Open fracture infection following combat trauma:
Defining the problem and evaluating novel treatments

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Surgeon Commander
Jowan G Penn-Barwell
Royal Navy

Institute of Cellular Medicine
University of Newcastle

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Abstract

The British military was engaged in combat operations in Iraq and Afghanistan over a 12-year period from 2003 to 2014. It has been asserted that over this time survival after combat injury improved generating a cohort of patients with complex limb injuries, including open fractures, which are prone to infection and challenging to reconstruct.

Using an anatomic measure of injury severity I demonstrate an improvement in survival after combat injury. I further tested this finding by devising a military specific version of an anatomic-physiological injury scoring system, which confirmed the survival improvement.

The UK military trauma registry was used to determine that the most frequently fractured bone was the Tibia and 65% of these fractures were open. Of these, 23% were surgically treated for infection in the first year and *S. aureus* bacteria was the causative organism in 60%. Infection was significantly associated with amputation or unplanned revision surgery.

To further investigate open fracture infections in a controlled setting, an established rodent model of a stabilised, *S. aureus* contaminated, femoral defect was refined. This model was used to investigate the relationship between timing of treatment and infection. The results of this study indicate that delaying antibiotics administration has a greater effect on infection rates than delaying surgery and that early antibiotics can reduce the greater infection seen with surgical delay but not negate its effect entirely.

Novel treatments with potential to reduce infection in open fractures were then evaluated. Chlorhexidine was found to be similar to saline for wound irrigation with respect to preventing infection. A novel biodegradable antibiotic gel proved to be superior at preventing infection in the model than the existing clinical standard local antibiotic delivery vehicle: bone cement (Polymethylmethacrylate) beads. Finally Bismuth Thiols were demonstrated to potentiate the effect of antibiotics in preventing infection.

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Chapter One: Introduction

1.0   Introduction

The assertion that medical advances are one of the few benefits of war is often repeated[1,2]. There is a logic to this idea: armed conflicts typically injure large numbers of previously fit young people in a concentrated place and time and the society to which they belong have an instinctive desire to provide optimal care for them. This results in both incentive and opportunity for improvements in trauma care to be developed.

The United Kingdom was continuously involved in over a decade of conflict, from the invasion of Iraq on 19th March 2003 to the cessation of combat operations in Afghanistan on the 27th October 2014. During this time all injured service personnel were evacuated to a single medical facility at the Royal Centre for Defence Medicine, Birmingham, UK.

As the design of body armour[3,4] and advances in resuscitation techniques[5,6] evolved throughout the 12 years of conflict, it was speculated that casualties were surviving who previously would have succumbed to their injuries. As a result, it was asserted that surgeons were faced with survivors with injuries of a severity not previously encountered. In effect, morbidity had been increased as a result of the reduction in mortality. The greatest recovery and rehabilitation for these injured survivors, requires their wounds to heal free from infection[6].

In this chapter, I will outline how trauma registries allow the study of injury patterns across a large number of patients, and how these are implemented in the military context. I will then discuss the nature and character of combat injuries and explain the rationale for focusing research on extremity injuries and infection.

Overall, this thesis seeks to characterise the combat injuries and define the burden of extremity injuries particularly open fractures complicated by infection. Furthermore, this work will explore potential strategies to reduce infection following combat open fracture.

1.1 Combat Injury

Unlike most traumatic injuries in peacetime, those sustained in combat are a result of a deliberate application of technology that aims to injure or kill. This is achieved by the transfer of energy from chemical form i.e. explosives or propellants, to the kinetic energy of bullets and blast fragments after detonation[7]. This kinetic energy is the ability to do *work*, in this case work equates to the shearing and laceration of body tissues when the kinetic energy is transferred into the tissues.

1.1.1 Trauma Registries

In addition to this heterogeneous pattern of injuries seen in trauma, the typical treatment pathway of a trauma casualty adds a further layer of complexity. Multiple healthcare professionals deliver care at numerous stages as the patient transitions from the prehospital environment, through the emergency department to the operating theatre and finally to critical care. As a result of these factors, determining the outcomes of patients across this complex system is challenging.

In the 1960s the United States' Academy of Sciences recognised that trauma care in the US was poor and recommended the improved collection of statistics as a basis for improving treatment[8]. As a result of this, the first trauma registry was established in Chicago in 1969[9]. The stated aims of this registry were:

> (1) to facilitate and improve patient care by rapidly locating and accurately reproducing significant amounts of clinical information germane to the patient's present clinical problem;
>
> (2) to provide on-line clinical summaries of diagnostic and therapeutic methods;
>
> (3) to establish a data source for developing at-risk factors for accidental events;
>
> (4) to define the variables on which patient morbidity and mortality depend;
>
> (5) to determine logistical and manpower requirements for a given community's trauma needs;
>
> (6) to estimate cost expenditures for certain injuries and their comprehensive care requirements;

(7) to provide continuous monitoring of project planning for the care of the critically injured.

Other cities and states developed similar registries and in the 1980s the American College of Surgeons initiated the Major Trauma Outcome Study, which led to the amalgamation and standardisation of local registries into the US National Trauma Data Bank (NTDB)[10].

Parallel to the US efforts to improve patient outcomes after trauma, in the UK in 1988 Sir Miles Irving published his analysis of 1,000 trauma deaths and highlighted the inadequacies in British trauma care[11]. This led to the establishment of the UK's own trauma registry, the Trauma Audit Research Network (TARN).

1.1.2 Military Trauma Registries

Collection of statistics on the sick and injured in the military has pre-dated efforts in the civilian setting. In 1780, Sir Gilbert Blane, Royal Navy physician to the West Indies squadron, insisted on monthly reports from all the Ships' surgeons on the health and mortality of their crews[12]. Similar efforts to collect information on, and effect improvements in, casualty care have occurred in nearly all major conflicts since.

In the UK, the Hostile Action Casualty Survey was developed in the Northern Ireland conflict in the 1970s, predominantly to examine the mechanisms and patterns of wounding and the efficacy of body armour[13]. Even though it was not designed for this purpose, HACS was sufficiently detailed to allow analysis of the performance of the combat casualty care system in the Falklands war.

The first recognisably modern military trauma registry was set up by Brigadier Tim Hodgetts in 1999 during UK peace-keeping operations in Kosovo[14]. This registry was designed specifically to measure combat casualty care and was used as the basis for the UK's Joint Theatre Trauma Registry (JTTR) for operations in the 2000s[15]. It differed from previous compilations of injury statisics and was a modern trauma registry in the sense that it contained integral statistical tools in order to measure the performance of the trauma system.

The JTTR was in place in an embryonic form for the start of combat operations in Afghanistan in 2001 and was fully functioning for the invasion of Iraq[16] in 2003. Data is prospectively captured on all trauma cases admitted to deployed UK military medical facilities that trigger a 'trauma alert', or that are subsequently repatriated to the UK for treatment of their injuries. This data collection is performed by two sets of trained 'Trauma Nurse Coordinators' (TNCs): one team of TNCs is in the deployed Medical Treatment Facility (MTF) and the other is based back in the Royal Centre for Defence Medicine (RCDM) in the UK. The database is administered by UK Defence Statistics (UKDS) agency.

The TNCs collect data on patient demographics and mechanism of injury, along with anatomic coding of injuries as per the Abbreviated Injury Scale (AIS) described in detail below, and physiological data i.e. vital signs and conscious level. The JTTR was established by the Academic Department of Military Emergency Medicine (ADMEM): as such, treatment details are focused on resuscitation e.g. blood products and the use of haemostatic dressings. Details on reconstructive surgery are not captured. Similarly, the only outcome recorded is mortality: there is no capture of patient reported outcomes, retention in military service, subsequent completion of military fitness test or any other measure of recovery.

1.2 Combat limb injuries and their treatment

As stated previously, injuries sustained in combat differ to those sustained in a civilian setting. Battlefield mechanisms of injury include explosions and high-energy gunshot wounds that are rarely encountered in the civilian setting. Unlike civilians, service personnel are normally wearing body armour when they are injured, potentially changing the distribution of their injuries.

1.2.1 Anatomic Distribution of Injuries

In conflicts since the Second World War, the majority of wounds sustained in combat have affected the extremities as shown in **table 1.1** below:

	Falklands [17]	US-Vietnam [18]	US-Korea [19]
Extremity wounds	75%	61%	65%
n	233	17,726	111,716

Table 1.1: anatomic distribution of injures in historic conflicts.

The clear preponderance of wounds affecting the extremities is demonstrated in these reports from previous conflicts. However, it is not possible to ascertain if these historical reports involve all casualties or only survivors. Similarly it is not clear how many of these wounds involved open fractures, nor what infection rates were.

Given the focus on improving body armour for the head and torso in the recent conflicts[20,21], it is reasonable to speculate that the proportion of injuries affecting the limbs will be greater compared to previous conflicts since the Second World War. If this assumption is correct, then the trend is likely to continue since personal protection continues to improve[22,23]. Therefore, improving the ability to treat and reconstruct limb injuries will be an ongoing requirement in the care of those injured in combat.

1.2.2 Open Fractures and their Treatment

Combat wounds do not occur in sterile, controlled surroundings and are universally contaminated. The extent of this contamination varies, and depends on multiple factors including the clothing the casualty was wearing, the environment in which they were operating, and the nature of the munitions that injured them.

Infection after combat injury has been a focus of significant attention since the birth of modern surgery in the 17th and 18th centuries. Key figures in the development of the discipline, Hunter and Pare-both experienced military surgeons, wrote extensively of the difficulties and dangers of wound infection [24,25]. In the First World War, fought largely in the heavily manured agricultural fields of Belgium and France, the wound infection incidence and aggression surprised military surgeons[26].

A fracture is a disruption in the continuity of a bone's macroscopic structure: it is classed as 'open' where there is a breach in the skin and soft tissue envelope

overlying the fracture. The preponderance of combat wounds to affect the extremities is clear, and given the high-energy mechanisms causing these injuries, it is not surprising that extremity injuries from the modern battlefield commonly result in fractured bones.

A study by the Owens *et al*. examined 1,566 US military casualties wounded or killed in the period from 2001 to 2005 as a result of enemy action. Their findings revealed that 26% of extremity wounds involved fractures, of which 82% were open[27]. This study was based on the earliest form of the US JTTR which was not regarded as highly reliable[28]. The US JTTR at that time used 9th edition of the International Classification of Disease (ICD-9) published in 1978 rather than trauma specific classifications systems like the Abbreviated Injury Scale (AIS)[29] more commonly used by Trauma Registries.

In an open fracture, there is a communication between the fracture and the external environment, leading to inevitable microbial contamination of exposed, injured bone tissue. The main difference between open and closed fractures is the increased risk of infection due to this contamination. While it is accepted that some wound infections are caused by fungal pathogens, this is relatively rare compared to bacterial infection which is therefore the focus of this work[30].

The current treatment of open fractures is aimed at preventing infection through two means: removal of contamination and necrotic tissue, and optimisation of the host immune system to eradicate pathological microbes.

The removal of contaminated and necrotic tissue is usually performed as part of a surgical procedure with two components. Firstly, the surgeon identifies and removes necrotic tissue and any frank contamination in a process known as *excision* or *debridement*. Secondly, *irrigation* is performed, whereby a liquid is used to rinse both macroscopic and microscopic contamination from the wound[31].

The relationship between the timing of surgical and antibiotic treatment of open fractures is not understood. It has been established that bacteria are capable of exponential growth in wounds[32] and therefore it is reasonable to assume that the earlier that either treatment is initiated then the lower the infection rate will be.

However, proving this via a clinical trial would be un-ethical and to date animal research in this area has looked at the antibiotic and surgical treatments separately and so the relationship between the two treatments is not understood.

Optimising the host immune system's ability to eradicate pathological bacteria from an open fracture wound is multifactorial and not fully understood. The use of systemically administered antibiotics to create an environment within the wound bed where contaminating bacteria are unable to thrive reduces infection[33]. Furthermore it has been shown that increased stability in a fracture, and therefore the wound bed, reduces infection in an animal model [34]. Swelling in an enclosed or partially enclosed muscular compartment can compromise blood flow and cause further necrosis, a process prevented by surgical *decompression* or *release* of surrounding fascial tissue.

The reason that musculoskeletal infection can be resilient to antibiotics is increasingly understood to be due to the ability of some bacteria to form biofilm[35]. In basic terms this is the process by which bacteria adhere to a surface, secrete a 'slime' of hydrated polymeric matrix and then enter a dormant state[36]. This then allows the bacteria to resist antibiotic concentrations in orders of magnitude greater than would be toxic in their planktonic form.[37]

1.3 Outstanding Questions

This thesis will attempt to improve the knowledge and understanding of combat injuries, in particular those involving open fractures of the limbs, by concentrating on a number of outstanding questions:

1.3.1 Has survival after combat injury improved and are we measuring it accurately?

British military forces were on engaged in conflict for 12-years between the invasion of Iraq on the 19th March 2003 until the end of combat operations in Afghanistan on 27th October 2014. Over the course of this period, it was asserted that combat casualty care system performance had improved significantly, resulting in larger numbers of seriously injured casualties requiring complex medical care and rehabilitation[38]. This situation can be summarised as a lowering in mortality after

combat trauma has resulted in a rise in morbidity as casualties were surviving with injuries that would have been fatal in the early stages of the conflicts.

However, even confident assertions to this effect by the National Audit Office were followed by the admission that accurately quantifying survival improvement is extremely challenging[38]. Previously, no academic study examining survival in UK service personnel have been published, nor official statistics released that allow such assertions to be examined. Whereas organisations like the UK TARN and the US NTDB provide external oversight of respective hospital's performance in terms of survival after injury, the UK DMS is largely responsible for monitoring its own performance, it is therefore important to clearly demonstrate that its performance is measured as accurately as possible.

It is unclear how common extremity wounds were in the recent conflicts and whether they frequently involved open fractures.

This thesis will seek to determine whether survival after combat injury has improved over the duration of the conflicts in Iraq and Afghanistan 2003-2014, and examine the best way to measure this improvement. Furthermore, whether this survival improvement has resulted in large numbers of surviving casualties with open fractures will be established.

1.3.2 How common is infection after open combat fractures, and what are the consequences?

Combat open fracture wounds are assumed to be universally contaminated, however in the antibiotic era it is not clear how common wound infections are, nor how serious are their consequences are. It is possible that this is no longer a common or serious problem.

This thesis will determine the infection rate after combat open fracture, identify causative pathogens and establish the consequences of infection on fracture healing and patient outcome.

1.3.3 How does the timing of surgical and antibiotic treatment of open fractures affect infection?

It is possible that early administration of systemic antibiotics might mitigate the effect of delaying surgery. However, if the is not the case, then there are implications for military medical logistics since casualties with open fractures will require transport across the battle space to receive surgical treatment without delay in order to avoid higher infection rates.

This work will attempt to explore the relationship and effect of timing of surgical and antibiotic treatment on infection in open fractures.

1.3.4 Are there novel treatments that might reduce infection in open fractures?

The last development in the prevention of infection in open fractures was the routine use of antibiotics for prophylaxis. There have since been three areas where novel treatments with the potential to reduce infection rates might emerge:

A. Since Lister first described irrigating open fractures with Carbolic acid in 1867[39], various antiseptic chemicals have been assessed for their ability to reduce infection in open fracture wounds but none have been shown to be superior to saline[40]. Chlorhexadine however is a relatively new antiseptic[41] which is frequently used clinically as it is toxic to micro-organisms while being relatively benign to mammalian tissue[42]. Chlorhexadine has not be evaluated as a potential irrigation solution for open fractures either clinically or in an animal model. If this readily available solution is superior to saline as a wound irrigation fluid at reducing infection in open fractures then this would provide a simple way for clinicians to reduce infection rates.

B. For antibiotics to be effective they have to reach a concentration in the wound sufficient to be toxic to the bacteria present there. If they are administered systemically e.g. intravenously or orally, in order for them to be effective their concentration throughout the body, not just the wound, must exceed this threshold. Not only is this inefficient, but it risks unintended adverse systemic effects. Administering antibiotics locally and directly to the wound could potentially avoid systemic side effects while achieving greater

concentrations in the wound. Identification of an effective vehicle for delivering local antibiotics into open fracture wounds could improve the efficacy against pathological bacteria in wounds while avoiding systemic toxicity.

C. The behaviour of bacteria in forming biofilm and the role this plays in resilience to antibiotics and therefore the development of recalcitrant musculoskeletal infection is increasingly understood[35]. Treatments that address this defensive bacterial behaviour therefore have the potential to increase bacterial susceptibility to antibiotics.

1.4 Conclusions

This thesis will define the nature of combat injuries from the recent conflicts in Iraq and Afghanistan. The question of whether survival has improved over the period of the conflict will be examined. Furthermore, the rate and consequences of open fracture infection will be determined.

An animal model of contaminated open fractures will be used to improve the understanding of the relationship between the timing of treatment and infection and evaluated potential novel treatments.

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Chapter Two: Combat injury and survival

2.0 Introduction

Death in combat is an unambiguous metric and was used historically to measure the human 'cost' of war.[1] However mortality is only part of the human cost, as examining death rates ignores those who survive with combat injuries. It has been argued that over the last two centuries, with the emergence and application to war of modern medicine, survival after combat injury has improved.[2]

If this assertion is correct then survivors with combat injuries will increasingly present a relatively larger need for medical and rehabilitative care. As such, improvements in the understanding and treatment of combat injuries will have even greater potential benefit for those surviving with combat injuries.

The assumption that survival following combat injury has improved over the last two centuries has inherent logic given the medical advances over such a long period. However, demonstrating a significant improvement over the twelve years that the UK was involved in major combat operations in Iraq and Afghanistan is more challenging.

In order to plan the surgical and rehabilitative care of those injured in combat, it is important to understand the nature of the injuries that casualties are surviving with. This thesis concerns the field of orthopaedic trauma and will therefore be limited to examining injuries to the limbs and pelvis that are managed by this surgical speciality.

2.1 Historic measurements of combat survival

As stated previously, it is reasonable to assume that over the last two centuries survival after combat injury has improved. There are two possible methods for testing this assumption: firstly by examining the mortality from a specific injury type in different conflicts over history; and secondly, by looking at the ratio of those killed to those injured - the Case Fatality Rate - in different conflicts in history.

2.1.1 Survival following a specific injury

This is the simplest method for measuring changes in survival after combat injury. It involves establishing the mortality rate in a sample of casualties with a similar injury type and in a specific conflict or time period. This is then compared with the mortality rate in a cohort from a different conflict or time period but with the same injury.

This methodology relies on two assumptions: firstly that all types of a specific injury are of the same severity, e.g. a 1915 German Imperial Army bullet traversing an abdomen will produce similar wounds to a 2008 AK-47 bullet fired by an Afghani insurgent.

The second assumption is that survival from that particular injury type is a valid surrogate of survival from all other injury types, e.g. improvements in survival following a head injury develop at the same pace as those following abdominal injury.

An obvious example of this metric is the open femur fracture, which was an area of considerable focus in the First World War. In his book on surgical care in the First World War *The early treatment of war wounds*, the Royal Army Medical Corps surgeon Sir Henry Grey stated that in the first two years of the war, mortality from a gunshot wound causing an open femur fracture was 80%[3]. This is consistent with the account of James McBean Ross MC, a doctor serving with a Royal Marine Battalion in the First World War:

"*It is an exceedingly common impression amongst both officers and men that a compound fracture of the upper half of the femur is necessarily fatal. My experience, perhaps unfortunate, is that a very large number of such cases die of shock, either at the regimental aid post, the field ambulance or the casualty clearing station.*"[4]

Assuming then that an open femur fracture resulting from a gunshot wound had a high mortality in the First World War, how does this compare with mortality from similar injuries today?

To answer this question, I used data from the Joint Theatre Trauma Registry (JTTR). As described in chapter one, the JTTR is an electronic database that prospectively gathers data on casualties sustained overseas and is administered by Defence

Statistics. Data are gathered on all casualties either killed immediately or who are injured and trigger a trauma alert on arrival at a deployed medical facility, or those whose injuries subsequently require repatriation to the UK. The data is collected by research nurses at deployed medical facilities and at the Royal Centre for Defence Medicine (RCDM), Birmingham, United Kingdom[5]. Research nurses use the Abbreviated Injury Scale (AIS) anatomic injury system to classify all injuries.[6]

Approval to search this JTTR was granted after formal application to the Joint Medical Command under number RCDM/Res/Audit/1036/14/0427.

The registry was searched for AIS codes that described bony injuries of the hip, femur or knee sustained between the invasion of Iraq on 19 March 2003 and 31 December 2012. There were no records of fatality cases with an isolated open femur fracture in that period. Conversely there were 48 open femoral shaft fractures amongst survivors, half of which (25/48) were as a result of gunshot injury [7].

Using this methodology, I was able to demonstrate a clear difference in survival between the early years of the First World War and the recent conflicts a century later. The same injury in the earlier conflict was associated with a high likelihood of fatality, whereas in the recent conflicts all patients survived this type of injury.

Despite its illustrative utility, this example also demonstrates the weaknesses inherent in this methodology. First, there is heterogeneity within this injury type: there is clearly a difference in injury severity between an open fracture from a gunshot wound where the femoral artery is severed and one where it is not, however such distinction is not made with this methodology.

The second weakness is the assumption that the mechanism of injury in the First World War and the recent conflicts are the same: this assumption is unlikely to be valid. Weapons used by the Imperial German Army were different to those used by insurgents in Iraq and Afghanistan, and therefore there will invariably be differences in the injuries that these weapons caused. Similarly, the wounds produced by artillery shells and those by buried improvised explosive devices are likely to be different.

However, perhaps the most important weakness in this methodology is the questionable validity of extrapolating improvements in survival from this one type of injury across all combat trauma. Specifically, open femur fractures were subject to particular attention in the First World War in an attempt to reduce mortality. Considerable efforts were made to train and equip medics to apply Thomas splints and dressings to open femur fractures and rapidly extricate injured patients[8]. Therefore survival from this injury is likely to have improved faster than other combat injuries.

Though measuring survival after a specific injury can indicate trends in survival it is very limited in its ability to accurately measure improvements in survival after combat injury. Furthermore, although it has the advantage of being applicable in a context of limited casualty statistics, it is unlikely to be sensitive to subtle changes.

2.1.2 Case Fatality Rate

The ratio between the number of fatalities from a conflict and the overall number of casualties (survivors and fatalities) is known as the Case Fatality Rate (CFR). This methodology has the advantage of relying on very simple casualty statistics, which are available even from conflicts in the 19th Century: this allows CFRs from different conflicts to be compared.

