease in hydrophobicity was observed using the BATH technique which was particularly striking for S. aureus 178 and somewhat smaller for S. aureus 262. Although apparently contradictory, it is possible that these results may be compatible. Onaolapo and Klemperer (1986) reported a similar phenomenon in an isolate of Proteus mirabilis with and without R-plasmid RP 1 in which surface hydrophobicity was greater in the plasmid-containing bacteria when measured using BATH, but greater in the plasmid-lacking bacteria when measured by contact angle. The two methods employed to compare the bacteria may reflect different aspects of bacterial cell wall hydrophobicity, the contact angle technique involving the most superficial properties of the wall. Van Oss (1978) related surface hydrophobicity as measured by contact angle to the degree of susceptibility of bacteria to phagocytosis.

In view of the positive correlation between contact angle and phagocytosis, it is interesting that preliminary results (Section 7.4) suggest that S. aureus 178 may have been more susceptible to whole blood killing than S. aureus 262.

The cell wall is a three-dimensional matrix and changes in its structure caused by growth in the presence of flucloxacillin may affect its hydrophobic properties non-uniformly at different loci in the matrix. If, indeed, S. aureus 178 was more susceptible to whole blood killing than S aureus 262, then it is possible that contact angle measurement may provide a better indication of susceptibility to phagocytosis than the BATH technique.

8.2 Whole Blood Killing

No increase was observed in the rate of whole blood killing after growth of S. aureus 262 and 178 in the presence of flucloxacillin 0.05μg ml^{-1} even though both isolates had previously shown an increase in contact angle. Both strains were very sensitive to the effects of whole blood, with a kill in excess of 90% occurring within 60 minutes. Extensive further investigation into the action of blood during the first few minutes of the blood-bacteria interaction would be needed to detect any changes in the rate of kill of those bacteria grown in the presence of flucloxacillin.

An unexpected finding was the initial increase in viable count when whole blood was added to S. aureus 178 grown in the presence of flucloxacillin 0.05μg ml^{-1}. It is possible that clusters of cells may have developed as a result of growth with flucloxacillin and that these clusters may have been broken up on addition of the whole blood, resulting in an apparent rise in the number of viable cells.

No useful information could be obtained from the observations on the association of bacterial cells with neutrophils and this is perhaps not surprising in view of the high rate of kill. The observations made by microscopy, may suggest that there was a balance between the rate of cells adhering to the neutrophils and the rate of digestion, or even that a considerable part of the fall in viable count was independent of phagocytosis. Further investigations e.g. serum killing, would be required in order to assess the proportion of bactericidal effect attributable to phagocytosis. These investigations were not performed due to a limit on laboratory time.

8.3 Choice Of Sub-inhibitory Concentration

Throughout these investigations a single sub-inhibitory concentration of flucloxacillin was employed, 0.05μg ml^{-1}, and this represented approximately one half of the MIC for both isolates. If different concentrations of antibiotic had been used, the results obtained may have been somewhat different. In the clinical situation, the concentration of an antibiotic in the tissues changes constantly, and a more complex model would be required to imitate such conditions.

It is difficult to make direct comparisons between the two isolates regarding the effects of flucloxacillin at 0.05μg ml^{-1} i.e. approximately one half of the MIC, as the MIC only indicates the concentration required to achieve a bacteriostatic action on the most resistant cells in the population and cannot be measured with great accuracy.

8.4 Future Work

The bacteria employed in these investigations were cultured in suspension, in a standard growth medium and with a rapid rate of growth in a typical laboratory environment. Bacteria growing in animal tissues are exposed to a totally different environment in which the bacterial cells tend to grow on surfaces rather than in suspension, certain nutrients may be relatively unavailable and the growth rate is slow. It would be useful in future work to study bacterial properties during or after growth in living animal tissues as this may provide a model more akin to the clinical situation in which small numbers of bacteria are introduced into soft tissues through a lesion.

Further work will be required in the future to determine which method of measurement of bacterial cell wall hydrophobicity, if any, provides the best indication of susceptibility to phagocytosis.