Using the CFR to compare survival rates from different conflicts relies on a number of assumptions. Firstly, the CFR is a ratio with a numerator and denominator; the numerator is the number of military battlefield fatalities, which is clearly defined and, at least in modern conflicts, unambiguous and meticulously recorded. The definition of the denominator i.e. the total number of casualties, is more complicated as there is ambiguity about what constitutes a wounded casualty i.e. an injured survivor, specifically, what severity of injury qualifies as 'wounded'. Multiple different definitions have been used throughout history, with terminology varying not only between conflicts, but between militaries for the same conflict.

Comparisons of the CFR between different conflicts, or over the course of long conflicts, rely on the assumption that the injury profiles are the same with an identical distribution of injury severity. This is hard to quantify but seems highly unlikely. For example, if a conflict involved more weapons designed to wound rather than kill e.g.

anti-personnel mines, then the CFR would appear lower than if a greater number of lethal weapons were used.

Despite these confounders, the CFR was used by the US military to justify their assertion that survival after combat wounding improved over the course of their involvement in the wars of Iraq and Afghnistan[9].

2.2 Quantifying injury severity

Traumatic injuries are heterogeneous, in order to allow valid analysis, injuries need to quantified.

2.2.1 Anatomic measures of injury severity

As previously stated, traumatic injuries are heterogeneous and confound attempts to correlate, cohort and analyse patterns of wounding. In the 1960s the American Association of Automotive Medicine (AAAM) devised a system for quantifying injury severity based on the anatomic nature of the injury. The AAAM intended to use the score to allow the correlation of specific injuries with collision types, and measure the injury severity associated with certain car designs.

Subsequently known as the Abbreviated Injury Scale (AIS), this system divides the body into eight anatomic regions i.e. lower limbs, upper limbs, head, face, neck, thorax, abdomen and spine, and then assigns a numerical code from one to six to each specific injury based on the threat to life from that injury. A score of one is minor and six is regarded as untreatable and unsurvivable. This system allowed the overall injury burden to be calculated for both individual regions and the whole casualty: patients with similar overall injury burdens could be grouped, even if their actual injuries were different[10]. Since 1969 the AIS has been expanded and refined periodically by the AAAM[11-14] with the most recent version published in 2015[6].

The AIS was used as the basis of an anatomic measure of injury severity by the Injury Severity Score (ISS), developed by Prof. Susan Baker in the 1970s using hospital and coroner's data from Boston in 1968-9[15]. The ISS used a simple methodology: the highest AIS values from the three most severely injured regions

were squared and summed, with the resultant score capped at 75. In their original series, no patient survived with an ISS of greater than 50.

The use of only a single worst AIS score from an individual anatomic region was recognised as an arbitrary feature of ISS and a potential flaw. In practice this meant that a double lower limb amputee was regarded as having the same lower limb injury burden as a single lower limb amputee. This was resolved with the New Injury Severity Score (NISS), which refines the original methodology by summing the squares of the three highest AIS values, even if they occur in the same region[16].

2.2.2 Anatomic/physiological measures of injury severity

The obvious criticism of the purely anatomic measure of injury severity was that is does not take into account a patient's physiological response to the traumatic insult. To address this, in the 1980s Champion and colleagues[17,18] proposed the Trauma Score (TS), and then the Revised Trauma Score (RTS), as physiological measures of injury severity. This approach used conscious level, along with cardiac and respiratory observations, to quantify physiological derangement in an attempt to predict death after injury.

Inevitability a system developed that used both anatomic scores and physiological parameters combined into a single trauma severity scale, and in the early 1980s the Trauma Injury Severity Score (TRISS) evolved[19-21].

TRISS not only combined anatomic scores of injury severity with a measure of physiological derangement, but also included the patient's age and whether they were injured by a blunt (e.g. motor vehicle collision or fall) or penetrating (e.g. stabbing or shooting) mechanism.

The TRISS methodology uses weighted co-efficients which were based on data from the Major Trauma Outcome Study (MTOS) established in 1982 and running to 1987[22]. These coefficients allowed for the probability of survival (PS) to be calculated. It was the intention of those who developed TRISS that: *'As improvements in trauma care over time result in decreased mortality, these MTOS coefficients can be expected to change'.*

The PS following an injury is calculated using the following TRISS equation:

$$PS = 1/(1 + e^{-\beta})$$

Where $\beta = b_0 + b_{ISS} + b_{Age} + b_{GCS} + b_{RR} + b_{SBP}$

ISS: Injury Severity Scale, GCS: Glasgow Coma Scale, RR: Respiratory Rate, SBP: Systolic Blood Pressure. The weighting of the coefficients 'b' vary depending on whether the mechanism of injury was blunt or penetrating.

The use of PS allowed trauma systems to be evaluated and compared: if a patient dies but their PS is >0.5 they are an unexpected fatality. Similarly, if a patient lived despite a PS <0.5 they are an unexpected survivor. A well performing trauma system would have more unexpected survivors than unexpected fatalities.

TRISS is the most widely adopted methodology to measure trauma system performance and is used by the US NTDB[23], the US military trauma registry[24] and the UK JTTR[25].

2.3   Measuring survival using an anatomic measure of injury severity

In order to test the assertion that survival following combat injury has improved over the course of the conflicts in Iraq and Afghanistan, I originally intended to use TRISS as a widely used, combined anatomic and physiological tool for predicting survival. However, during discussions with the UK JTTR and UK Defence Statistics it became apparent that the original TRISS coefficients based on 1980s US civilian data were being used. Furthermore it was not apparent if injuries from explosive weapons were being coded as penetrating or blunt.

Therefore I decided to rely initially on the NISS figures from the UK JTTR to measure if survival had improved. I intended to revisit the TRISS methodology and revise the coefficients later to: a) accurately reflect the contemporary standard of care and b) develop a coefficient for the mechanism of explosive injury.

It was apparent that due to the heterogeneous nature of the data, sophisticated modelling would be required to determine if the NISS predictive of survival increased over the study period. Therefore I requested the assistance of Dr Jon Bishop, a biostatistician from the University of Birmingham Clinical Trials unit, was engaged. Dr

Bishop developed the statistical modelling described below on the basis of the analytical questions framed for him.

2.3.1 Methods: data

The project was approved and registered with the Joint Medical Command RCDM/Res/Audit/1036/12/0319. The JTTR was searched for all UK casualties injured or killed in Iraq and Afghanistan between 2003 and 2012. New Injury Severity Scores were used as an anatomic measure of injury[16].

In order to provide a denominator to the casualty figures, population years at risk (PYAR) were calculated between 2008-12 from UK Defence Statistics data. This was based on computerised records of every day spent in either of the two operational theatres by each service person. These figures were summed for each calendar year and divided by 365 to give the PYAR i.e. the equivalent number of personnel deployed for 12 months. For 2003-7, detailed personnel records were not available: rather the information was extrapolated from Ministry of Defence (MoD) figures on troop levels contained in memoranda to the UK Parliament and is regarded as less precise[26,27]. All PYAR figures exclude Special Forces (SF), as details on SF deployments are not released by the MoD.

2.3.2 Methods: analysis

Data were grouped into calendar year cohorts according to the date of injury, and logistic regression used to examine relationships between year of injury and specific variables. In all models year of injury was coded as a continuous variable on the range 1-10 corresponding to years 2003-12.

A model was developed where the NISS score was included as a continuous variable as both a main effect and as part of an interaction term with year of injury. The interaction between year of injury and NISS was statistically significant (p = 0.009). The year of injury was modelled using restricted cubic splines to allow for flexible relationships. Model selection was based on Akaike Information Criterion[28]. Logistic regression was used for this analysis and fitting was performed using maximum likelihood estimation. The reference level of the outcome variable was coded as 'Fatality' (vs. 'Survival').

Analyses were conducted using *R* and the libraries: *stats* [29], *rms* [30], *effects* [31] and *nnet* [32].

2.3.3 Results

The JTTR recorded 2,792 UK casualties injured or killed during service in Iraq and Afghanistan. The mean age was 25.7 years (SD=5.9) and 2,746 (98%) were male. The majority of casualties (2,227, 80%) were a result of hostile action, with the remaining 565 (20%) resulting from incidents not involving enemy forces e.g. road traffic collisions. There were 608 fatalities (22% of all casualties) during this decade. The distribution of casualties and fatalities throughout the 10-year study period is shown in **Table 2.1**.

	Fatalities	Injured Survivors	PYAR	CFR
2003	50	43	17,820	54
2004	23	47	10,483	33
2005	24	76	10,767	24
2006	68	109	13,000	38
2007	88	320	13,300	22
2008	55	214	13,513	20
2009	109	435	11,909	20
2010	104	418	11,657	20
2011	46	301	11,771	13
2012	41	221	11,488	16
Total	**608**	**2184**	**125,708**	**22**

Table 2.1: Number of fatalities and injured survivors per year. PYAR = personnel years at risk; CFR = case fatality rate expressed as a percentage.

The odds of surviving a given injury severity was examined by analysing NISS as a continuous variable in a logistical regression model of NISS score and year, the output of which is shown in **Table 2.2**:

Model 2		
NISS	**OR**	**95% CI**
10	1.67	(1.39, 2.00)
25	1.55	(1.35, 1.77)
50	1.36	(1.25, 1.49)
75	1.20	(1.07, 1.36)
Observations	2762	(Fatalities: 590)
R^2	0.830	
LR χ^2	2121.81	Pr(>χ^2)<0.0001

Table 2.2: Output from the NISS model – Odds ratios (ORs) obtained from logistic regression of NISS score and Year (main effects and interaction) on survival. ORs denote odds ratio of a unit change in year for a fixed NISS value (e.g. the odds ratio of survival increased by a factor of 1.67 per year for a casualty with a NISS of 10).

Analysis demonstrated a consistent improvement in survival year-on-year over the decade of the study as shown in **Figure 2.1**.
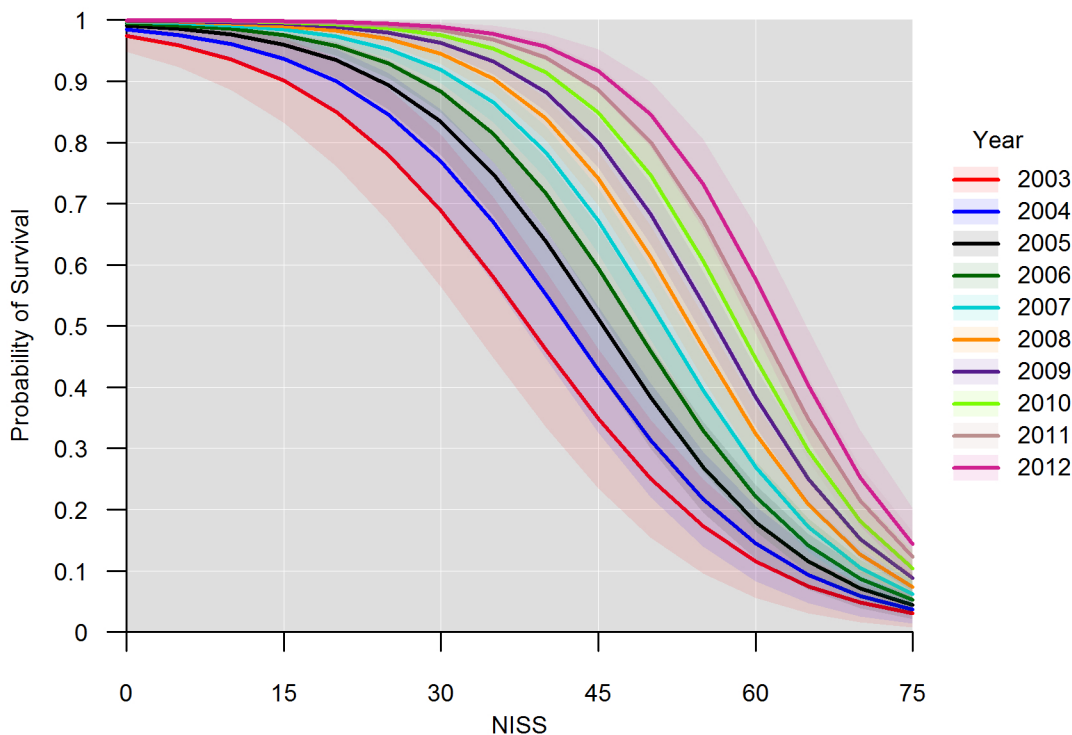
Figure 2.1: Plot of predicted probability of survival by NISS value for each year of study. Shaded regions indicate the 95% confidence intervals for the predicted values obtained from the logistic regression model summarized in **Table 2.2**.

The NISS model can be used to estimate the NISS value associated with a 50% probability of fatality for each year in the study period. The estimated probabilities of survival were obtained from the model for every possible NISS value in each year. The smallest NISS value with a corresponding 95% confidence interval lower limit

that exceeds 50% was identified in each year. This 50% survival NISS value rose from 32.5 in 2003 to 59.6 in 2012 **(Figure 2.2)**.
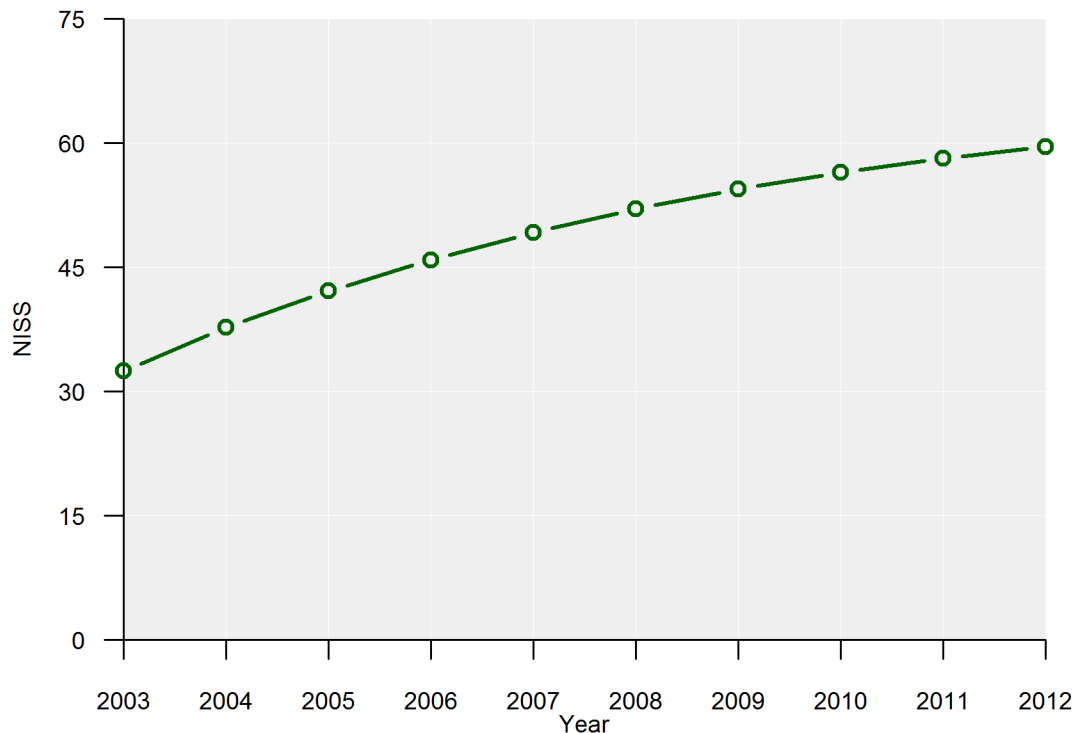
Figure 2.2: NISS values associated with a predicted 50% or greater probability of survival predicted by the logistic regression model summarized in **Table 2.2**.

This analysis, using an anatomic measure of injury severity, indicates that over the 10-years of the study period, survival improved significantly.

2.4 Deriving an anatomic/physiological measure of combat injury severity

As described in section 2.3, it was initially my intention to use TRISS to measure improvements in survival, as it has the advantage of being a combined anatomic and physiological method of measuring injury severity. However, as previously stated the version of TRISS the UK JTTR used was based on clinical outcomes from the 1980s and lacked a clear method for coding injuries from explosive mechanisms using either blast or penetrating coefficients.

The aim of this part of the work was to adapt the TRISS coefficients in two ways. Firstly, to reflect the contemporary standard of military clinical care, and secondly to allow for mechanisms of injury relevant to the military, specifically explosive injury, to be accurately coded.

2.4.1 Deriving contemporary, combat relevant TRISS coefficients: data set

This phase of work was approved by the Joint Medical Command as an extension to the previously described phase on survival using NISS (section 2.3.1). Due to the time taken to analyse the previous data set it was possible to expand the previously described 10 years of data to 12 years.

The JTTR was searched for all UK Casualties injured or killed in Iraq and Afghanistan by explosive or gun-shot mechanisms between 1st Jan 2003 and 31 Dec 2014. This calendar period encompassed the invasion of Iraq in 19 March 2003 until the cessation of major combat operations in Afghanistan on the 12th December 2014.

There were 3,043 UK casualties identified within the JTTR in the study period: 622 (20%) were injured by mechanisms other than explosive munitions or gunshot injuries, and were excluded. Of the 2,421 remaining cases, a further 319 (13%) were excluded due to inadequate data leaving a study population of 2,102 (**Figure 2.3**).
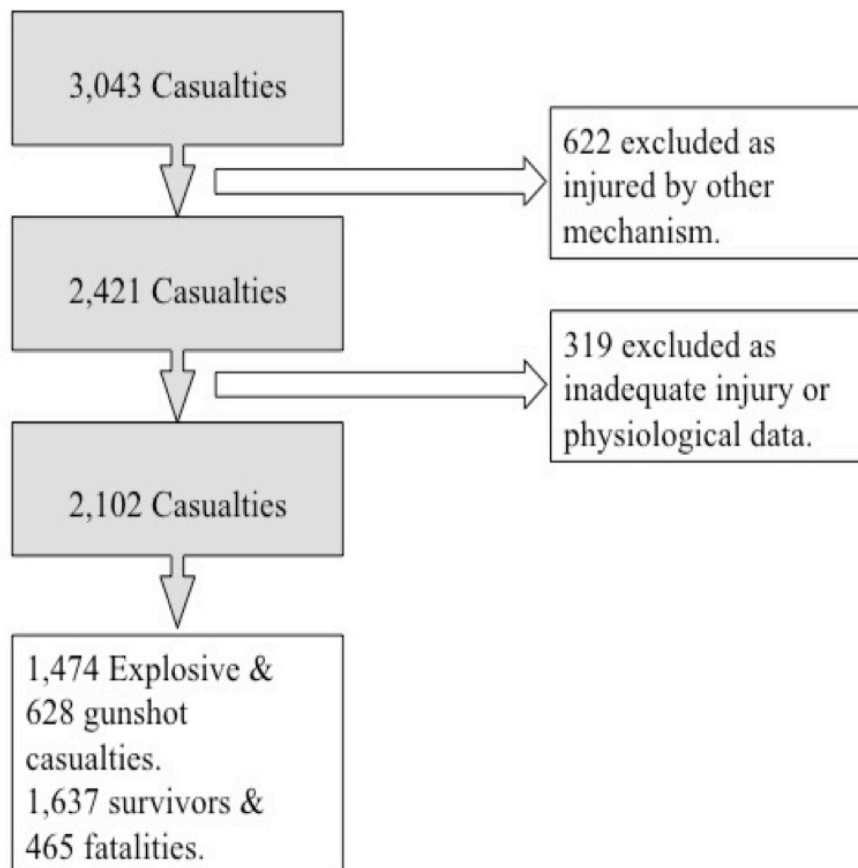
Figure 2.3: Flow diagram showing study recruitment and exclusion of casualties documented within the UK military Joint Theatre Trauma Registry (JTTR) resulting in 2,102 casualties available for analysis.

The study population contained 1,637 survivors and 465 fatalities; further details relating to the casualties can be found in **Table 2.3**.

	Explosive	Gun Shot Wound	Total
Survivors	1,163	474	1,637
Fatalities	311	154	465
Total	1,474	628	2,102

Table 2.3: Details of the UK combat casualties used to determine military relevant Trauma and Injury Severity Score (TRISS) coefficients.

2.4.2 Deriving contemporary, combat relevant coefficients: methods

In order to perform this phase of study, again I requested Dr Bishop to develop another multivariable logistic regression model. The variables were selected to mirror the construction of the original TRISS model[21]. Each component of the Revised Trauma Score (RTS, described above in **section 2.2.2**) was included in the model as an independent predictor variable with a coded value between 0 and 4, as determined by the RTS thresholds.

Age at injury is normally included in TRISS models as a binary variable (assigned 0 if age <55, 1 if age ≥55). In our dataset fewer than five casualties were aged 55 or over at time of injury: therefore, we had insufficient data to estimate the parameter corresponding to age and excluded this variable from our model.

The standard TRISS formula was used:

$$Ps = {1}/{(1 + e^{-\beta})}$$

Where $\beta = b_0 + b_1(ISS) + b_2(GCS_c) + b_3(RR_c) + b_4(SBP_c)$ and the c subscript denotes the use of the coded value (0 to 4) rather than the observed value.

A variable corresponding to mechanism of injury (either gunshot or explosive) was then included and the parameters for each of these levels estimated.

Model accuracy was assessed by evaluating the area under the Receiver Operating Characteristic (ROC) curve, with reporting of the Area Under the Curve (AUC) and 95% confidence intervals.

2.4.3 Deriving contemporary, combat relevant coefficients: results

The logistic regression analyses produced the updated coefficients given in **Table 2.4**.

Variable		Explosive Injury	Gun Shot Wound
TRISS coefficient	α_1	2.1361	-1.0996
RR (breaths/min)	β_{RR}	0.2149	-0.1261
SBP (mmHg)	β_{SBP}	1.0968	1.0761
GCS	β_{GCS}	0.6818	1.1228
ISS	β_{ISS}	-0.1053	-0.0490

Table 2.4: New Trauma and Injury Severity Score (TRISS) coefficients derived from contemporary UK combat casualty data.

If a predicted probability of survival (PS) of 0.5 or above is taken as denoting survival and a probability of less than 0.5 as denoting fatality, then the accuracy of the revised TRISS model can be measured. The results are given in **Table 2.5**.

		Predicted Outcome (TRISS Blast Model)		Predicted Outcome (TRISS GSW Model)	
		Fatality	**Survived**	**Fatality**	**Survived**
Observed Outcome	**Fatality**	303	8	149	5
	Survived	16	1147	9	465

Table 2.5: The performance of the new Gunshot Wound (GSW) and Blast Trauma and Injury Severity Score (TRISS) models.