P A R T 3

General Conclusions

1. The results of the clinical trial suggest that for the prevention of infection in accidental wounds, a five day course of oral flucloxacillin can safely be replaced with a single dose of 1g provided that adequate wound toilet is employed and 'Triplopen' is given.

2. The incidence of adverse effects associated with a 1g dose of flucloxacillin was no greater than for a five day course.

3. Preliminary experimental work in vitro showed that sub-inhibitory concentrations of flucloxacillin affected the surface properties of two isolates of Staphylococcus aureus. Low concentrations of flucloxacillin in vivo may make bacteria more susceptible to body defence systems.

4. The literature reviewed does not define the most advantageous way of using antibiotics either for prophylaxis of wound infection following minor accidental injuries or for the prevention of tetanus. Further investigation of surgical wound toilet with a view to reducing or eliminating the use of prophylactic antibiotics may be desirable.

5. If all aspects of accidental wound treatment were to be investigated together, considerable resources would be required. It seems that useful information can be gained from relatively small investigations such as this one.

Appendix 1a

PHARMACY DEPARTMENT, STAFFORD DISTRICT GENERAL HOSPITAL

FLUCLOXACILLIN TRIAL PROTOCOL

Title: A randomised, double blind, single centre study to investigate the comparative efficacy and tolerance of single oral dose (1g) flucloxacillin versus five day (250mg six hourly) oral flucloxacillin in prophylaxis of soft tissue infections in contaminated wounds requiring anti-staphylococcal antibiotic cover

Trial Organiser: P.S. Smith
Pharmacist, Stafford D.G.H./West Midlands Regional Health Authority

Trial Physician: Mr. A.V. Kumar, F.R.C.S.
Consultant Surgeon, Accident and Emergency Department, Stafford D.G.H.

Trial Advisers: Dr. B.M. Hynam
District Pharmaceutical Officer, Stafford D.G.H.

Dr. R.M.M. Klemperer
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Estimated Start Date: 6th March 1986

1. INTRODUCTION

Flucloxacillin, a gastric acid-stable and penicillinase resistant penicillin, is widely prescribed for the prophylaxis of soft tissue infections in wounds presented at hospital casualty departments.

A commonly used regimen is 250mg flucloxacillin orally, six hourly for five days.

Contaminated wounds are normally cleaned with antiseptics before suturing if necessary. Patients are given intramuscular penicillin injection for its anti-clostridial action and a five day course of oral flucloxacillin for its anti-staphylococcal action.

There appears to be little clinical evidence to demonostrate the effectiveness of flucloxacillin in this usage.

There are an increasing number of reports showing that single dose therapy with antibiotics can be as effective as more prolonged courses both in the treatment of infections and in prophylaxis e.g. treatment of urinary tract infections and prophylaxis in surgery.

This trial will compare the efficacy of single large oral dose flucloxacillin (1g) with a five day oral course (250mg six hourly) in preventing secondary wound infection.

Advantages of single dose therapy include convenience to the patient, guaranteed compliance and reduced cost of therapy.

2. OBJECTIVES

To assess the comparative efficacy and tolerance of single oral dose (1g) flucloxacillin versus five day oral (250mg six hourly) flucloxacillin in prophylaxis of soft tissue infection in contaminated wounds requiring anti-staphylococcal antibiotic cover.

3. PATIENTS

(i) Numbers

Approximately 400

(ii) Selection Criteria

Male or female patients aged >15 and <65 years, presenting with contaminated wound or wounds of one or more of the following types and requiring anti-staphylococcal antibiotic prophylaxis:-

(a) Laceration

(b) Dog, cat, human or other mammal bite

(c) Puncture wound (including those caused by firearms)

(d) Crush injury

(e) Grazing

continued ...

3. PATIENTS (continued)

Exclusions

(a) Current antibiotic therapy

(b) Known intolerance to penicillins

(c) Patients not receiving intramuscular Triplopen injection for any reason

(d) Signs of an established infection in the wound(s) under treatment

(e) Wounds sustained more than 24 hours prior to presentation

(f) Patients unable to take capsules

(g) Patients taking oral contraceptives

(h) Telephone contact with the patient for the purpose of wound assessment is not possible

4. METHOD

(i) Study Design

A randomised, double blind, single centre study.