Comparing the predictions from the GSW TRISS model to the observed outcomes, it demonstrates a sensitivity of 98.1% (465/474) and a specificity of 96.8% (149/154) giving an overall accuracy of 97.8% (614/628). With respect to the explosive TRISS model, there is a sensitivity of 98.6% (1147/1163), a specificity of 97.4% (303/311), giving an overall accuracy of 98.4% (1450/1474).

The ROC curve for the updated GSW TRISS model is shown in **Figure 2.4** with a c-statistic of 0.9966 (95%CI: 0.9940, 0.9992). The ROC for the explosive TRISS model is shown in **Figure 2.5** with a c-statistic of 0.9979, (95%CI: 0.9966, 0.9991).
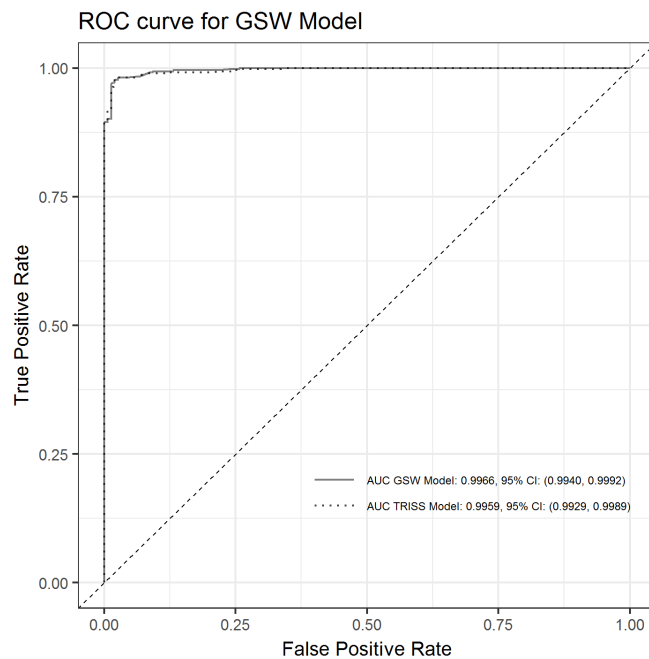
Figure 2.4: Receiver Operator Characteristic (ROC) curve for the updated Gun-Shot Wound (GSW) Trauma and Injury Severity Score (TRISS) model (grey) compared to the original TRISS model (dotted)[21].
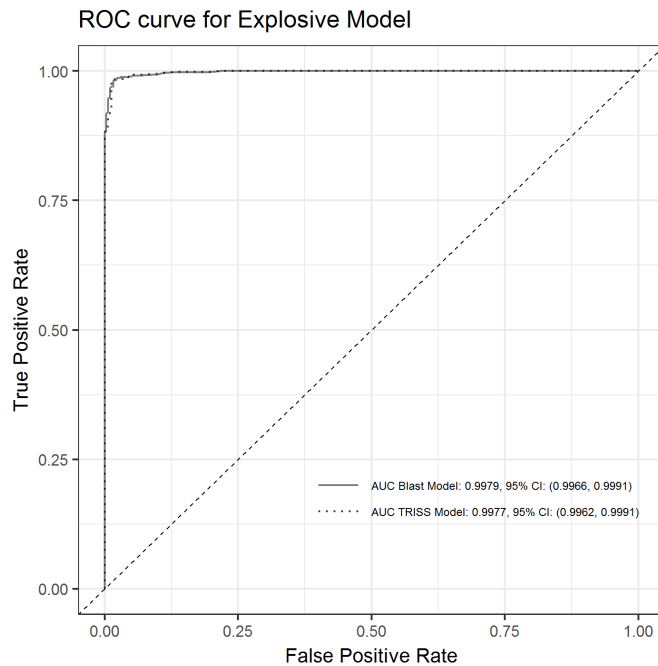
Figure 2.5: Receiver Operator Characteristic (ROC) curve for the updated Blast Trauma and Injury Severity Score (TRISS) model (grey) compared to the original TRISS model (dotted)[21].

Global goodness-of-fit can be assessed through the le Cessie-Van-Houwelingen-Copas-Hosmer test statistic, which gives a p-value of 0.799 for this model. This indicates there is no evidence that the available data was a poor fit for this model.

Model calibration can be assessed through estimating the uniform shrinkage factor or optimism of the model[33]. Models that are well calibrated produce predicted probabilities that closely match observed probabilities and have calibration slopes close to 1. Following bootstrap resampling using 999 replicates, the revised TRISS model has a calibration slope of 0.9357, suggesting a high level of calibration with some small level of over-fitting.

The overall accuracy of predictions can be assessed through the Brier score, which measures the average prediction error. In well-specified models a Brier score of 0 denotes perfect prediction and a score of 0.25 denotes the worst possible performance. The revised TRISS model has a Brier score of 0.014 suggesting a high level of predictive accuracy.

2.4.4 Measuring survival with revised coefficients: methods

The updated TRISS coefficients for both the explosive and gunshot models were used to calculate new TRISS values for casualties wounded by either mechanism of

injury. These updated TRISS values were used in logistic regression analyses to model survival outcomes using the methodology previously described in section 2.3.2[34]. Data were grouped into calendar year cohorts according to date of injury and logistic regression was used to examine the relationship between year of injury and updated TRISS. TRISS was modelled as a continuous variable both a main effect and as part of an interaction term with year of injury. Year of injury was modelled using restricted cubic splines to allow for flexible relationships. Model selection was based on Akaike's Information Criterion[28] and fitting was performed using maximum likelihood estimation.

All model analysis was conducted using R and the libraries: *stats*[29], *rms*[30], *effects*[35] and *ggplot2*[36].

2.4.5 Measuring survival with new methodology: results

The relationship between survival and time was examined by plotting the smallest TRISS value at which the lower limit of the 95% CI for the predicted probability of survival exceeded 0.5 in each year **(Figure 2.6)**. With the original TRISS scoring system, the performance of combat casualty care appears to peak in 2009 and then decline slightly with calculations in 2013 and 2014 not marked as the lower 95%CI remains greater than 0.5. When the new explosive and GSW coefficients are used to model survival over the study period the improvement in survival is shown to 'plateau' from 2010 onwards.
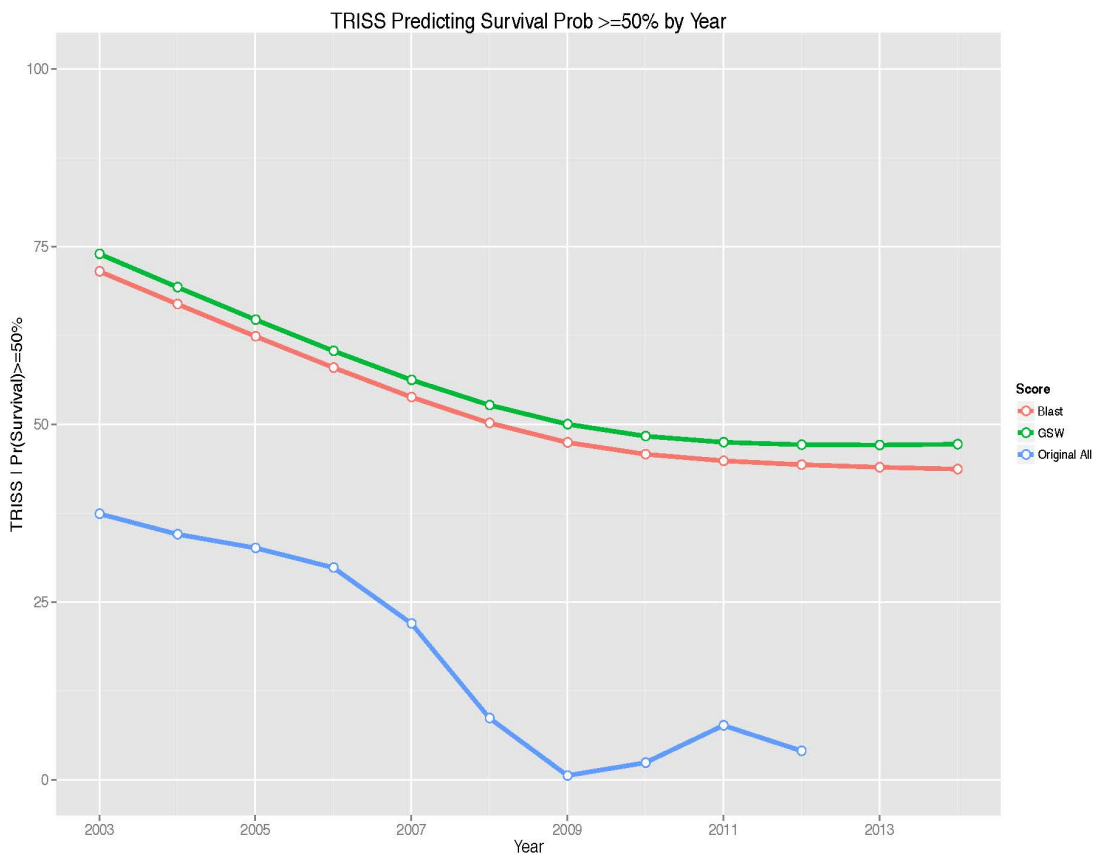
Figure 2.6: TRISS associated with a 50% chance of survival by calendar year, as determined by logistic regression. Calculations based on original 1987 TRISS methodology for all casualties shown, along with modified TRISS values for separate groups of casualties injured by explosive or GSW mechanisms. The plot for the original TRISS methodology is not shown for 2013 and 2014 as the 95% CI span 0 and 50.

2.5 Injury mechanism

The mechanism of injuries seen in combat is distinct from that seen in the civilian healthcare setting, but it is not homogenous and changes over time as the character of conflicts evolve.

I performed a sub-analysis of the examination of survival patterns in order to characterise the mechanism of injury during the Iraq and Afghanistan conflicts and demonstrate any changes in injury pattern.

The dataset from the UK JTTR described in detail in **section 2.3** and covering the 10 years of conflict 2003-12 was used. This data set involved 2,792 UK casualties injured or killed during service in Iraq and Afghanistan. The majority of these

casualties (2,227, 80%) were a result of hostile action, with the remaining 565 (20%) resulting from incidents not involving enemy forces e.g. road traffic collisions.

The most common mechanism of injury was explosive weapons, causing 1,592 casualties, representing 56% of total casualties and 65% of those from hostile action. Gun-shot wounds (GSW) were the next most significant mechanism of injury, being the cause of 684 casualties, 28% of total casualties and 31% of those from hostile action. Aside from the increased proportion of GSWs in 2003 during the invasion of Iraq, the relative proportion of casualties of GSW to injuries from explosive weapons remained approximately consistent as the conflicts in Iraq and Afghanistan evolved at approximately 1:3. This relationship between wounding patterns is represented graphically below in **Figure 2.7** below:
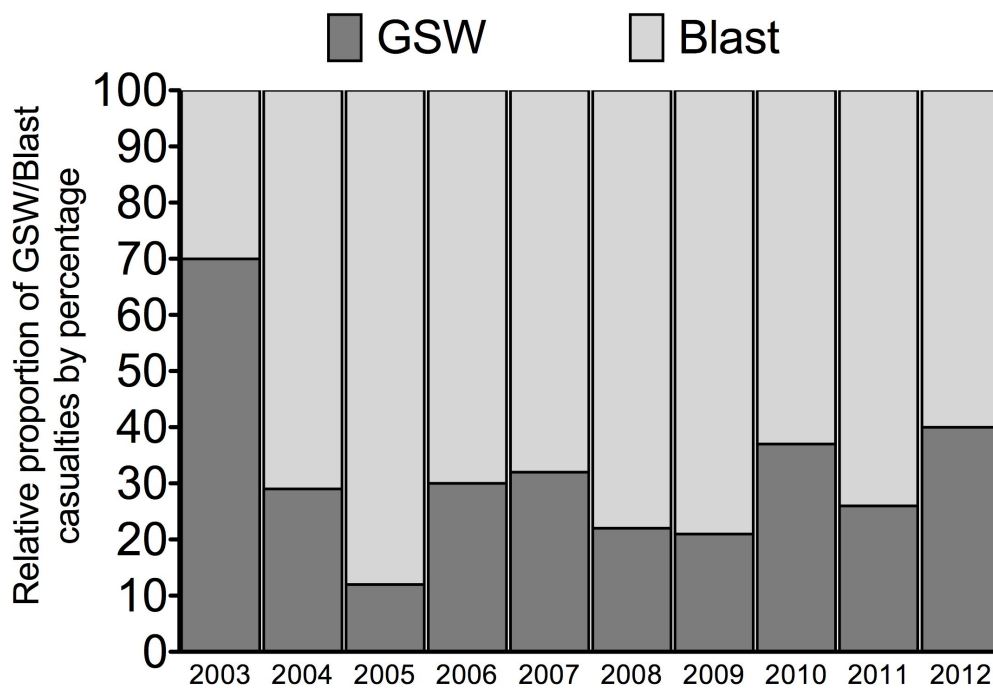
Figure 2.7: The relative proportion of injuries caused by gunshot wounds (GSW) or blast (explosive munitions) encompassing Improvised Explosive Devices, mortars, rockets mines etc.

2.6 Anatomic injury patterns

As outlined in **section 1.1** of Chapter One, it is believed that several factors, including improvements in body armour, may have resulted in an increased proportion of injuries involving the extremities. This phase of this work sought to test

whether the anatomic regions injured changed over the course of the conflicts in Iraq and Afghanistan.

2.6.1 Have anatomic injury patterns changed? Methods

In order to determine whether anatomic injury patterns changed over the course of the Iraq/Afghanistan conflicts, I requested that Dr Bishop build a similar multinomial log-linear regression model using the same 10 year dataset from the UK JTTR as previously described in **section 2.3.1**.

The model was used to examine the distribution of total recorded injuries across the eight AIS body regions by year of injury. The categorical outcome variable, body region of injury, was coded with 'Abdomen' set as the reference level as the proportion of injuries affecting this region was stable over the study period. Year of injury was modelled using a restricted cubic spline to allow for flexible non-linear relationships between time and region of body injury. Model fitting of this complex data was performed using a quasi-Newton optimization method due to the potential for convergence problems using standard optimization algorithms. Injuries to body regions were assumed to be clustered by individual: standard errors were estimated using a cluster bootstrap approach based on 10000 samples to account for this.

Observed proportions of injuries by body region and year of injury were analysed for temporal trend using the Cochran-Armitage test. Although there is evidence that the proportion of injuries by body region is associated with year (p<1e-11) the Cochrane-Armitage test is restricted to testing for linear monotonic trends. We wished to examine how the relative distribution of injuries across body regions changed over time. Models that included non-linear functions of time provided an improved fit to the data and were used for the analyses.

2.6.2 Have anatomic injury patterns changed? Results

During the ten year study period, 14,071 injuries were sustained by the 2,792 casualties, distributed across body regions as shown in **Table 2.6**. The extremities were the most commonly injured body regions, comprising 43% of all injuries. The relative distributions of injuries affecting the abdomen, thorax, spine, face, neck and upper extremity remained relatively constant over the study period.

	Total Injuries	Head (%)	Face (%)	Neck (%)	Thorax (%)	Abdomen (%)	Spine (%)	Lower Extremity (%)	Upper Extremity (%)
2003	320	43 (13)	25 (8)	15 (5)	78 (24)	41 (13)	15 (5)	59 (18)	25 (8)
2004	263	54 (21)	25 (10)	16 (6)	40 (15)	16 (6)	12 (5)	45 (17)	37 (14)
2005	241	13 (5)	19 (8)	20 (8)	27 (11)	26 (11)	10 (4)	50 (21)	58 (24)
2006	741	140 (19)	75 (10)	36 (5)	118 (16)	57 (8)	35 (5)	116 (16)	86 (12)
2007	1,964	284 (14)	224 (11)	65 (3)	228 (12)	179 (9)	91 (5)	490 (25)	321 (16)
2008	1,503	126 (8)	143 (10)	29 (2)	233 (16)	191 (13)	74 (5)	465 (31)	199 (13)
2009	3,320	282 (8)	422 (13)	95 (3)	414 (12)	470 (14)	225 (7)	842 (25)	501 (15)
2010	2,599	196 (8)	299 (12)	85 (3)	279 (11)	368 (14)	161 (6)	758 (29)	404 (16)
2011	1,787	140 (8)	194 (11)	45 (3)	156 (9)	177 (10)	75 (4)	649 (36)	326 (18)
2012	1,333	117 (9)	134 (10)	47 (4)	204 (15)	91 (7)	58 (4)	393 (29)	250 (19)
Total	14,071	1,395 (10)	1,560 (11)	453 (3)	1,777 (13)	1,616 (11)	756 (5)	3,867 (27)	2,207 (16)

Table 2.6: Injured regions as defined by AIS system[37] per year. NB more than one injury possible per region and casualty.

The relative risk ratio (RR) of sustaining an injury to the head, relative to sustaining an injury to the abdomen (which remained relatively constant), changed by a factor of between 0.87 95%CI (0.78, 0.97) and 0.79 (0.68, 0.92) per year from 2006 to 2010. Relative risk ratios for head, face and neck injuries for all unit changes in year are presented in **Figure 2.8**.
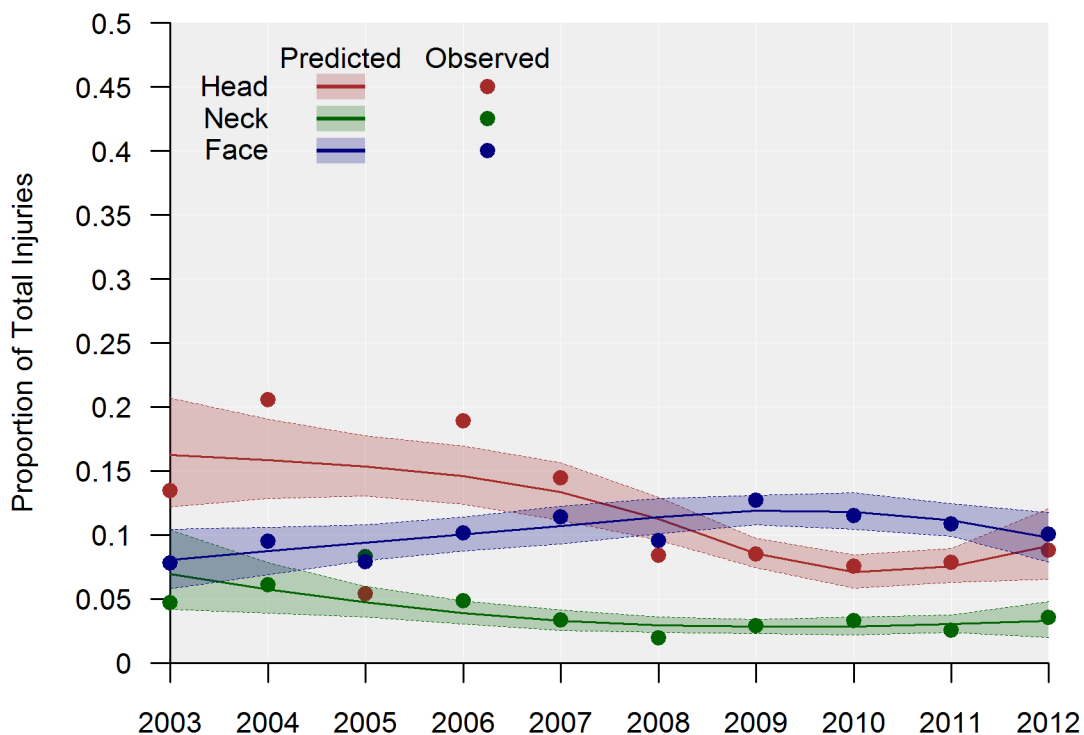
Figure 2.8: Distribution of head, face and neck injuries over time as proportion of total injuries. Shaded regions denote 95% confidence intervals about the predicted values obtained from the multinomial logistic regression model. Dots denote observed proportions.

The relative risk (RR) of sustaining an injury to the lower extremity, relative to the abdomen, remained statistically indistinguishable from zero between 2003 and 2010. The RRs changed by 1.44 (95%CI 1.25, 1.68) from 2010 to 2011 and by 1.80 (95%CI 1.41, 2.30) from 2011 to 2012. RRs for lower extremity injuries for all unit changes in year are presented in **Figure 2.9**.
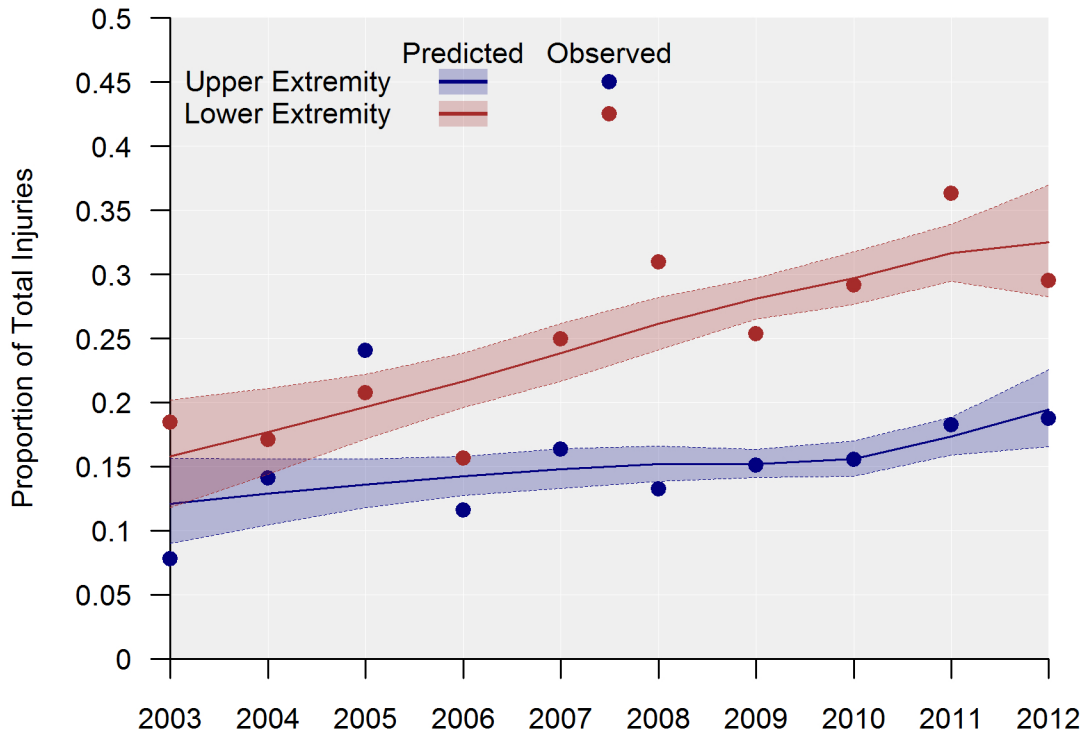
Figure 2.9: Distribution of upper and lower extremity injuries over time as proportion of total injuries. Shaded regions denote 95% confidence intervals about the predicted values obtained from the multinomial logistic regression model 1. Dots denote observed proportions.