(ii) Materials

Flucloxacillin 250mg capsules and matching placebo

Supplies will be pre-packed according to a random allocation code and numbered.

Issue will be from the Accident and Emergency Department, Stafford District General Hospital.

(iii) Trial Entry Procedure

To enter a patient into the trial who fulfils the inclusion criteria and none of the exclusion criteria the prescriber should:-

(a) Explain the nature of the trial to the patient and ask him/her to sign the consent form

(b) Mark the Accident and Emergency Department patient record card FLUCLOXACILLIN TRIAL

(c) Ensure that intramuscular penicillin (Triplopen) has been given and recorded on the card

(d) Ensure that the record card has been marked with a telephone number and times of availability

(e) Write a prescription for FLUCLOXACILLIN TRIAL PACK and record on the prescription the code number of the pack supplied

(f) Ensure that the patient takes the four capsules in Bottle 1 before leaving the department

continued ...

4. METHOD (continued)

(iv) Treatment

Patients will be allocated to the next available number and corresponding supply of capsules.

Each patient will receive a supply pack consisting either:-

A. Four placebo capsules for immediate supervised administration and twenty 250mg Flucloxacillin capsules as a five day oral course for self administration .

or

B. Four 250mg Flucloxacillin capsules for immediate supervised administration and twenty placebo capsules as a five day oral course for self administration

Each supply pack will consist of Bottle 1 labelled "Four capsules to be taken immediately" and Bottle 2 labelled "One capsule to be taken every six hours for five days.

(v) Prescription Charges

Patients entered into the trial will not be charged for flucloxacillin. The prescription charge will normally be payable for any other item(s) on the prescription.

5. ASSESSMENT

Patients will be assessed by structured telephone questionnaire seven days after presentation (or with reference to the state of the wound after seven days if interview is not possible at that time).

After discussion of the state of the wound in terms of swelling, redness, pain, exudation and colour of exudate, patients' wounds will be graded as:-

(a) Wound probably infected
(b) Wound probably not infected
(c) Information inconclusive

6. ADVERSE REACTIONS

All adverse reactions are to be recorded on the ADVERSE REACTIONS RECORD including the type, severity, date of onset, duration in days and relationship to flucloxacillin (remote, possible or probable).

7. WITHDRAWALS

Whilst every effort should be made to encourage patients to comply with the protocol, the prescriber or patient's general practitioner is free to withdraw a patient from the study at any time for any reason.

Patients are free to withdraw themselves from the study at any time.

Establishment of non-compliance on assessment is not a reason for withdrawal as this is an intrinsic risk of the treatment.

continued ...

8. ETHICS

(i) Consent

Written informed consent shall be obtained from each patient.

(ii) Trial Cessation

The trial shall be terminated if there is evidence of an unacceptably high rate of wound infection in any group of patients.

Appendix 1b

FLUCLOXACILLIN TRIAL - CONSENT FORM

We are carrying out a study to try to improve the treatment of your wound. At this stage in your treatment you would normally be prescribed a course of medicine to help your wound to heal. The usual course is one capsule (flucloxacillin 250mg) every six hours for five days. We hope to show that taking a single large dose of the medicine would be as effective and more convenient than the five day course.

You are invited to help us in our study and take four capsules immediately followed by one capsule every six hours for five days. You will receive either a single dose of medicine (four flucloxacillin 250mg capsules) followed by an inert five day course or an inert single dose followed by a five day course of medicine (flucloxacillin 250mg capsules).

You would be contacted in a week's time in order to assess the progress of your wound.

You would be free to withdraw from the study at any time.

A.V. Kumar
Consultant Surgeon

P.S. Smith
Pharmacist

I HAVE READ THE ABOVE STATEMENTS AND I AM WILLING TO TAKE PART IN THE STUDY DESCRIBED.

Signed .....

Date .....