The predicted probabilities from the logistic regression model display the negative trend in the proportion of head injuries (**Figure 2.8**), and the positive trend in the proportion of lower extremity injuries (**Figure 2.9**) over the study period. Predicted probabilities, and corresponding 95% CIs, for receiving an injury to a specific body region for each year are presented in **Table 2.7** below:

	Head % (95% CI)	Face % (95% CI)	Neck % (95% CI)	Thorax % (95% CI)	Abdomen % (95% CI)	Spine % (95% CI)	Lower Extremity % (95% CI)	Upper Extremity % (95% CI)
2003	16 (12, 21)	8 (6, 10)	7 (4, 10)	19 (14, 24)	9 (6, 13)	4 (3, 6)	16 (12, 20)	12 (9, 16)
2004	16 (13, 19)	9 (7, 11)	6 (4, 8)	18 (14, 21)	9 (7, 12)	4 (3, 6)	18 (14, 21)	13 (10, 16)
2005	15 (13, 18)	9 (8, 11)	5 (4, 6)	17 (14, 19)	9 (8, 11)	5 (3, 6)	20 (17, 22)	14 (12, 16)
2006	15 (12, 17)	10 (9, 11)	4 (4, 6)	15 (14, 17)	9 (8, 11)	5 (4, 6)	22 (20, 24)	14 (13, 16)
2007	13 (11, 16)	11 (9, 12)	3 (3, 4)	14 (12, 16)	10 (8, 11)	5 (4, 6)	24 (22, 26)	15 (13, 16)
2008	11 (10, 13)	11 (10, 13)	3 (2, 4)	13 (11, 14)	11 (10, 13)	5 (5, 6)	26 (24, 28)	15 (14, 17)
2009	9 (7, 10)	12 (11, 13)	3 (2, 3)	11 (10, 13)	14 (12, 15)	6 (5, 7)	28 (27, 30)	15 (14, 16)
2010	7 (6, 8)	12 (10, 13)	3 (2, 4)	11 (9, 12)	14 (13, 16)	6 (5, 7)	30 (28, 32)	16 (14, 17)
2011	8 (6, 9)	11 (10, 12)	3 (2, 4)	12 (10, 13)	11 (9, 12)	5 (4, 6)	32 (29, 34)	17 (16, 19)
2012	9 (7, 12)	10 (8, 12)	3 (2, 5)	13 (10, 17)	6 (4, 8)	4 (2, 5)	32 (28, 37)	19 (17, 23)

Table 2.7: Predicted proportions of injuries for each body region by year. Predicted values and 95% confidence intervals obtained from multinomial log-linear model using cluster bootstrap estimates of standard errors.

2.7 Extremity injuries in survivors

The findings of the previous sections establish that survival increased over the course of the conflicts in Iraq and Afghanistan, and furthermore that injuries to the lower extremity became proportionately more frequent during that time. The next phase of this work is to look at extremity injuries in greater detail.

The underlying theme of this work is the surgical treatment of combat casualties. Although many fatalities in war are killed immediately, some survive long enough to require surgical treatment before succumbing to their injuries[38]. Therefore I wanted this section of work to focus solely on those surviving their injuries, and exclude those who received some surgical treatment before dying of their wounds.

The aim of the following study was to characterise the range of extremity injuries sustained by surviving UK military casualties over the 12 years of conflict in Iraq and Afghanistan. Furthermore, this study aimed to define the treatment burden (as a feature of length of hospital stay and number of operative procedures) associated with extremity injuries sustained on the modern battlefield.

2.7.1 Characterising extremity injuries in survivors: methods

All cases recorded in the JTTR in the twelve years between the invasion of Iraq on the 19th March 2003 and cessation of combat operations in Afghanistan on the 27th October 2014 were examined. Casualties who were killed in action or died from their wounds were excluded. No distinction was made between casualties injured as a result of hostile or non-hostile action. Survivors with AIS codes for extremity injuries

(including pelvic fractures) were included. Survivors with spinal injuries but no extremity injury were excluded. All extremity injury codes were included, capturing patients with soft tissue injuries but no fracture or amputation in addition to those with bony injuries.

Data were gathered from the JTTR on patient demographics, injury pattern, surgical management and length of hospital stay (LOS). LOS is expressed as median with interquartile range (IQR) throughout. Amputation was defined as traumatic-complete or nearly complete at time of initial coding and did not include amputation following failed salvage or reconstructive surgery. Only the loss of an extremity at or more proximal to the wrist or ankle was included as amputation; i.e. digit loss was not regarded as an amputation for the purpose of this study. Fractures of the carpals, metacarpals, phalanges, tarsals and metatarsals respectively were each grouped together and counted as a single fracture. Bilateral amputations were counted as separate injuries, as were fractures in the same limb as an amputation but at a different level, e.g. a femoral fracture in a limb with a trans-tibial amputation.

LOS data included the time spent in deployed medial treatment facilities and in the Royal Centre for Defence Medicine (RCDM), but did not include re-admission or time spent as an inpatient at rehabilitation facilities. A surgical episode was defined as a single episode in an operating theatre undergoing surgery and not as separate surgical procedures. Surgical episodes were only counted if they addressed an extremity injury and only during the initial hospital admission.

As previously, I calculated Personnel Years at Risk (PYAR) between 2008-14 from UKDS data. This was based on computerised records of every day spent in either of the two operational theatres by each service person. These figures were summed for each calendar year and divided by 365 to give the PYAR i.e. the equivalent number of personnel deployed for 12 months. For 2003-7, detailed pay records were not available, therefore the information was extrapolated from Ministry of Defence (MoD) figures on troop levels contained in memoranda to the UK Parliament and is regarded as less precise[26,27].

Descriptive data are given as medians with inter-quartile range (IQR). Continuous data (e.g. NISS) was analysed by Mann-Whitney testing using Graphpad Prism 6 (San Diego, CA, USA) with a threshold for significance set at 0.05.

2.7.2 Characterising extremity injuries in survivors: results

During the twelve years of combat in Iraq and Afghanistan, UK personnel were deployed for 137,174 PYAR, from which 2,348 were injured and survived. Of these, 1,813 (77%) had extremity injuries, of which 205 (11%) had at least one amputation at the level of, or proximal to the carpus or ankle. In the same period 1,530 fractures were recorded: 501 (33%) involved the upper limbs and 1,029 (67%) involved the lower limbs and pelvis with the remaining injuries involving just the soft-tissues. Three hundred and forty four (344/501, 69%) upper limb fractures were open, compared to 597 (597/1,029, 58%) lower limb fractures. The temporal distribution of these injuries is shown in **Table 2.8** and **Figure 2.10,** and the anatomic distribution in **Figure 2.11**.

Year	PYAR	Survivors with extremity injuries	Survivors with long bone fractures	Survivors with major amputations
2003	17,820	22	4	*
2004	10,483	31	9	*
2005	10,767	49	11	*
2006	13,000	70	20	10
2007	13,300	254	57	19
2008	13,513	181	58	16
2009	11,909	329	89	41
2010	11,657	329	93	52
2011	11,771	252	67	35
2012	11,488	179	52	24
2013	7,679	98	20	*
2014	3,787	19	6	0
Total	**137,174**	**1,813**	**486**	**205**

Table 2.8: Incidence of extremity injury, long bone fracture and major amputation per calendar year of conflict with Population year at risk (PYAR) shown. Years with identifiably low numbers of amputees omitted and marked with '*' to maintain patient confidentiality.
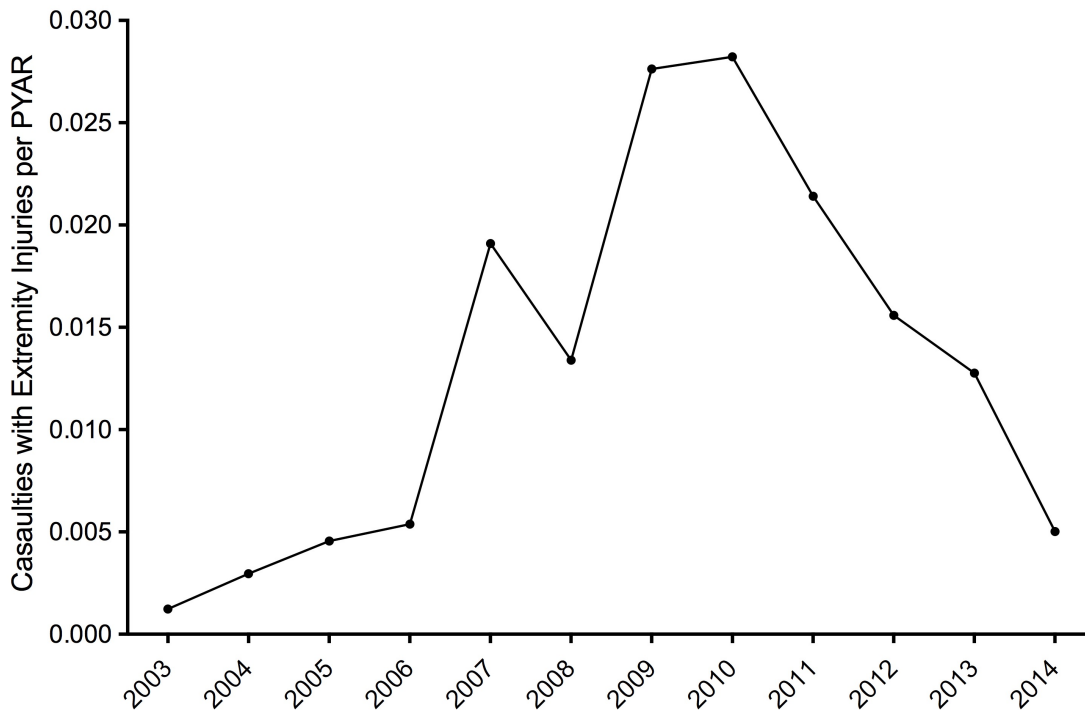
Figure 2.10: Survivors with extremity injury sustained per Population Year at Risk (PYAR), per year.

Closed fractures, n=589		Open fractures, n=941	
Clavicle	16 (2.7%)	Clavicle	8 (0.9%)
Scapula	17 (2.9%)	Scapula	25 (2.7%)
Humerus	14 (2.4%)	Humerus	68 (7.2%)
Radius	36 (6.1%)	Radius	35 (3.7%)
Ulna	27 (4.6%)	Ulna	58 (6.2%)
Carpals	9 (1.5%)	Carpals	7 (0.7%)
Metacarpals	22 (3.7%)	Metacarpals	22 (2.3%)
Phalanges (fingers)	16 (2.7%)	Phalanges (fingers)	70 (7.4%)
Pelvis	57 (9.7%)	Pelvis	57 (6.1%)
Femur	31 (5.3%)	Femur	97 (10.3%)
Tibia	85 (14.4%)	Tibia	159 (16.9)
Fibula	93 (15.8%)	Fibula	93 (9.9%)
Calcaneum	80 (13.6%)	Calcaneum	45 (4.8%)
Tarsals	38 (6.5%)	Tarsals	59 (6.3%)
Metatarsals	42 (7.1%)	Metatarsals	49 (5.2%)
Phalanges (toes)	4 (0.75)	Phalanges (toes)	17 (1.8%)

Figure 2.11: Schematic showing anatomic distribution of closed and open fractures. NB More than one fracture can occur in a single casualty.

Explosive munitions were the most common mechanism of injury, seen in 1,090 cases (60%) with further detail in **Table 2.9**.

Mechanism of injury	n (%)
Explosive	1090 (60)
GSW	420 (23)
Fall	86 (5)
MVC	78 (4)
Other	139 (8)
Total	**1813**

Table 2.9. Mechanism of injury. GSW = gunshot wound, MVC = motor vehicle collision

Total LOS following extremity injury was 24,486 days, or 67 years and 1 month; a total of 2,908 surgical episodes were performed on extremities.

Patients with at least one major amputation had a LOS of 51 days (IQR 30-65) and underwent a median of seven extremity surgical procedures (IQR 5-9). One hundred and three patients lost a single limb, of which twelve were unilateral upper limb amputations. Eighty-five casualties lost two limbs, the majority of which (82/85, 96%) lost both lower limbs. Seventeen lost three limbs representing 0.7% of all survivors with further detail show in **Table 2.10**. There were no UK survivors who lost all four limbs at or proximal to the wrist or ankle. Levels of limb loss are detailed in **Figure 2.12**.

Unilateral lower limb (n=91)	
Trans-tibial	64
Knee disarticulation	11
Trans-femoral	16

Bilateral lower limb (n=97*)	
Bilateral trans-tibial	12
Trans-tibial / knee disarticulation	8
Trans-tibial / trans-femoral	22
Bilateral knee disarticulation	12
Knee disarticulation / trans-femoral	17
Bilateral trans-femoral	26

Table 2.10. Patterns of amputation. *This figure includes lower limb amputation for triple amputees, all of whom lost both legs and an arm.
NB: not all amputation patterns are shown in this table due to identifiable low numbers of some patterns of limb loss.
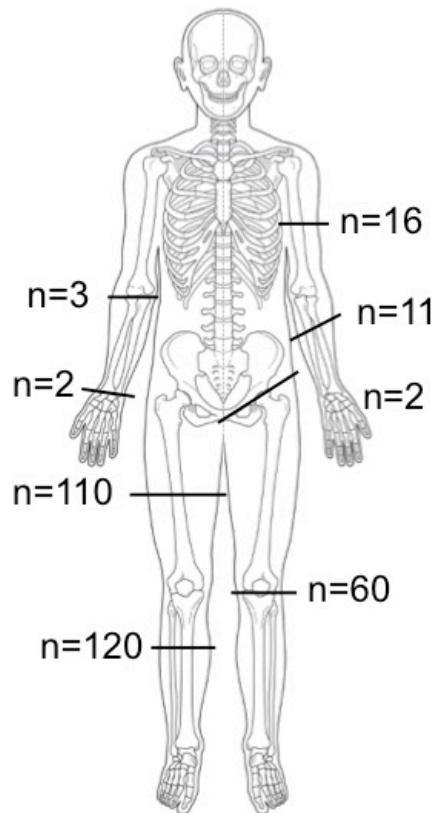
Figure 2.12: Schematic showing level of amputation. NB more than one amputation can occur in a single casualty; this schematic does not represent laterality of amputation.

For casualties with long bone fractures without amputation, LOS was 13 days (IQR 6-25) with a median of two surgical procedures on their limbs (IQR 1-4). In closed fractures LOS was 11 days (IQR 6-32). In open long bone fractures by contrast, LOS was 24 days (IQR 13-46) (p<0.0001, **Figure 2.13**). For closed fractures, median surgical episodes was 1 (IQR 0-3) compared to 3 (IQR 2-5) for open fractures (p<0.001).
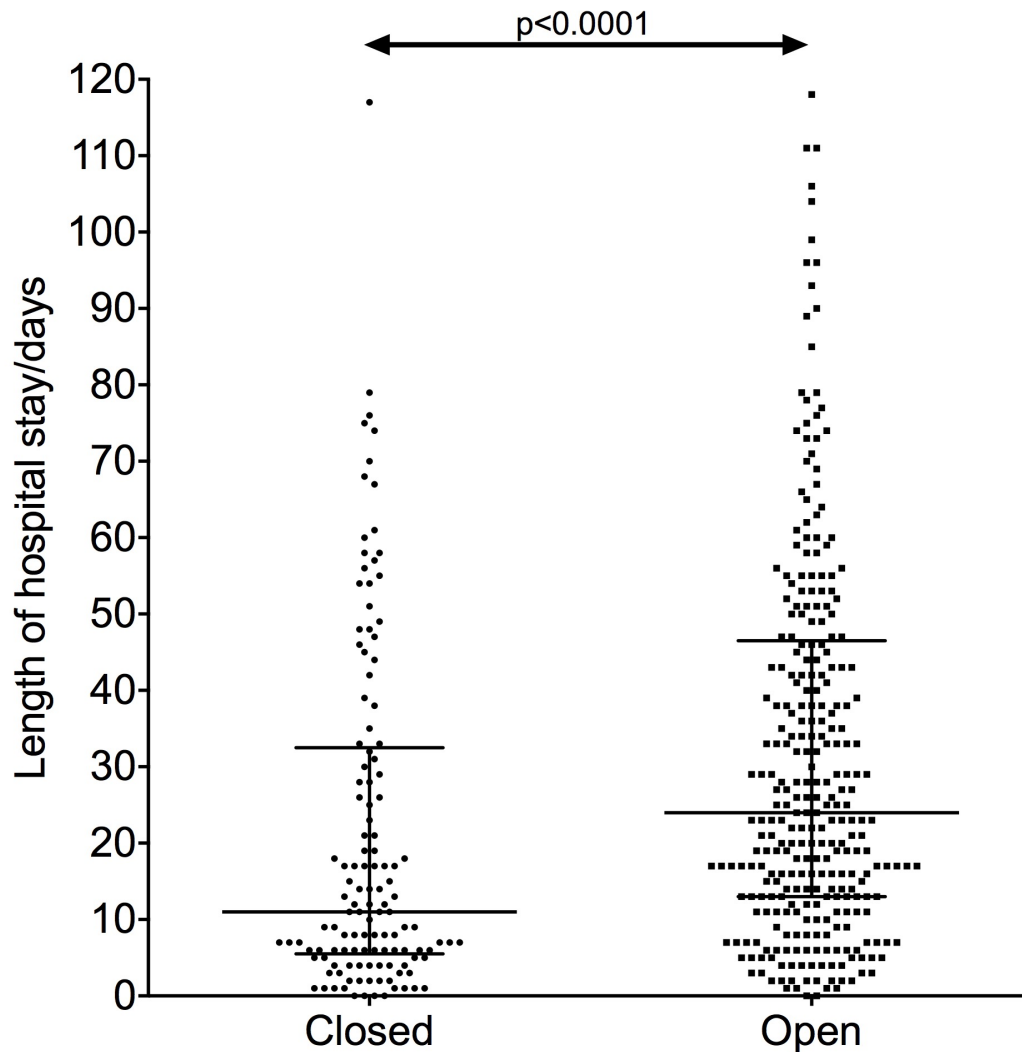
Figure 2.13: Scatter plot showing length of hospital stay for patients with open versus closed long bone fractures. The horizontal bars represent the median and interquartile ranges. Statistical difference as per Mann-Whitney analysis.

2.8 Infection in open tibia fractures

My findings from the analysis of JTTR data in Figure 2.11 of **Section 2.7** shows that the tibia is the most commonly fractured bone, and that 65% of these injuries are open. These findings are based on registry data and therefore lack clinical detail.

Open tibia fractures remain a considerable challenge to the orthopaedic surgeon. They are recognised as having the highest rate of infection following open fracture compared to other long-bones[39], thought due to the limited soft-tissue envelope. Open tibia fractures sustained on the battlefield have been reported to have an infection rate of 20-30%[39], with infection shown to be associated with poor outcomes and late amputation[40,41].

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The aim of this phase of work was to move from registry data to clinical notes in order to characterise these injuries in greater detail. Specific objectives were to:

i) accurately determine the number of open tibia fractures in UK casualties,

ii) establish the rate of infection, and

iii) determine factors associated with infection.

2.8.1 Determining the infection rate in open tibia fractures: methods

The JTTR was searched for cases of survivors with AIS codes encompassing bony injuries of the knee, tibia or ankle sustained between the 10 years of the invasion of Iraq in 2003 and 31 December 2012. A wide range of codes was chosen to allow cases to be individually reviewed in order to avoid missing cases due to coding inaccuracies.

The clinical records, X-rays and microbiological results of patients with open diaphyseal tibia fractures were reviewed. The following were excluded: cases not involving diaphyseal tibia fractures (AO/Muller type 42); cases other than those graded by the operating surgeon as grade III in the Gustilo-Anderson (GA) classification[42], and patients who were managed with a primary amputation within the first three surgical episodes.

Data were gathered on demographics, injury and surgical management. For the purposes of this study, an injury was deemed to have been infected if the infective episode required surgical treatment. A consultant microbiologist analysed all microbiological results and determined the causative microorganism.

The influence of the following factors on infection was examined:

A. New Injury Severity Score (NISS).
B. Mechanism of injury i.e. explosive versus gun-shot injury.
C. Bone loss[43] (**Table 2.11**).
D. Requirement for tissue transfer (both local and free flaps).
E. Initial stabilisation with an external fixator.
F. Smoking during initial treatment.

Grade	Definition
0	*None*
1	*Minimal*: some bone loss but less that 1 cm longitudinally around at least 50% of the circumference of the shaft but with some cortical contact
2	*Moderate*: bone loss between 1 and 2 cm around at least 50% of the circumference of the shaft but with some cortical contact
3	*Severe*: bone loss greater than 2 cm around at least 50% of the circumference of the shaft but with some cortical contact
4	*Segmental bone loss:* no cortical contact

Table 2.11: Bone loss grading system [41]

Descriptive data are reported as medians with inter-quartile range (IQR). Continuous data (i.e. NISS) was analysed by Mann-Whitney analysis. The remaining five factors were analysed by Fisher's exact test. In interpreting the levels of statistical significance, a Bonferroni correction was applied to avoid the increased risk of a Type 2 error inherent in multiple comparisons, hence significance was set at $p=0.0083$.

2.8.2 Determining the infection rate in open tibia fractures: results

The JTTR search identified 445 cases of casualties with bony injury affecting the knee, tibia or ankle. Following case note and X-ray review, 353 cases were excluded according to the pre-defined criteria. Ninety two patients with 100 severe open tibial fractures were therefore eligible for inclusion (eleven patients with bilateral fractures). The median age was 25 years (IQR 21.3 – 29.0, mean = 26.0, SD = 5.1).

Three patients were not followed-up for a minimum of 12 months and therefore were excluded from the outcome analysis. In the remaining 97 fractures intra-medullary nailing was the most common technique for definitive treatment, used in 42 fractures, with other fixation methods detailed in **Table 2.12**.

Management	*n*	Time to definitive fixation/median days (IQR)
IM nails	42	3 (2-4)
Plates/screws	30	3 (3-5)
External Fixation	7	2 (1-2)
Frame	5	52 (33-81)
ORIF with External Fixation	5	3 (2-8)
Conservative	8	-

Table 2.12: Definitive fracture fixation techniques.

Limb salvage failed in eleven patients necessitating amputation: in three cases this was due to on-going infection. Initial definitive fixation was subsequently revised in 33 cases (34%), twelve of which were revised to a circular frame for non-union with union eventually achieved.

Twenty two cases (22/97, 23%) were complicated by infection. The most common causative micro-organism was *Staphylococcus aureus* with further detail given in **Table 2.13**.

Organism	n	
S. Aureus	13	(1 MRSA)
Acinetobactor	3	
Pseudomonas Sp.	2	
Coag. Neg. Staph	1	
Enterobacter Sp.	2	
Unknown	1	

Table 2.13: Causative microorganisms in the 22 cases requiring surgical treatment of infection.

A. Injury severity

The median overall NISS was 17 (IQR 12-22). In the 22 infected cases the median NISS was 20 (IQR 15-29) and 17 (IQR 11-22), in the 75 uninfected fractures (p=0.0469, Mann-Whitney) as shown in **Figure 2.14**. This difference was not regarded as statistically significant due to the Bonferroni correction that was applied.
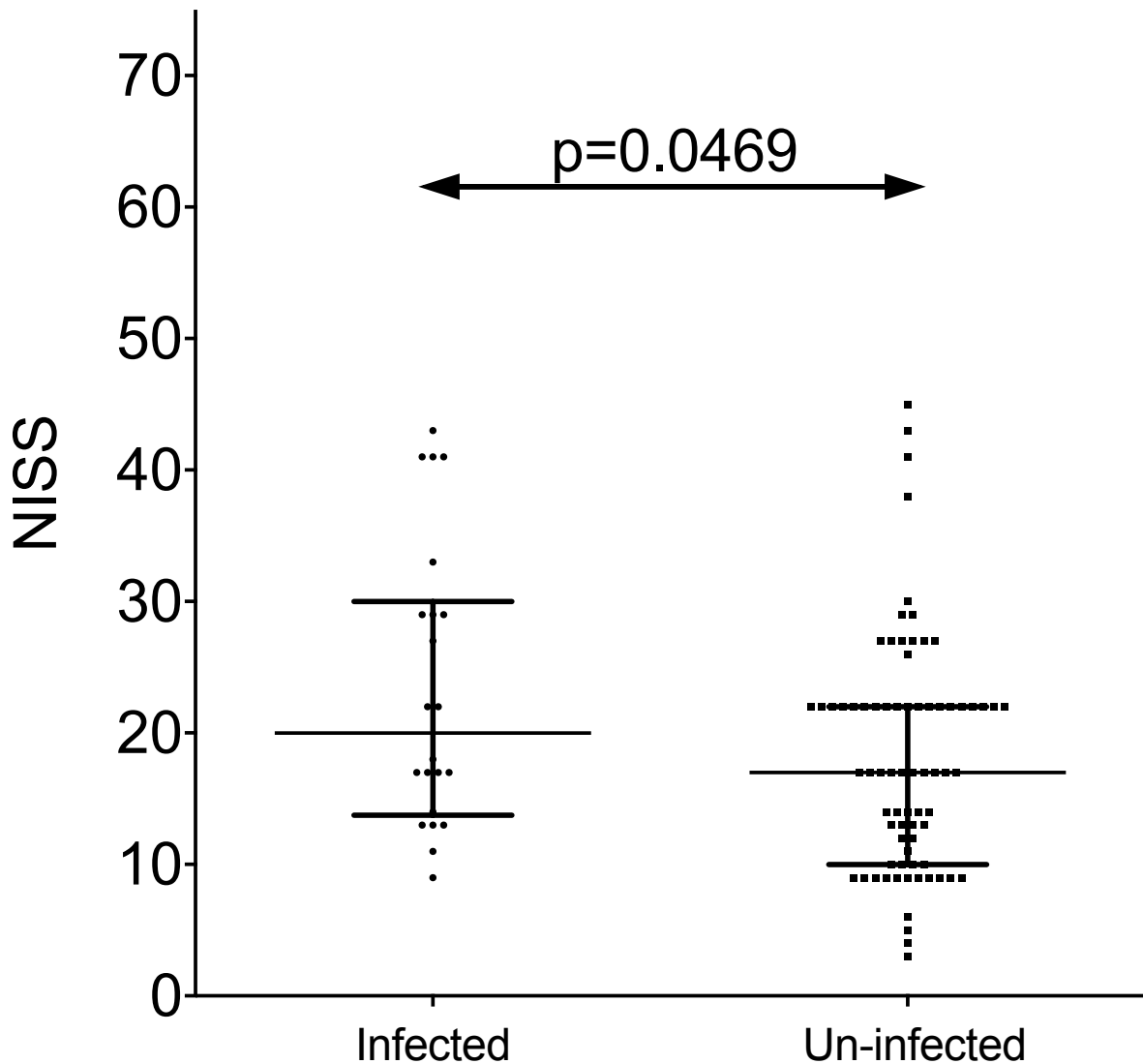
Figure 2.14: Scatter plot showing the New Injury Severity Score by infected and un-infected groups. The horizontal lines denote the median and the inter-quartile range.

B. Mechanism of Injury

The majority of fractures in this series were a result of explosive weapons (69/97, 71%) with the remainder being injured by gunshot wounds (GSW). The proportion of injury types was statistically similar in both the infected cohort (GSW = 5/22) and uninfected cohort (GSW = 18/75) (p=0.6014, Fisher's exact test).

C. Bone Loss

The degree of bone loss after debridement is shown in **Figure 2.15**. There were four cases of segmental bone loss in the infected cohort and none in the uninfected group. Bone loss was significantly associated with subsequent infection (p<0.0001, Fisher's exact test).
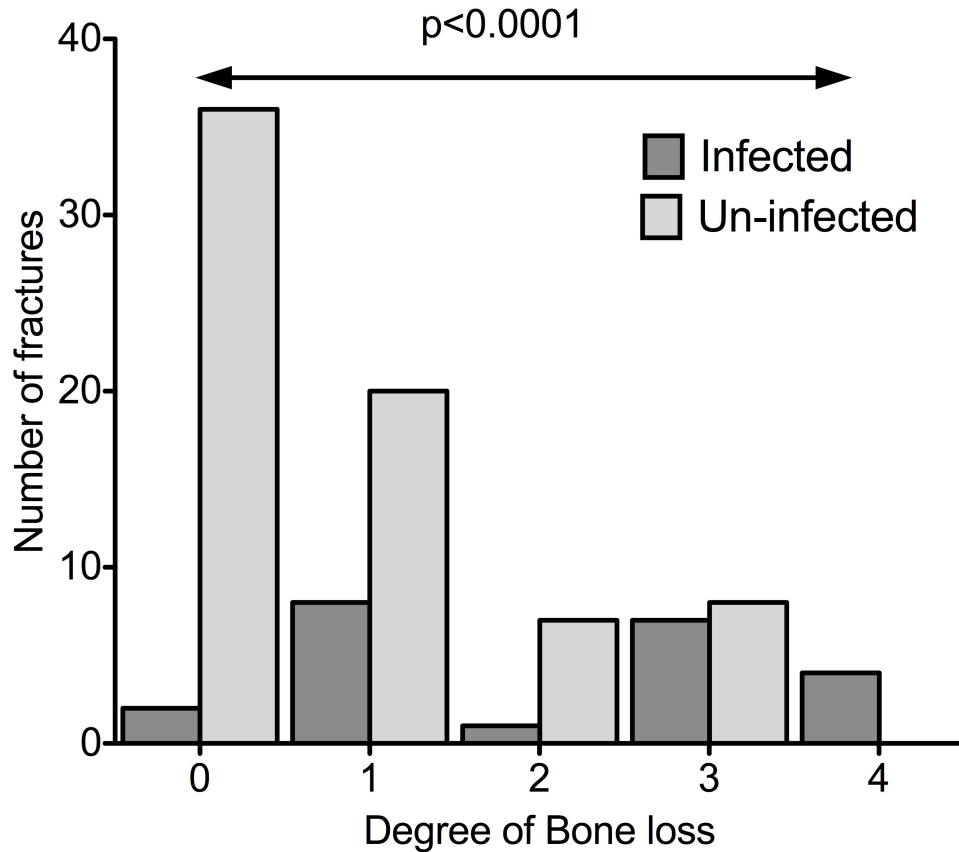
Figure 2.15: Bar chart showing bone loss and infection.

D. Vascularised tissue transfer

Approximately one third of injuries (31/97, 32%) were covered with vascularised tissue transfer i.e. both local and free flaps. Twelve of these were performed in cases that subsequently became infected (12/31, 39%, p=0.0177, Fisher's exact test). This association was not significant with the application of the Bonferroni correction.

E. External fixation

An external fixator was applied in the initial phase of management in 61 fractures (61/97, 63%). Eighteen of the 22 cases which subsequently developed infection (18/22, 82%) and 43 of the 75 (43/75, 57%) uninfected cases were initially stabilised with an external fixator (p=0.0443, Fisher's exact test). This association was not significant with the application of the Bonferroni correction. In 46 cases external fixation was converted to internal fixation. The median delay until this occurred was 3 days (IQR 2-5).

F. Smoking

Reliable data on smoking status was available for 69% of fracture cases. Five of 17 infected cases (29%) with a recorded smoking status smoked, compared to 18 of 50 uninfected cases (36%). This difference was not significant (p=0.7704, Fisher's exact test).

2.8.3 Determining the consequences of infection

A previously published analysis performed earlier on four years of patients from the same cohort described in this section found 44 patients with 52 open tibia fractures with 12-months of follow-up[41]. In this series, 19 tibias required revision surgery at point of follow-up, with seven requiring amputation, an overall revision rate of 50% (26/52).

Infection was defined, as it was in **section 2.8.1** as a clinical diagnosis requiring surgical treatment. There was a statistically significant association between deep infection requiring surgical treatment and poor outcome (p = 0.008, Fisher's exact test)[41].

2.9 Conclusions

The findings of the analysis of the casualty data from the JTTR demonstrates a sustained improvement in survival amongst UK military personnel injured whilst deployed in Iraq and Afghanistan over the years of conflict. This trend is seen using both an anatomic (NISS) or combined anatomic/physiological (TRISS) measure of injury severity. Unsurprisingly it appears that the greatest improvement in survival occurred in the first half of the conflicts compared to the more modest improvements in the second half.

My analysis of injury mechanisms and anatomic distribution reveal that the majority of injuries were the result of explosive weapons, and that 77% of survivors had injuries to their extremities.

The tibia was the most commonly fractured bone amongst survivors, of which 65% were open. An open fracture was associated with a significantly greater number of surgeries and length of hospital stay.

The infection rate for battlefield open tibia fractures was 23%, with 60% of these infections caused by *S. aureus*. Infection in open tibia fractures was strongly associated with fixation revision surgery or amputation.

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Chapter Three: Methodology and Model Development

3.0 Introduction

High-quality clinical studies examining factors that affect infection following open fractures are challenging due to substantial variation amongst patients, specifically with respect to their injuries and their treatment. This heterogeneity risks confounding clinical research. To overcome this, clinical studies have to recruit hundreds of patients and can take many years to report findings which might help guide clinical practice[1,2].

While randomised clinical trials will likely remain the highest standard of clinical research, the gap between the level of understanding required in order to design safe clinical research studies and the knowledge produced by *in vitro* or 'lab-top' work is vast.

Animal models provide translational research between *in vitro* studies and clinical studies: they allow broad principles to be tested, dosages to be refined and research questions to be clarified[3]. Although there is a compromise between reality and reproducibility, they allow testing of interventions not sanctioned for clinical trials and save research money for the pursuit of only the most promising therapies. Furthermore, there is a versatility in animal models that allows rapid progress in developing themes as well as the direct comparison of multiple treatment modalities.

Studies using animal models of open fracture offer the potential to test concepts and evaluate novel treatments or treatment algorithms in a reproducible, standardised setting where all variables other than those under investigation can be controlled and results obtained rapidly.

3.1 Ethical and Legal Framework for Animal Research

This research work was completed in the US Army Institute for Surgical Research (USAISR) in San Antonio, Texas. USAISR is the Department of Defence's principal facility for conducting research aimed at improving combat

casualty care. It employs approximately 700 people and includes a large animal laboratory licenced to conduct research using a range of species from rodents to large animals including primates.

All research involving mammals at the USAISR is governed by US Animal Welfare Act of 1966 in accordance with the Guide for the Use and Care of Laboratory Animals[4]. Each individual study was registered with the USAISR Institutional Animal Care and Use Committee (IACUC).

3.2 Model Selection

Using an animal to model infection after open fracture accepts limitations due to differences in anatomy and physiology and the restrictions in creating a contaminated 'traumatic' injury in a controlled and reproducible way. All medical research using animals represents modelling an injury or pathology rather than perfectly recreating it. It is important that these limitations and compromises are acknowledged and understood.

With respect to species selection, the closer the species to *Homo sapiens*, then the smaller anatomic and physiological differences will be. However, it is a principle of scientific animal work that research should be performed on the 'lowest' animal species that would still permit valid conclusions to be drawn from the data generated by the study[4]. For this reason animal models of bone healing and infection have typically used mice, rats and rabbits, although sheep and dogs have also been used.[5,6]

The planned studies that make up the animal proportion of this body of work are either testing concepts or screening potential treatments. Therefore, although higher primates might present the ideal theoretical choice, that degree of very close approximation is unlikely to be scientifically necessary and is not ethically or financially justified.

Aside from the ethical imperative already described, the advantages of smaller animals e.g. rats, are cost, simpler animal husbandry requirements,

and more rapid recovery after surgical procedures. The disadvantages associated with smaller animals are the constraints on venepuncture due to animal morbidity and pain, which means that intravenous sampling or drug administration is extremely limited. There are technical limits due to size on the complexity of surgical procedures that it is possible to perform on small animals, limiting the nature of some investigations.

Animal models of musculoskeletal infection have predominantly attempted to mimic four clinical situations:

i) Osteomyelitis
ii) Infected prosthesis
iii) Traumatic wound/open fracture infection
iv) The presence of a co-morbidity e.g. diabetes

Creating an animal model to study infection in a traumatic wound/open fracture is challenging for the following reasons:

i) **Severity of injury**. The outcome of interest is usually infection after injury. i.e. the presence or absence of infection at a minimum of 48 hours and usually several days after injury. This requires a balance to be established between the severity of the injury created, and the need for the animal to be pain-free and relatively mobile until euthanasia. For example, it is inhumane to leave an animal with an un-stabilised fracture, therefore a fracture must either be stabilised (internally or externally), or the bony injury must be limited e.g. a drill hole through a single cortex.

ii) **Open or closed wounds**. By definition, traumatic wounds are open to the environment. However, in order to standardise the amount of bacterial inoculation between animals and to avoid cross-contamination, wounds in animal models have to be sealed with dressings or primarily closed with sutures immediately after inoculation.

Clearly this is different from the clinical reality that the model is attempting to mimic.

iii)   **Non-human immunology and healing**. Non-human species differ from humans in ways that are poorly understood in their response to infection and their ability to heal following an injury e.g. the size of a bone defect that will not heal spontaneously.

3.3 Methodology of Contaminated Rat Femur Segmental Defect

The USAISR has adapted a rat model of a contaminated femur defect first described by Chen *et al.* in 2002[7]. This model has the versatility of being able to examine infection and bone healing as an outcome either separately or concurrently in the same study. As a small animal model it is well suited to translational work examining treatments hitherto based on *in vitro* work or proof of concept studies.

3.3.1 Surgical Procedure

Immunologically intact adult male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, Indiana) weighing between 350 and 400g on the day of surgery were anesthetised with 2% isoflurane. The fur over their right leg was shaved and the skin prepared with povodine iodine and 70% isopropanol. An incision was made in the skin originating over the greater trochanter and continuing distally towards the knee, centred over the femoral shaft. The femoral shaft was exposed and stabilized with a bespoke contoured polyoxymethylene plate, secured with six threaded Kirschner wires (K-wires) inserted under power. This allowed the humane stabilisation of a bony defect, and had the effect of introducing an implant to lower the bacterial inoculum required for infection[8].

A 6mm defect was created in the mid-shaft of the femur with a reticulating saw (Series 1000, Microaire, Charlottesville, US) under continuous saline irrigation as shown in **Figure 3.1**. A 6mm defect has previously been shown to be critical, i.e. sufficiently large that healing will not occur spontaneously[7].
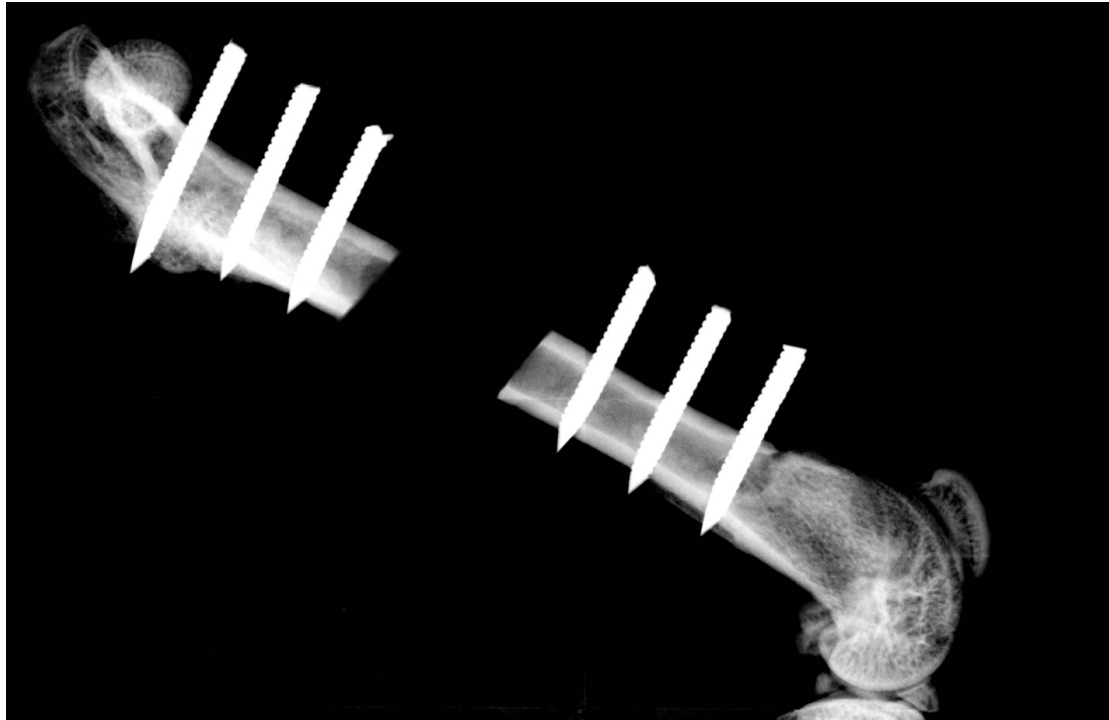
Figure 3.1: A microradiograph showing a proximally disarticulated rodent femur with 6mm segmental defect. Also visible are six threaded Kirschner wires (K-wires) used to fix the radiolucent polyoxymethylene 'plate' conferring stability despite the defect.

The bony defect was contaminated with 30mg of sterile bovine collagen soaked with 1×10^5 Colony Forming Units (CFU) of *Staphylococcus aureus* in 0.5mls of saline. The (Xenogen 36) strain of *S. aureus* used derived from ATCC strain 49525, originally from a septic human patient and modified to emit photons as shown in **figure 3.2** below. (Caliper LifeSciences, CA, USA). This strain of bacteria has been regarded by other investigators as being an appropriate choice for modelling musculoskeletal infection[9]. The wound was then closed with continuous nylon sutures to the fascial layer and surgical clips to skin. Animals were recovered and allowed unrestricted mobility.
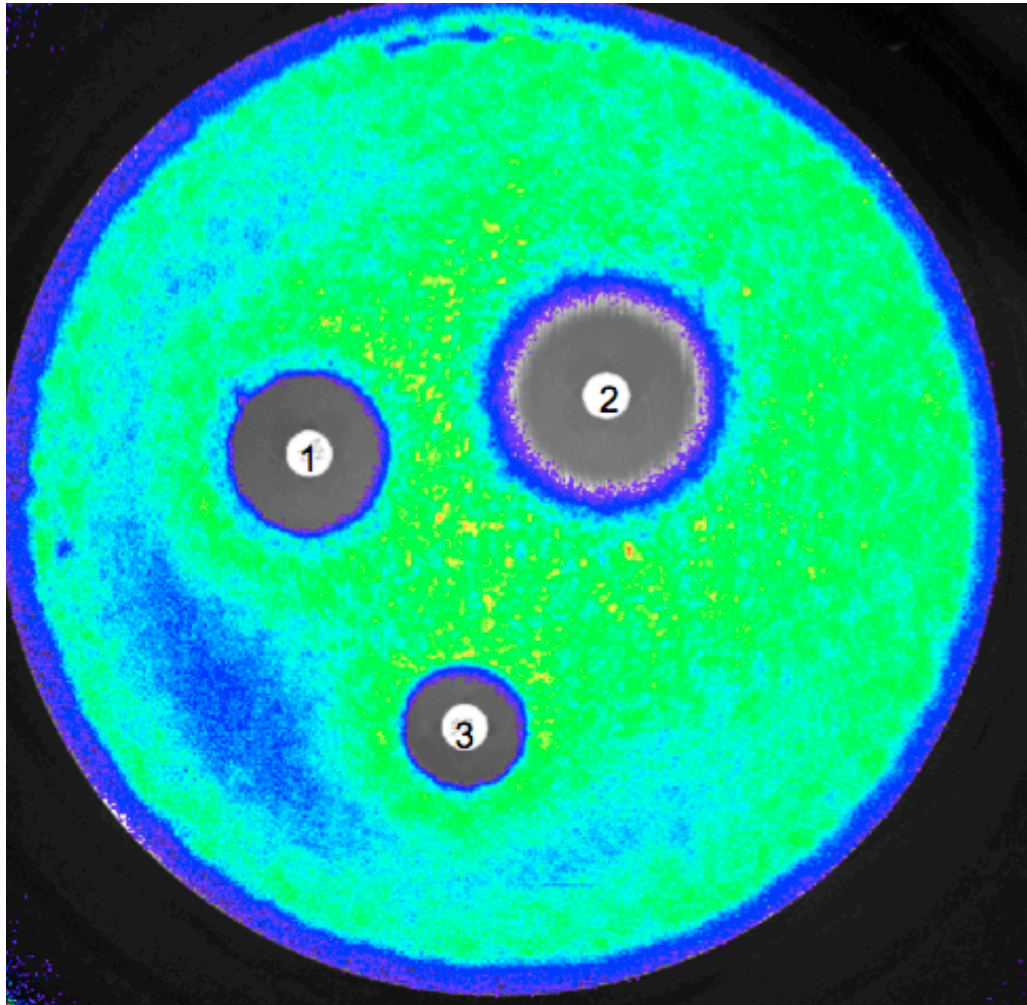
Figure 3.2: A limited spectrum photograph of a Kirby–Bauer antibiotic sensitivity test of the Xenogen-36 photon-emitting strain of *S. aureus* used in this model. Disc 1 = tobramycin; Disc 2 = gentamicin; Disc 3 = vancomycin.