Appendix 1c

FLUCLOXACILLIN TRIAL - QUESTIONNAIRE

QUESTIONNAIRE TO ESTABLISH THE PRESENCE OR ABSENCE OF WOUND INFECTION SEVEN DAYS AFTER ATTENDING THE ACCIDENT AND EMERGENCY DEPARTMENT

Surname:		Casualty No:	
Other Names:	SEX M.F.	Age:	D.O.B.
Address:		Occupation:	
Phone No:		Date of Attendance:	a.m. p.m.
G.P.		M. O. Treating	

Brief Description of Injury

Telephone Availability	Assessment Complete

INTRODUCTION

Hello. My name is Peter Smith, I am a research pharmacist at Stafford District General Hospital.

I understand that you were treated at the Accident and Emergency Department at the hospital about a week ago and kindly agreed to join in our study on the medicine flucloxacillin.

As you are probably aware, the study is comparing the effectiveness of a single dose of medicine with a five day course in helping wounds such as yours to heal.

I would like to ask you some questions about how well your wound is healing.

1.	(a)	Was your wound swollen immediately after the injury?			NO		YES
	(b)	Is your wound swollen now? OR Was your wound swollen a week after being treated?			NO		YES
		IF YES					
	(c)	How does this compare with the initial swelling, is it more swollen, less swollen or about the same?	LESS	(1)	SAME	(2)	WORSE (3)
2.	(a)	In the last week has the area around the wound been red?			NO		YES
	(b)	Is the area around the wound red now? OR Was the area around the wound red a week after being treated?			NO		YES
		IF YES					
	(c)	How does this compare with the earlier redness, is there more redness now than before, less redness or is it about the same?	LESS	(1)	SAME	(2)	WORSE (3)
	(d)	How far does the redness go from the edge of the wound?	¼"	(0)	¼" to 1"	(1)	1" (3)
3.		I would like to ask you about any pain associated with the wound.					
		Since your wound was treated has it become more painful, less painful or remained about the same.	LESS	(0)	SAME	(1)	WORSE (2)
4	(a)	Is your wound weeping? OR Was your wound weeping a week after being treated?			NO	(0)	YES (1)
		IF YES					
	(b)	What colour is this liquid?	COLOURLESS	(0)	WHITE	(2)	YELLOW/ GREEN (3)

Wound Assessment Score .....

Wound infection probable (Score 5 or more) ☐

Wound infection unlikely (Score 4 or less) ☐

Information inconclusive (assessment incomplete) ☐

5. (a) Did you need to return to the Accident & Emergency department at the hospital or visit your own doctor in connection with your wound during the last week? NO YES

IF YES

(b) Why?

6. (a) Have you had any illnesses or symptoms during the last week? NO YES

IF YES

(b) How severe was the problem?

(c) When did you start to .....?

(d) For how long did you .....?

7. (a) During the last week have you suffered from:

Diarrhoea NO YES

Feeling sick NO YES

Actually being sick NO YES

Any skin rashes No YES

IF YES to any of the above

(b) How severe was the problem?

(c) When did you start to .....?

(d) For how long did you .....?

8. (a) Many people find it difficult to remember to take their medicines.

When you were taking your capsules did you find it easy to remember to taken them? No YES

(b) For how many days did you take the capsules? .....

(c) How many capsules did you take each day? .....

(d) About how many capsules do you have left? .....

That is all I have to ask you.

Thank you very much for your help

Appendix 2

STAFFORD DISTRICT GENERAL HOSPITAL

Pharmacy Department Tel: Stafford 57731 Ext. 4140

Dear Patient,

Thank you for agreeing to participate in our study.

You will see that your medicine has been arranged in two containers and should be taken as follows:-

(i) Take the FOUR capsules in Bottle 1 before leaving the Accident and Emergency Department.

(ii) As soon as you return home start taking the capsules in Bottle 2.

(iii) You should take ONE capsule every six hours, which means FOUR capsules evenly spaced through each day.

(iv) Continue taking the capsules for FIVE DAYS until you have finished the course.

(v) The medicine is most effective if taken when your stomach is empty, for example, one hour before a meal or two or more hours after your last meal.

You will be contacted by telephone in a week's time in order to assess the progress of your wound.

Yours faithfully,

P.S. Smith

P.S. Smith
Pharmacist

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