Six hours after the original procedure animals were re-anesthetised. The wounds were opened and all the contaminated collagen was meticulously removed: any necrotic or soft tissue of dubious viability was excised. The wound was then irrigated with 60ml of sterile saline delivered at low pressure from a hand-held syringe held at 10cm from the surgical field. At this stage any local treatments were applied: wounds were then similarly closed in layers. The animals were recovered and allowed full mobility, food and water.

Fourteen days after simulated injury the animals were euthanised after isoflurane anaesthetic, with an intra-cardiac injection of potassium chloride. At this stage, the hip joint was disarticulated. Under sterile conditions the wounds were opened through the healed incision, and the bone defect exposed. The

wound was photographed with a photon-count camera (Xenogen IVIS Imaging System 100 series, Caliper Life Sciences, Hopkinton, MA). Samples of tissue were taken for histological analysis at this stage.

The femur and implant material were stripped of soft tissue and separated. The bone tissue was snap frozen in liquid nitrogen and crushed. Bone and implant samples were sent separately for standard quantitative microbiological analysis. Crushed bone samples are homogenized with 10ml sterile saline in an agitator; implant specimens were rinsed with 10ml of sterile saline in an agitator. Aliquots from individual specimens were sequentially diluted and spread onto Tryptic-Soy-Agar plates. After overnight incubation at 37˚C, bacterial colonies were counted and recorded: the threshold of detectability was 30 CFU/g. Confirmation that the bacterial colonies being counted were from the original, contaminating *S. aureus* was obtained by further imaging using the photon count camera.

3.3.2 Outcome Measures

Depending on the study, different outcome measures can be examined:

 i. The presence of detectible bacteria in bone;
 ii. The quantity of bacteria in bone tissue in CFU/g;
 iii. The presence of detectible bacteria on the implant material (polyoxymethylene plate and K-wires);
 iv. The quantity of bacteria on the implant material in CFU/g;
 v. The photon-count of the wound;
 vi. Radiological evidence of defect healing (micro CT or radiograph);
 vii. Histological evidence of defect healing;

There is clearly a spectrum between contamination (presence of bacteria), colonisation (persistence and multiplication of bacteria), and infection (pathological effect of bacteria multiplying bacteria and resulting inflammation). Just as there is no clear definition of infection in the clinical setting, neither is there one in animal research[10,11].

The widely used US Centre for Disease Control criteria for defining infection still relies in part on subjective clinical diagnosis of infection i.e. a case is infected when the treating physician judges it to be so. For the purposes of these studies using this rodent model it was decided to adopt an unambiguous definition of infection as the primary outcome measure: the persistence of detectable bacteria in the bone or on the implanted hardware after 14 days from 'injury'. This definition was used to avoid the obvious problems with a subjective 'diagnosis' of infection in an animal model. The secondary outcome measure was the quantity of recovered bacteria measured separately from the bone and implant samples.

3.3.3 Statistical Analysis

The categorical data for the presence of bacteria in the bone and on the hardware from study groups were compared using Fisher's exact test. The quantity of bacteria in the bone and on the hardware for each animal was summed and log transformed before being averaged across each group. Pairs of groups were directly compared using a Mann-Whitney test while ANOVA of results across a range of groups was calculated using a student Newman-Kuells test.

3.4 Model Development

The model described in Section 3.3 has previously been used in USAISR to examine infection in open fractures[12]. However, in previous studies a bacterial inoculation of 1×10^5 CFUs of *S. aureus* with surgical treatment 6 hours later produced universal infection in all animals. Investigators therefore used the differences between the quantity of bacteria recovered from animals in different treatment arms to determine the possible effects of each treatment. Furthermore, the primary outcome measure used in the rodent model at USAISR had been the photon count, i.e. measuring photons produced by the Xenogen 36 strain of *S. aureus* to quantify the amount of bacteria present in the wound.

I identified that the previous use of this model had the following problems:

1. The rodent model did not mimic the clinical scenario it was attempting to model as closely as it could.
   The modelled 100% presence of infection is markedly different from the clinical setting where infection rates of less than 50% are typically seen after open fracture[13,14]. With previous use of the model, investigators had to judge the potential efficacy of treatments based on differentiating between 'infected' and 'very infected' wounds.

2. The reliance on photon counts of the wound bed was vulnerable to confounders: the wound had to be opened and a 3-dimensonal structure rendered into a 2-dimensional image to be measured by the photon camera.

To maximise the potential for the results of the animal studies to translate to clinical trials, the model needed to mimic the clinical reality of infection more closely, by:

1. Establishing a more clinically appropriate balance between infected and uninfected wounds;

2. Incorporating systemic antibiotic treatment within the model.

To achieve this, the following objectives were established:

1. Determine the bacterial inoculation resulting in infection rate of 50%;

2. Establish a systemic antibiotic treatment regime that results in a infection rate of 50%.

3.5 Bacterial Inoculation

In order to determine the relationship between initial bacterial inoculation and subsequent infection a model development study was performed.

3.5.1 Bacterial Inoculation: Methods

The standard model described above in **Section 3.3** was used. Fifty rats were divided into five groups of ten rats each. Each group was inoculated with a different quantity of bacteria, increasing in orders of magnitude from 1×10^1 to 1×10^5 CFUs.

Animals were treated with surgical debridement including irrigation with 60mls of sterile saline at 6 hours from time of injury; no antibiotics were given. Animals were euthanised 14 days after initial inoculation: the plate and K-wires were retrieved and separated from the femur. Both samples were processed separately as described above.

3.5.2 Bacterial Inoculation: Results

No bacteria were detectable in animals inoculated with 1×10^1 CFU, whilst all animals inoculated with 1×10^3 CFUs and greater had bacteria detectable on both hardware and bone samples as shown in **Figure 3.3** below.
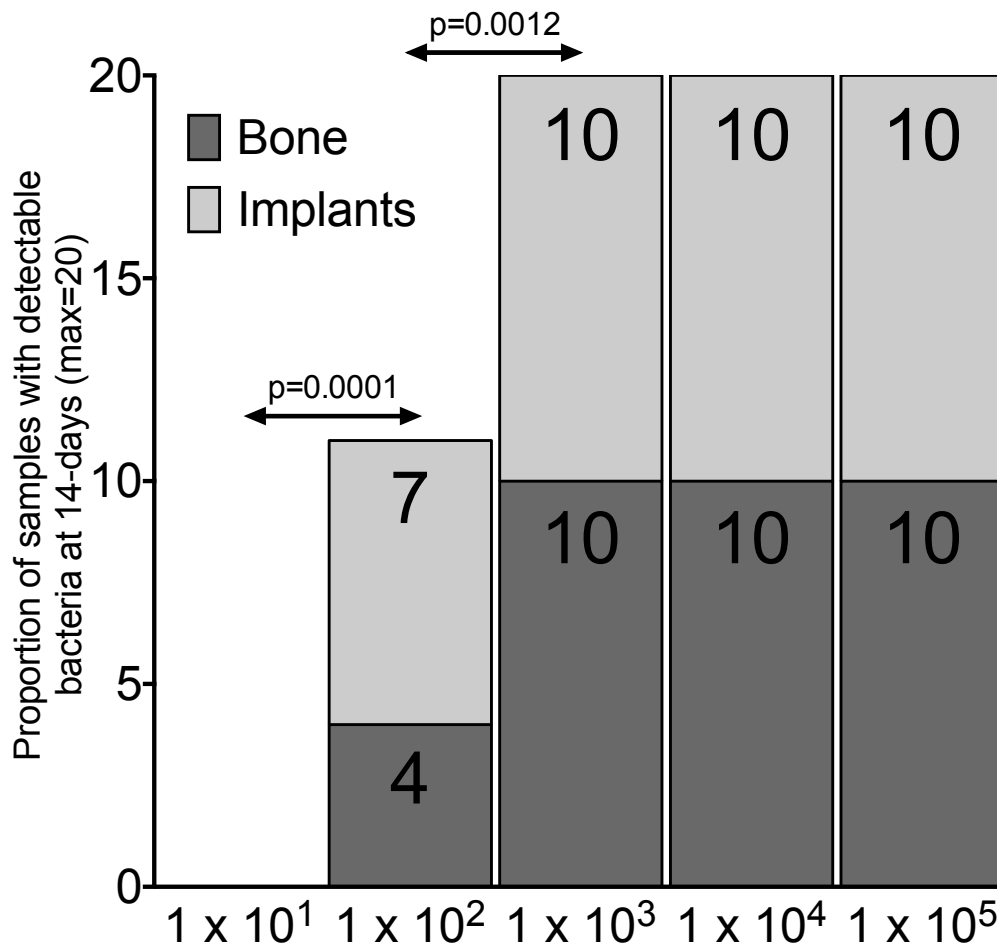
Figure 3.3: Proportion of samples with detectible bacteria in each group of 10 animals (maximum of 20 samples per group) 14 days after inoculation with various quantities of bacteria, given in colony-forming units (CFUs). P-values calculated with Fisher's exact test shown.

In the group inoculated with 1×10^2 CFUs approximately half of the animals had detectable bacteria. This group had a mean bacterial level of several hundred CFU/g. All groups inoculated with more bacteria had similar levels of bacteria, of an order of magnitude greater than the group inoculated with 1×10^2 CFUs, as shown in **Figure 3.4** and **Table 3.1**.

When the model was inoculated with 1×10^2 CFU, bacteria were recoverable from approximately 50% of animals at 14 days, equating to a 50% infection rate.
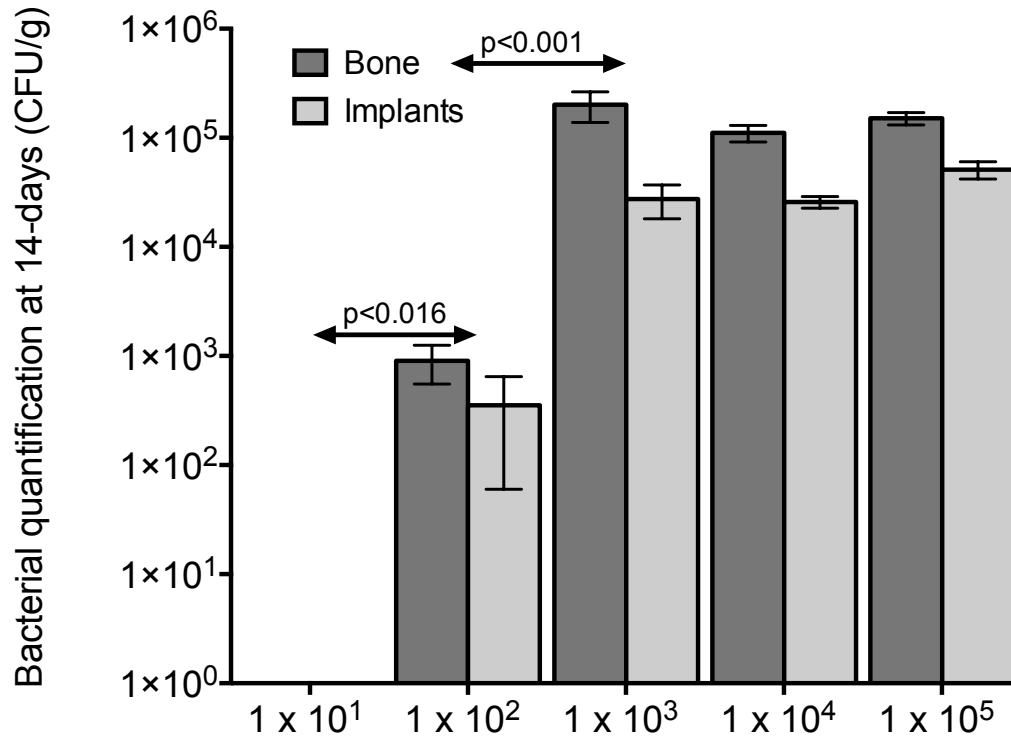
Figure 3.4: Quantification of bacteria recovered from animals (10 per group) 14 days after inoculation with various quantities of bacteria, given in colony-forming units (CFUs). Error bars show standard error of the mean, p-values by Mann-Whitney test given.

	Quantity, CFU				
	1×10^1	1×10^2	1×10^3	1×10^4	1×10^5
Bone mean	0	7.21×10^4	7.83×10^5	1.11×10^5	1.51×10^5
Bone SEM	0	4.40×10^4	1.35×10^5	1.92×10^4	1.95×10^4
Implant mean	0	9.82×10^4	1.65×10^5	2.58×10^4	5.11×10^4
Implant SEM	0	5.10×10^4	1.61×10^5	3.16×10^3	9.11×10^3

Table 3.1: Quantification of bacteria recovered from animals 14 days after inoculation with various quantities of bacteria, given in colony forming units (CFUs). SEM = standard error of the mean.

3.6 Systemic Antibiotics

In order to incorporate a systemic antibiotic regime into the model and better understand the relationship between systemic antibiotics and infection, the following study was performed.

3.6.1 Systemic Antibiotic Regime: Methods

The standard model described above in **Section 3.3** was used. Eighteen rats were divided into three groups of six rats each. Each animal was inoculated with 1×10^5 CFUs of *S. aureus,* and had their wounds surgically treated with debridement and irrigation with 60mls of sterile saline. At the time of debridement each animal was treated with subcutaneous cephazolin. The initial dose range of antibiotic to be given was based on an assumed weight of 400g per rat. The dose of antibiotic given varied between each group: $2mgKg^{-1}$, $5mgKg^{-1}$ and $10mgKg^{-1}$. Further administrations of the same dose were repeated at 12 hourly intervals for 72 hours, so that each rat received a total of 7 doses.

Animals were euthanised 14 days after initial inoculation: the plate and K-wires were retrieved and separated from the femur. Both samples were processed separately as described above.

3.6.2 Systemic Antibiotic Regime: Results

All samples in animals treated with 2 mgKg^{-1} had detectible levels of bacteria: no animals treated with 10 mgkg^{-1} of cephazolin had any bacteria detectible on their bone or hardware. In the group treated with 5 mgKg^{-1} of cephazolin, approximately half of the animals had samples positive for bacteria, as shown in **Figure 3.5** below.
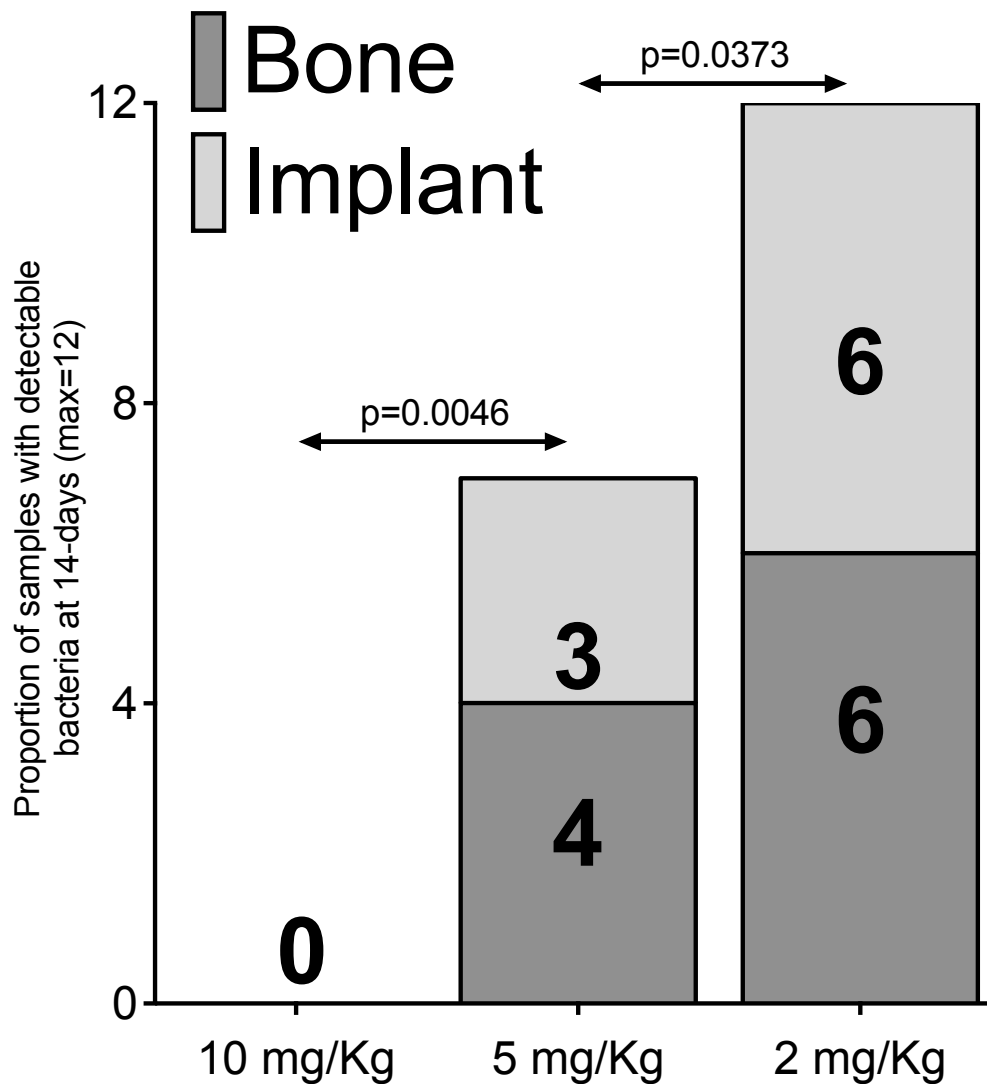
Figure 3.5: Proportion of samples with detectible bacteria in each group of six animals (6 animals per group therefore a maximum of 12 samples per group) 14 days after inoculation with 1×10^5 CFU of *S. aureus* and treatment with various doses of systemic cephazolin for 72 hours. P-values by Fisher's exact test shown.

The relationship between antibiotic treatment dose and and subsequent bacterial quantification is shown in **Figure 3.6** and in **Table 3.2** below.
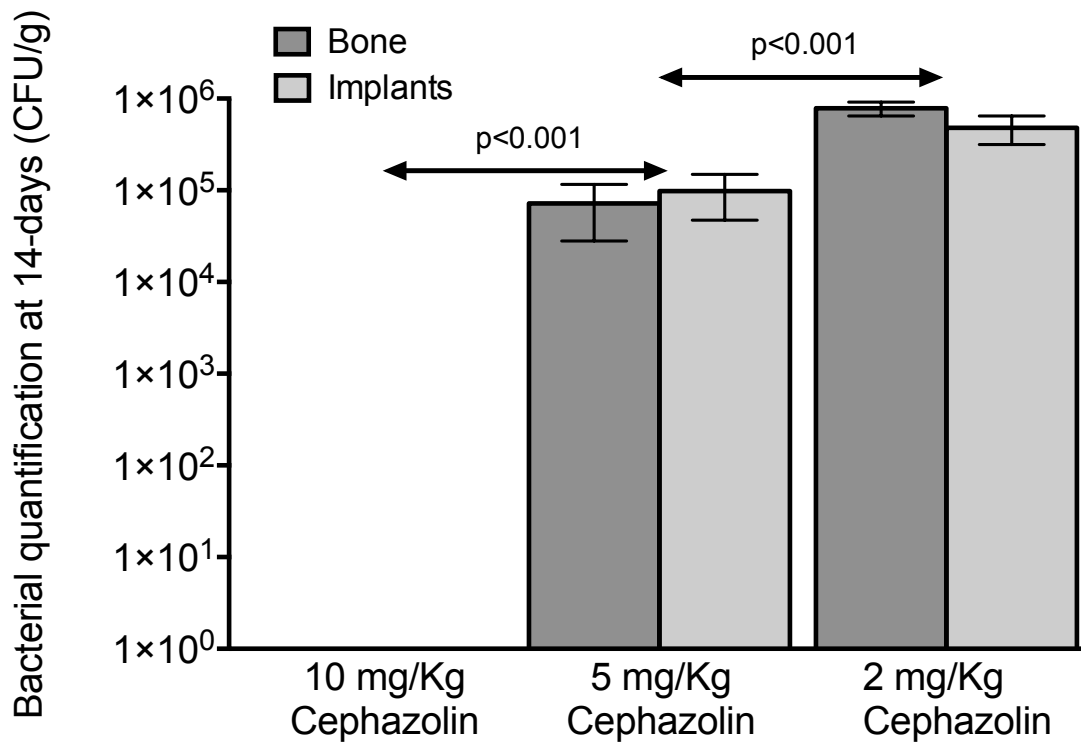
Figure 3.6: Quantification of bacteria recovered from animals (n=6 in each group) 14 days after inoculation with 1×10^5 CFU of *S. aureus* and treatment with various doses of systemic cephazolin for 72 hours. Error bars show standard error of the mean. P-values by Mann-Whitney analysis are shown.

	10 mg/Kg Cephazolin	5 mg/Kg Cephazolin	2 mg/Kg Cephazolin
Bone mean	0	7.21×10^4	7.83×10^5
Bone SEM	0	4.40×10^4	1.35×10^5
Implant mean	0	9.82×10^4	1.65×10^5
Implant SEM	0	5.10×10^4	1.61×10^5

Table 3.2: Quantification of bacteria recovered from animals 14 days after inoculation with 1×10^5 colony-forming units (CFUs) of *S. aureus* and treatment with various doses of systemic cephazolin for 72 hours. Results are given in CFUs; SEM = standard error of the mean.

3.7 Conclusions

The contaminated rodent femur segmental defect model used at USAISR uses an established and well described methodology for examining bony infection in the context of an injury. The model has advantages of its ethical acceptability, versatility, short study time and relatively low cost.

The model development studies achieved the aim of incorporating a clinically appropriate systemic antibiotic treatment regime into the model. Furthermore the infection 'tipping point' at which approximately 50% of animals were infected was achieved both with and without systemic antibiotics.

3.8 References
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Chapter 4: Timing of Treatment

4.0 Introduction

As described in **Chapter one**, the debate about the urgency of debriding open fractures, and the relationship with subsequently developing infection, is on-going. Recently, practice has shifted away from the previous doctrine of debriding open fractures as an emergency, commonly referred to as the 'six hour rule'[1].

This shift is exemplified by the change in the clinical standards published by the British Orthopaedic Association (BOA) and British Association of Plastic, Reconstructive and Aesthetic Surgeons (BAPRAS) on the management of open tibial fractures. In 1997 they stated that, "The first orthopaedic procedure should be undertaken within six hours of injury"[2]. In the 2009 revision of these guidelines the 'six hour rule' had been explicitly laid aside in favour of debridement, "Within 24 hours of injury"[3].

This evolution was based on clinical evidence demonstrating that provided the patient received early antibiotics, the debridement could be safely delayed until it could be performed by a combined senior orthopaedic and plastic surgical team. This approach was felt to be preferable to an 'out-of-hours' emergency procedure by a junior orthopaedic trainee, with the potential for inadequate debridement and compromise of definitive reconstructive surgery.

The evidence from clinical and animal studies examining the relationship between timing of debridement and administration of antibiotics is inconsistent. The majority of clinical evidence comes from retrospective observational cohort studies, which suggest that surgery can safely be delayed by 12-24 hours following antibiotic administration without increasing the risk of infection[4-6]. Conversely, the animal studies that examine this relationship have concluded that delay to surgical treatment is closely related to the development of infection[7].

The contradictory conclusions reached by these two study types are likely due to their respective design weaknesses. Animal studies have looked at either the timing of systemic antibiotics[8] or surgery[9], and therefore do not examine the possibility that antibiotic drugs can render surgical delay 'safe'. Conversely, observational studies

are susceptible to selection bias with a tendency for clinicians to delay the debridement of cleaner, simpler fractures over contaminated, complex ones with the risk of masking the independent effect of surgical delay[1]. Unfortunately, the multiple confounders that exist in the clinical setting can never be ethically controlled for in a prospective study.

This aim of this study is to examine the relationship between the delay from injury to surgical debridement and antibiotic treatment, and subsequently developing infection.

4.1 Timing of Treatment: Methods

This study used the rodent model of a contaminated segmental femur defect described in **Chapter 3**. Inoculation with 1×10^5 Colony Forming Units (CFUs) of *Staphylococcus aureus (S. aureus)* was used to contaminate the segmental defect.

At a specified time after the initial 'injury' (2, 6, or 24 hours), the animals were re-anesthetised, their wounds opened, debrided and irrigated with 60 ml of sterile saline at low pressure.

Fourteen days after simulated injury the animals were euthanised. The femur and implants were stripped of soft tissue and separated. Bone and implant samples were sent separately for standard quantitative microbiological analysis.

4.1.1 Antibiotic Treatment

In addition to surgical debridement, the rats received antibiotic therapy (5 mg/Kg of cephazolin (Sigma-Aldrich, St Louis, MO, USA) sub-cutaneously). The animals received the initial dose of antibiotic at a specified time period after initial injury (2, 6, or 24 hours), and every twelve hours thereafter for a total of seven identical doses. This regimen was selected for the following reasons:

1) Approximation of clinical practice, where a 72 hour course of antibiotic therapy is recommend[3];
2) Model development studies described in Chapter 3 demonstrated a 50% infection rate when this dose of antibiotics and debridement occured six hours after injury.

4.1.2 Study Groups

Seventy rats were divided into seven groups of ten rats each. All groups were treated with identical surgery and antibiotic regimens initiated at different time points of 2, 6 and 24-hours. These time points were chosen to approximate clinical practice:

- o 2 hours: the time typical for surgical debridement in the military setting;
- o 6 hours: the previous clinical goal, and the time at which preclinical studies indicate biofilm formation and maturation in bone[10].
- o 24 hours: the proposed maximum operative delay that can safely be contemplated with the objective of ensuring sufficient surgical expertise.

The timings of surgical debridement and antibiotic administration for each study group are shown in **Table 4.1**. In an effort to minimise animal use it was decided not to study 24 hour antibiotic administration with 6 hour or 24 hour surgery for the following reasons:

1) Previous model development studies described in Chapter 3 indicated these groups would be statistically indistinct;
2) They did not represent clinically relevant treatment regimes.

	2hr Antibiotics	**6hr Antibiotics**	**24hr Antibiotics**
2hr Surgery	2hr Antibiotics, 2hr Surgery	6hr Antibiotics, 2hr Surgery	24hr Antibiotics, 2hr Surgery
6hr Surgery	2hr Antibiotics, 6hr Surgery	6hr Antibiotics, 6hr Surgery	
24hr Surgery	2hr Antibiotics, 24hr Surgery	6hr Antibiotics, 24hr Surgery	

Table 4.1: Metric showing the treatment timing combination of the seven study groups.

4.2 Results
Varying treatment times had a marked effect on bacteria levels as shown in **Figure 4.1**.

	2 hr Antibiotics 2 hr Surgery	2 hr Antibiotics 6 hr Surgery	2 hr Antibiotics 24 hr surgery	6 hr Antibiotics 2 hr Surgery	6 hr Antibiotics 6 hr Surgery	6 hr Antibiotics 24 Hr Surgery	24 hr Antibiotics 2 hr Surgery
■Bone mean (SEM)	0	5.92×10^3 (4.57×10^3)	4.10×10^5 (2.03×10^5)	4.25×10^5 (1.67×10^5)	9.15×10^5 (5.52×10^5)	1.60×10^6 (6.90×10^5)	5.50×10^5 (1.27×10^5)
■Hardware mean (SEM)	0	0	3.46×10^5 (2.48×10^5)	3.12×10^5 (1.25×10^5)	2.22×10^5 (1.16×10^5)	6.75×10^5 (2.12×10^5)	1.93×10^5 (4.50×10^4)

Figure 4.1: Mean bacterial quantification results of varying treatment timings with statistical groupings by combined bone and hardware results. Error bars showing standard error of the mean. Different letters signify a difference among groups, $p < 0.05$, and the same letter indicates no difference.

No animal that received both antibiotics and surgery at two hours after injury had detectible bacteria, whilst every animal in the group which had either treatment delayed for 24 hours had detectible bacteria in either hardware or bone or both as shown in **Figure 4.2**.
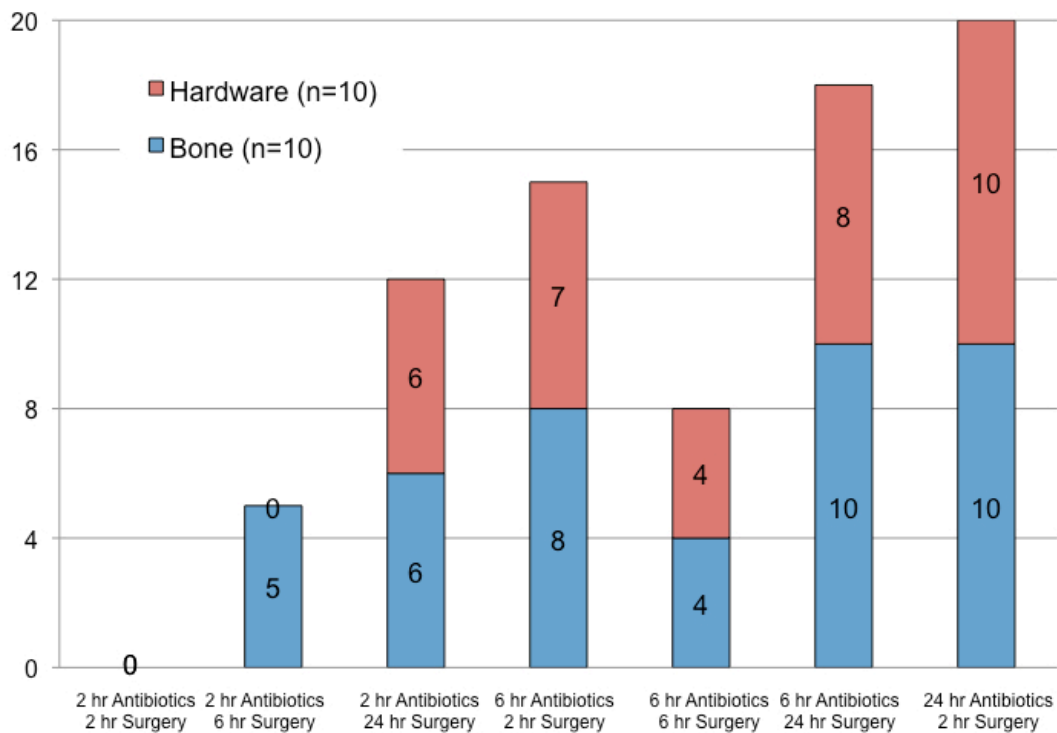
Figure 4.2: Proportion of samples from each treatment group with detectible bacteria. Y-axis shows the number of samples with positive bacterial, for each group of 10 animals, there were 10 hardware samples and 10 bone samples, a total of 20.

In the three groups of animals that received antibiotics at two hours, delaying surgery from two to six hours resulted in a significantly greater number of positive samples from 0 to 5 (p=0.047), however further delay to surgery from six to 24 hours, did not cause a significant increase in bacterial quantity or number of positive samples (5 vs 12, p=0.054) as detailed in **Table 4.2**. This was confirmed by ANOVA of bacterial quantification across these three groups.

If antibiotic administration did not occur within 6 hours of injury, delaying surgery from 6 hours to 24 resulted in a higher significant increase in proportion of samples that were positive for bacteria (p=0.002).

In order to determine whether antibiotics or surgery had a more temporally significant effect on bacteria, two pairs of 'mirrored' study groups were compared. The first was a pair of groups in which the timing of surgery and antibiotics was reversed around the two and six hour time points, the second in which treatments occurred at two and 24 hours. In both comparisons, earlier antibiotics had a significantly greater impact on the proportion of positive samples than earlier surgery. At the two and six hour

treatments, the p value was 0.004 and for the 6 and 24 timings it was 0.003 as shown in **Table 4.3**.

Antibiotics		2hr Antibiotics			6hr Antibiotics			24hr Antibiotics
	Surgery	2hr Group 1	6hr Group 2	24hr Group 3	2hr Group 4	6hr Group 5	24hr Group 6	2hr Group 7
2hr Antibiotics	2hr Group 1	-	0.047	0.001	0.001	0.003	< 0.001	< 0.001
	6hr Group 2	0.047	-	0.054	0.004	0.501	< 0.001	< 0.001
	24hr Group 3	< 0.001	0.054	-	0.501	0.344	0.065	0.004
6hr Antibiotics	2hr Group 4	< 0.001	0.004	0.501	-	0.054	0.408	0.00471
	6hr Group 5	0.003	0.501	0.344	0.054	-	0.002	< 0.001
	24hr Group 6	< 0.001	0.001	0.065	0.408	0.002	-	0.487
24hr Antibiotics	2hr Group 7	< 0.001	0.001	0.004	0.0471	< 0.001	0.487	-

Table 4.2: Metric table showing p-values of direct comparisons between treatment groups according to Mann-Whitney analysis of the presence of the quantification of bacteria from recovered samples. Each study group can be identified in the columns on the left and then matched to a comparator study group in the rows across the top.

		2 hr Antibiotics			6 hr Antibiotics			24 hr Antibiotics
	Surgery	2 hr	6 hr	24 hr	2 hr	6 hr	24 hr	2hr
2 hr Antibiotics	2 hr	-	*	*	*	*	*	*
	6 hr	*	-	NS	NS	NS	*	*
	24 hr	*	NS	-	NS	NS	NS	*
6 hr Antibiotics	2 hr	*	NS	NS	-	NS	NS	NS
	6 hr	*	NS	NS	NS	-	*	*
	24 hr	*	*	NS	NS	*	-	NS
24 hr Antibiotics	2 hr	*	*	*	NS	*	NS	-

Table 4.3: Statistical differences between treatment groups according to Fisher's exact test comparing presence of bacteria, NS=no significant difference, "*"=p<0.05.

4.3 Conclusions

The findings of this study demonstrate the importance of patients receiving antibiotic therapy *and* surgical debridement early.

These findings are consistent with previous work, which has described the progress of bacteria from its colonizing planktonic form to adherence to tissue and eventually the formation of biofilm, the so-called 'race to the surface'[11]. As bacteria progress through these stages, their vulnerability to conventional treatments of debridement, irrigation and antibiotics decreases; the six-hour time point appears to be significant[10].

Even when open fractures receive very early antibiotics, the findings of this study support the position that early surgical treatment can still reduce infection. However, beyond the first few hours the advantage offered by early surgery appears to be negated.

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Chapter Five: Novel Treatments of Open Fractures

5.0   Introduction

In **Chapter Three** the association between infection and poor outcome following open fracture was defined. Furthermore, it was established that the tibia is the most common open fracture seen in surviving battlefield casualties, and the infection rate following this injury is 23%.

Identifying novel treatments with the potential to reduce the infection rate following open fracture should improve outcomes for those injured in combat in the future.

5.1   Irrigation with Chlorhexidine Solution Versus Saline

As described in **Chapter one** open fracture wounds are *irrigated* or *rinsed* with a fluid to physically remove contamination and microbes. Since Lister first described irrigating open fracture wounds with carbolic acid solution in 1867[1], surgeons have experimented with a variety of antiseptic chemicals to irrigate wounds.

An antiseptic chemical is one that is toxic to microbes with the potential to cause infection. The rationale for their use in infected wounds is that those microbes not physically removed by the mechanical effect of inert fluids will be killed by the antimicrobial action of the antiseptic.

Irrigation of open fracture wounds with antiseptic solutions was performed on a massive scale fifty years after Lister's work during the First World War. As a Lieutenant in the Royal Army Medical Corps, Alexander Fleming studied the effect of irrigating battlefield wounds with antiseptic solutions.

Fleming took large numbers of microbiological samples from wounds before and after surgical treatment, and noted the solution used to irrigate those wounds. His findings from this purely observational work led him to believe that wounds irrigated with antiseptics had greater rates of infection than those irrigated with saline. He concluded that: *'All the great successes of primary wound treatment have been due to efficient surgery, and it seems a pity that the surgeon should wish to share his glory with a chemical antiseptic of more than doubtful utility.'*[2]

Despite Fleming's scepticism about the efficacy of antiseptic solutions which he shared in a 1919 lecture to the Royal College of Surgeons[2], the use of antiseptics in this way continued for the century from the end of the First World War until the present.

The use of antiseptic solutions in surgery has recently been the focus of much scientific interest and research. The authors of the Fluid Lavage of Open Wounds (FLOW) study aimed to determine North American orthopaedic trauma specialists' attitudes towards the use of irrigation fluids in contaminated wounds. They found that around half of the 984 respondents thought that irrigation with chlorhexidine solution would be more effective than irrigation with saline alone[3]. This survey was limited by a response rate of only 56%, and it is worth noting that although half the respondents said that that they regarded chlorhexidine as superior to saline, only 2% actually used it in their clinical practice.

The disparity between the perceived efficacy and actual utilisation of chlorhexidine is likely due to it being widely used by surgeons in an alcoholic solution to decontaminate or 'prep' skin prior to surgery[4]. However, there are no published animal or clinical trials that have evaluated its use in the irrigation of open fractures[5].

The purpose of this study was to compare the efficacy of irrigation with a variety of aqueous chlorhexidine solutions to saline in reducing infection in an animal model of a contaminated open fracture.

5.1.1 Irrigation Fluids: Study Groups and Methods

The animal model described in detail in **Chapter Three** was used with five groups of ten animals each. The standard injury was created in each animal before being contaminated with 1×10^2 Colony Forming Units (CFUs) of the Xen 36 strain of *Staphylococcus aureus (S. aureus)*.

Six hours after injury and temporary wound closure, the wounds were re-opened, gross contamination was excised and wounds were irrigated with a total of 60mls of fluid. The composition of this fluid varied according to the treatment group detailed in **Table 5.1**.

Group	Animals (n)	Treatment
Control	10	Irrigation with 60 ml 0.9% saline.
0.5% CHG	10	Irrigation with 60 ml 0.5% CHG aqueous solution.
0.05% CHG	10	Irrigation with 60 ml 0.05% CHG aqueous solution.
0.005% CHG	10	Irrigation with 60 ml 0.005% CHG aqueous solution.
0.05% CHG & Saline rinse	10	Irrigation with 50 ml 0.05% CHG aqueous solution followed by rinsing with 10 ml 0.9% saline.

Table 5.1: Study groups detailing irrigation fluids used following surgical debridement six hours after initial injury and contamination with 1×10^2 CFU of *S. aureus*. CHG: chlorhexidine

After irrigation, wounds were re-closed and the animals were recovered and survived for 14 days. At this point they were euthanized and bone and implants were recovered and analysed for the presence of bacteria.

5.1.2 Irrigation Fluids: Results

There was no statistical difference in the levels of bacteria detected on bone and hardware following irrigation with aqueous chlorhexidine at a range of concentrations as shown in **Figure 5.1** (p-values shown in **Table 5.2,** Fisher's Exact Test).

Similarly, there was no difference in the levels of bacteria recovered from animals following irrigation with aqueous chlorhexidine compared to saline alone as shown in **Figure 5.2**. P-values for the proportion of samples with detectable bacteria in each test group compared to the control group are shown in **Table 5.2**.

The results of the Kruskal-Wallis Test of the log sums of the bacteria recovered from the animal's wounds across all groups was not significant (p=0.21) and therefore pair-wise comparison between individual test groups and the control was not performed.
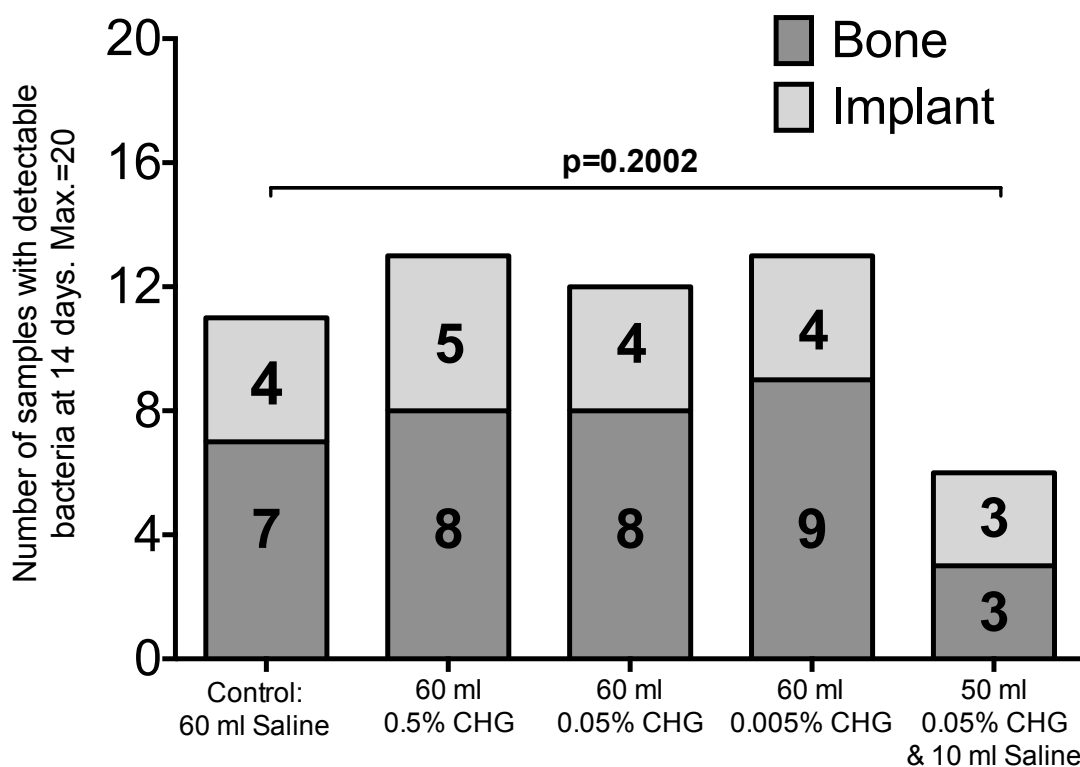
Figure 5.1: Proportion of bone and hardware samples with detectible bacteria from each group of ten animals 14 days after inoculation with 1×10^2 CFUs of *S. aureus* and irrigation with various fluids or combinations. No significant differences between groups. CHG: chlorhexidine.

Test Group	p-value of comparison with control
0.5% CHG	0.74
0.05% CHG	> 0.99
0.005% CHG	0.74
0.05% CHG & Saline rinse	0.20

Table 5.2: The similarity of the effect of various concentrations of CHG on the rate of detectable bacteria compared with control group (0.9% saline alone). P-values generated by Fisher's exact test.

	Control: 60 ml Saline	60 ml 0.5% CHG	60 ml 0.05% CHG	60 ml 0.005% CHG	50 ml 0.05% CHG & 10 ml Saline
■ Bone mean	8.18×10^5	4.33×10^5	7.88×10^5	1.91×10^6	3.60×10^5
Bone SEM	4.37×10^5	2.06×10^5	3.60×10^5	1.37×10^6	2.18×10^5
■ Implant mean	8.31×10^5	3.35×10^5	4.17×10^5	8.56×10^5	5.11×10^4
Implant SEM	5.13×10^5	1.69×10^5	1.54×10^5	$6.39x \times 10^5$	3.66×10^4
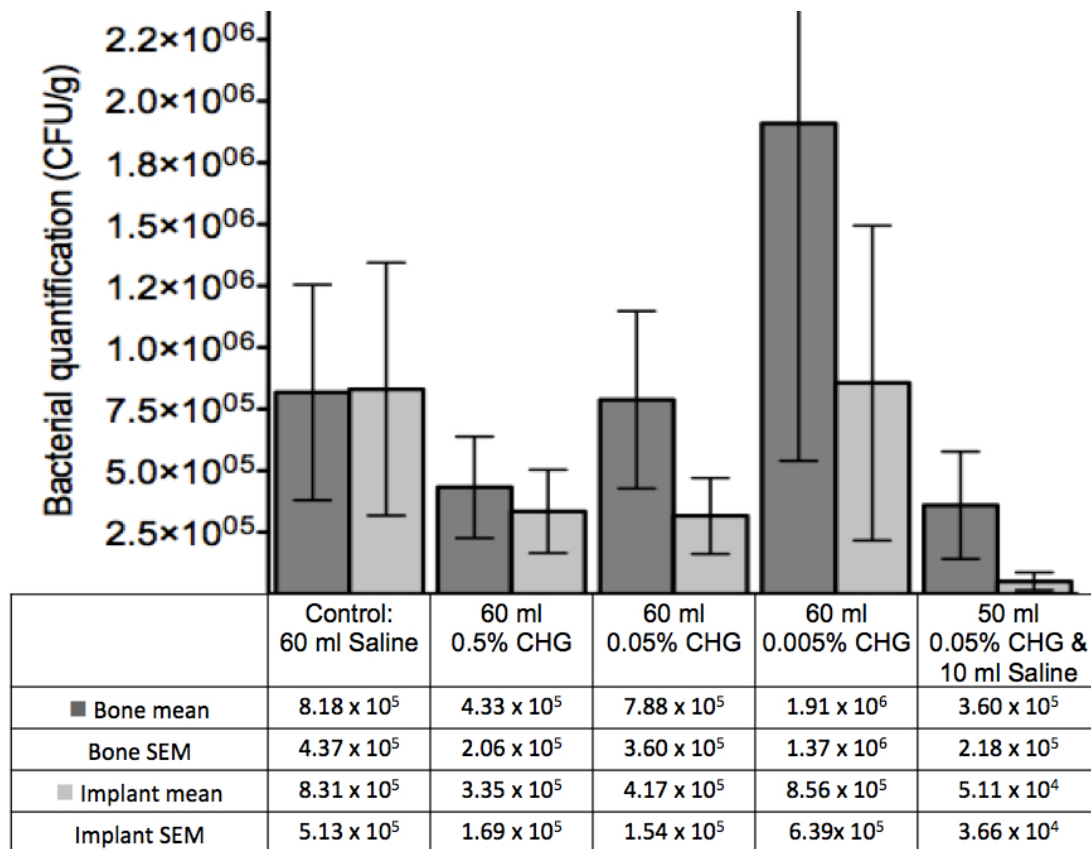
Figure 5.2: Mean bacterial quantification of bone and hardware samples 14 days after inoculation with $1x10^2$ CFUs of *S. aureus* and irrigation with various fluids or combinations. Ten animals in each study group. Error bars show the Standard Error of the Mean. No significant difference between groups.

5.2   Local Antibiotic Gel Versus Antibiotic Polymethylmethacrylate 'Beads'

As described in **Chapter One** in clinical practice antibiotic therapy is initiated as soon as possible after a casualty sustains an open fracture. Despite this, infections occur in 23% of open tibia fractures as described in **Chapter Three**.

Antibiotics are typically administered systemically, i.e. orally or intravenously. The main disadvantage of systemic antibiotics is that they require repeated dosages to achieve and maintain an effective concentration at the site of the wound. Additionally, an effective concentration of antibiotic in the wound is only achieved by establishing a similar concentration throughout the whole body. This limits the antibiotic concentration achievable at the wound to a level which is not toxic to other body tissues.

The alternative to systemic delivery is local delivery, where antimicrobials are administered directly into the wound, a technique being used in clinical practice even

before the widespread use of penicillin. Jensen *et al*. published their experience of local sulphanilamide powder in open fracture wounds in 1939[6]. In this retrospective study they attributed the reduction of infection rates from 6/22 (28%) in an historical group to 2/41 (5%) to the local administration of sulphanilamide. Although they did not present a statistical analysis of their findings, the difference appears to be significant ($p=0.0177$, Fisher's exact test).

Writing in 1945, Fleming cited the local use of penicillin as an important adjunct to systemic treatment [7]. However, after the Second World War the use of local antibiotics waned as new families of antibiotics were developed and experience with systemic administration improved.

It was not until the late 20th century that the advantages of local antibiotic delivery in achieving high concentrations of antibiotics within wounds without the need for repeated systemic dosing or the potential for causing systemic toxicity was re-popularised. Klem *et al* used polymethylmethacrylate (PMMA) cement blended with antibiotic powder as a vehicle for local delivery in the eradication of musculoskeletal infection[8,9]. The authors described the use of blocks or lumps of PMMA blended with antibiotic as a 'spacer' i.e. material used by surgeons to fill a void left after excision of normally necrotic or infected material to prevent the void being filled with a haematoma with potential to become infected.

Klem's experience was based on the treatment of established infection, where infected, necrotic bone seen in osteomyelitis is excised, and the resulting void filled with antibiotic PMMA 'spacers'.

Writing in 1990, Henry described how he had adapted this technique and used PMMA blended with antibiotics to mould beads around a thick suture material to facilitate their removal from a wound. He speculated that the larger surface area of the beads compared to a spacer allowed greater elution of active antibiotics from the PMMA, thereby delivering higher concentrations of antibiotics to the wound and reducing infection rates[10]. Henry advocated the routine use of local antibiotics in the form of antibiotic PMMA beads to prevent infection in open fractures.

Other methods for local antibiotic delivery include mixing them with either autologous[11] or synthetic bone graft, e.g. Tobramycin and calcium sulphate, commercially available as Osteoset T[©12]. These techniques are aimed at achieving two objectives: treating a bone defect and delivering antibiotics. This is a distinct clinical situation than purely the delivery of local antibiotics into a wound. Less than 10% of tibia fractures[13] involve bone loss that might require bone grafting, therefore this thesis focused on local antibiotic delivery vehicles that could be used in situations with or without bone loss possibly requiring grafting.

PMMA beads probably remain the most commonly used local antibiotic delivery vehicle in orthopaedic practice[14-16], though there are disadvantages associated with their use. The beads are bulky which complicates wound closure, and are not bio-absorbable thus necessitating surgical removal. Although some authors have advocated leaving them in situ[17], once they have eluted their antibiotics they act as an avascular foreign body and a site for potential bacterial colonization[18]. A conceptual disadvantage with antibiotic PMMA beads is that they are in essence an antibiotic depot requiring diffusion of the antibiotic from high concentrations close to the beads to the rest of the wound. This will potentially result in sub-therapeutic levels of antibiotics in areas of the wound furthest from the beads.

An idealised local antibiotic vehicle would therefore be:
- Bio-absorbable to avoid the requirement for removal;
- Degrade at the same rate as antibiotics are eluted to avoid persisting as a foreign body without the presence of antibiotics;
- Conform to the wound to permit easier wound closure;
- Allow similar levels of concentration to be achieved throughout the wound.

The pharmaceutical company Dr. Reddy's Laboratories, Inc., (Bridgewater NJ), (DRL), approached the United States Army Institute of Surgical Research (USAISR) after developing a bio-absorbable antibiotic gel that they had been developing for preventing surgical site infection in abdominal surgery. DRL claimed that their product performed closely to the idealised properties described above. Therefore, while working at USAISR, I established a Cooperative Research and Development Agreement (CRADA) to allow technology transfer and sharing of intellectual property between DRL and USAISR and to permit evaluation of the gel.

5.2.1 Local Antibiotic Treatments

The gel that DRL had developed is a sterile bioabsorbable phospholipid gel, designated DFA-02, containing 1.7% vancomycin and 1.9% gentamicin by weight. According to information provided by DRL the phospholipid gel was prepared by the addition of water to gentamicin sulphate and vancomycin hydrochloride, to allow complete dissolution of gentamicin sulfate and vancomycin hydrochloride. Then, lecithin (Phospholipon 90G, Phospholipid, GmbH, Cologne, Germany) and sesame oil was added, followed by high shear mixing at 5000 rpm for 60 min to obtain a uniform primary emulsion. The primary emulsion was placed in a microfluidizer (Microfluidics, Inc. Newton, MA) to produce a monophasic solution. The monophasic solution was lyophilized to remove water and obtain a dry paste. The dry paste was then mixed with dehydrated alcohol (6% w/w) and heated to form a viscous clear gel. The clear gel was filter sterilized by passing the entire mass through a 0.22 micron sterilizing filter (Sartorius Stedim, Inc. Bohemia, NY).

Antibiotic PMMA beads were manufactured under sterile conditions using Palacos R (Zimmer, Dover OH) arthroplasty cement. 40 g of MMA co-polymer powder was blended with 2.0 g of vancomycin (Sigma-Aldrich, St Louis, Missouri) and 2.4 g tobramycin sulphate (Sigma-Aldrich) then mixed with 20 ml of MMA monomer liquid. A 3mm mould was then used to create beads weighing approximately 20mg and containing 3.1% vancomycin and 3.7% tobramycin sulfate by weight. In each animal, four beads were placed in the wound, two in the bone defect, and two in the surrounding soft tissues; this was the number of beads that reasonably 'fit' into the wound. This delivered a dose of 2.5 mg of vancomycin and 2.9 mg of tobramycin.

5.2.2 Local Antibiotics: Study Groups and Methods

The model described in **Chapter Three** and above in Section 5.1.1 was used. Four group of ten animals each were studied, as shown in **Table 5.3**.

Group	Animals (n)	Treatment
Control	10	No treatment, wound closed after irrigation.
ABx PMMA Beads	10	Four 3 mm PMMA beads containing 2.5 mg vancomycin and 2.9 mg tobramycin were placed into the wound after irrigation and before closure.

ABx Beads/ ABx Gel	10	Two 3 mm antibiotic beads containing 1.25 mg vancomycin and 1.5 mg tobramycin and 0.5 ml of antibiotic gel containing 8.5 mg vancomycin and 8.5 mg gentamicin was placed into the wound after irrigation and before closure.
ABx Gel	10	1ml of phospholipid gel containing 19 mg vancomycin and 17 mg gentamicin was placed into the wound after irrigation and before closure.

Table 5.3: Study groups detailing different treatments and quantity of antibiotic received by each group. ABx: antibiotics, PMMA: polymethylmethacrylate.

All animals were contaminated with 1×10^5 Colony Forming Units (CFUs) of *Staphylococcus aureus* and treated six hours later with surgical debridement and irrigation with 60mls of saline. After this treatment, in the three of the four non-control groups, a local antibiotic was inserted into the wound as per the allocation described above in **Table 5.3**.

After fourteen days animals were euthanized: bone and implants were recovered and analysed for the presence of bacteria. The outcome measures were the presence and quantity of bacteria in the femur or attached to the implants (polyoxymethylene plate and K-wires).

5.2.3 Local Antibiotics: Results

Bacteria were recovered in all wounds in the control (no antibiotics received) and antibiotic-PMMA bead group. The group treated with antibiotic gel alone had significantly fewer animals with bacteria ($p \leq 0.004$) detectible in their wounds than these two groups, only half of the bone samples and 30% of the implant specimens had detectable bacteria as shown in **Figure 5.3**.
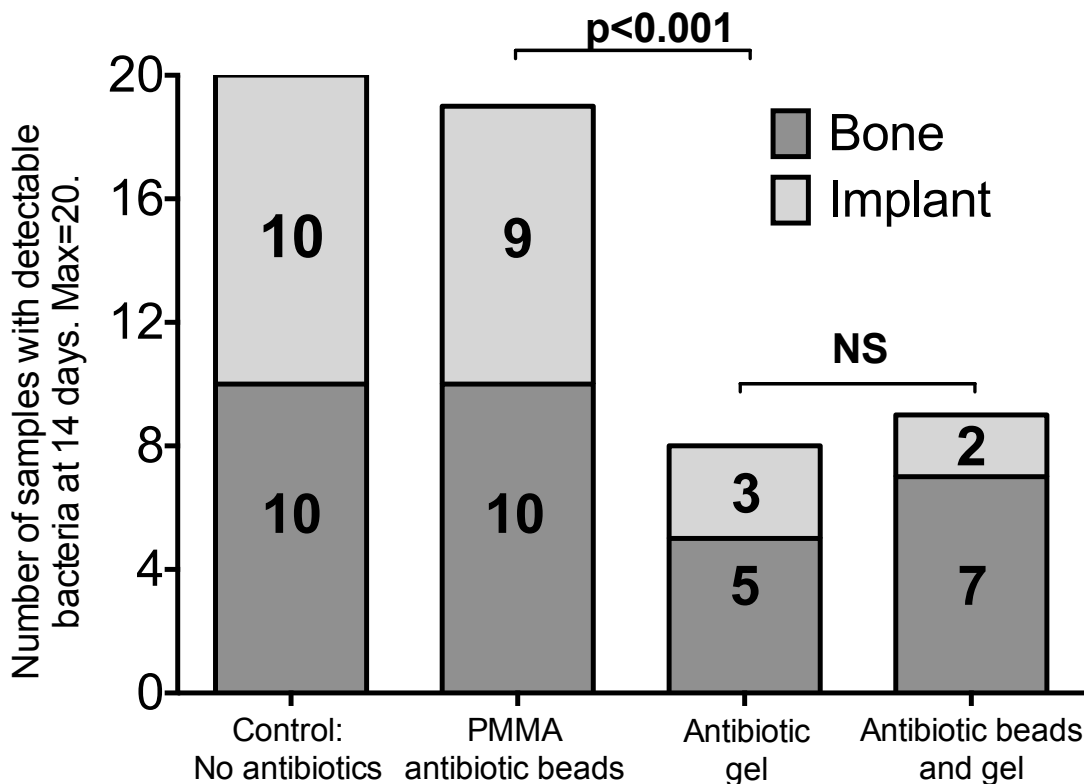
Figure 5.3: Proportion of 20 samples from each treatment group of 10-animals with detectible bacteria at 14 days. Statistical differences by Fisher's exact test are shown. *The antibiotic gel and antibiotic beads and gel group had a lower portion of the samples with bacteria that were recoverable than the other control and beads groups (p ≤ 0.0004). NS = no significant difference between adjacent groups.

Antibiotic gel was significantly superior to antibiotic-PMMA beads at reducing bacteria on both the bone (p=0.001) and the implant samples (p=0.004) as shown in **Table 5.4** below.

| Comparison | | Mann-Whitney Test | | Fisher's |
Group 1	Group 2	Bone	Implant	test
Gel	Beads	0.001	0.004	<0.001
Gel	Beads/Gel	0.310	0.820	1.00
Gel	Control	<0.001	<0.001	<0.001
Beads	Beads/Gel	0.001	0.007	0.001
Beads	Control	0.070	0.820	1.0000
Beads/Gel	Control	<0.001	0.002	<0.001

Table 5.4: P-value for comparison of groups by quantitative cultures of recovered bacteria from bone and implant samples (Mann-Whitney test) and presence or absence of bacteria in samples (Fisher's test).

Interestingly, there was no difference in the quantity of bacteria from the wounds of those treated with antibiotic-PMMA beads and those animals in the control group that received no treatment, with respect to either bone (p=0.07) or implant samples (p=0.82), as shown in **Figure 5.4**. The addition of antibiotic-PMMA beads to antibiotic gel did not reduce bacterial quantities when compared to gel alone (p>0.3).

	Control: No antibiotics	Antibiotic PMMA beads	Antibiotic gel	Antibiotic beads and gel
■ Bone mean	1.25×10^6	8.96×10^5	6.53×10^4	6.90×10^4
Bone SEM	2.62×10^5	4.70×10^5	6.19×10^4	6.53×10^4
▨ Implant mean	4.11×10^5	3.84×10^5	5.19×10^4	8.20×10^4
Implant SEM	1.01×10^5	9.62×10^4	4.92×10^4	7.77×10^4
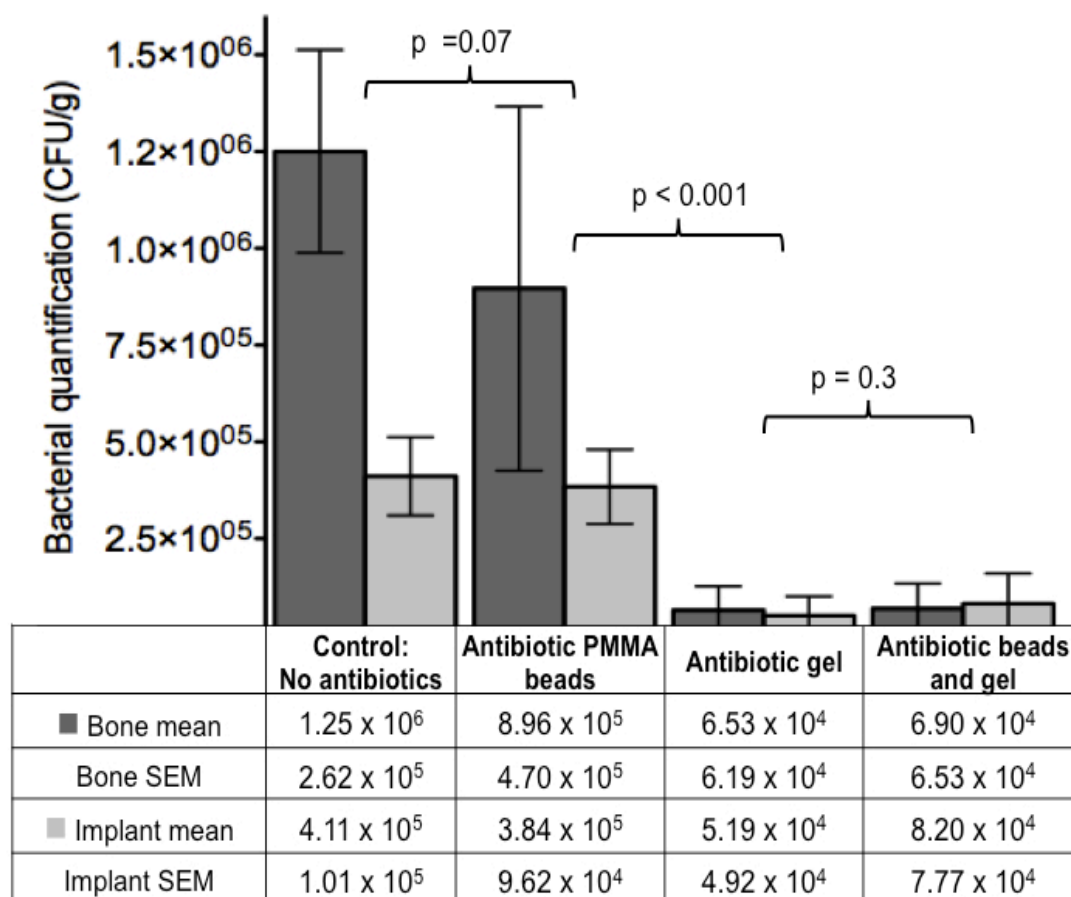
Figure 5.4: Mean quantity of bacteria recovered from bone and implants from different treatment groups of ten animals. Error bars show standard error of the mean (SEM). Statistical differences between quantities of bacteria recovered from bone samples calculated by Mann-Whitney analysis are shown.

5.3 Local Bismuth Thiol Gels with Systemic Antibiotics

Bacterial persistence in spite of standard treatment with systemic antibiotics is believed to be at least in part due to the ability of bacteria *in vivo* to form an adherent biofilm[19]. Biofilms are formed when bacteria adhere to a surface such as a surgical implant or bone, and they secrete an extracellular polymeric substance which acts both as a protective barrier and as a medium for intercellular signaling[20]. Compared to planktonic bacteria, those in biofilms are much more resistant to treatment with

antibiotics and can survive exposure to concentrations of antibiotics many times greater than are toxic to planktonic bacteria[20]. Clinically, biofilms persist in musculoskeletal infection despite prolonged antibiotic treatment[21].

Compounds made from the chemical element bismuth have been used for centuries in wound dressings[22] despite their relatively low antibacterial activity[23] and potential for toxicity[24]. Bismuth was used extensively in the First World War to pack heavily contaminated open fracture wounds in the form of bismuth iodoform paraffin paste (BIPP), a paste made from bismuth subnitrate combined with iodoform and liquid paraffin. Its mechanism of action was not understood, but it was regarded as an effective treatment of infection with no local toxicity and only occasional systemic toxicity[25].

When bismuth is chelated with thiol compounds, the antibacterial effect is enhanced and mammalian toxicity is reduced[26]. Bismuth thiols (BTs) have been shown in preclinical studies to inhibit bacterial biofilm formation and adherence to surfaces[27,28].

When used in combination with antibiotics, BTs have been shown to significantly reduce the antibiotic concentrations required to inhibit Gram-positive and Gram-negative bacteria, and in some cases synergize with certain specific antibiotics including tobramycin[29]. BTs, including MB-8-2 and MB-11, have also shown *in vitro* efficacy against MRSA and *Pseudomonas aeruginosa* biofilms when used alone or in combination with antibiotics[30].

The following study tested the hypothesis that combining BTs with systemic antibiotics will result in more effective reduction in rates of infection in an animal model of contaminated open fracture than systemic antibiotics alone.

5.3.1 Bismuth Thiols: Formulations

Three BT compounds were selected on the basis of their antimicrobial and antibiofilm activity,[28,30]: BisEDT (Bismuth-1,2-ethanedithiol), MB-8-2 (Bismuth-2,3-butanedithiol, 2-mercaptopyridine N-oxide), and MB-11(Bismuth-1,2-ethanedithiol, 2-mercaptopyridine N-oxide).

Hydrogel formulations of three different BTs were prepared in 50g lots, with each BT at 5mg/ml for the phase 1 study and MB-8-2 at 0.01, 0.1 and 1.0 mg/ml for the phase 2 study as follows. A surfactant mixture was prepared by mixing 12.5g of polypropylene glycol NF, 250mg polysorbate-20 NF (Tween 20), and 50mg methyl paraben NF in a glass beaker. The mixture was heated to 70 ^{o}C in a water bath with periodic stirring until completely dissolved. BT powder (0.5, 5, or 50mg of Bis EDT, MB-8-2, or MB-11) was added to the surfactant mixture, stirred, and sonicated in a fume hood using an ultrasound indenter for 45 to 60 seconds.

Immediately after sonication, 20g of hot (60° - 65 °C) purified water was added with mixing. In parallel, 150mg of xanthan gum (Kelco K9B310), 100mg of Ultrez 10 (carbopol NF), and 2.5g of glycerin were stirred together in a separate glass vessel until a homogenous paste suspension was formed, then 14.24g of hot (60° to-65 °C) purified water was added and continuously mixed until the polymers were fully hydrated. The hydrated polymer suspension was combined with the BT surfactant mixture, stirred with a spatula to blend, and then mixed using a high-shear mixer (IKA-12) at high speed to form a gel. The beaker was then covered and sonicated using an ultrasonic bath to eliminate air bubbles in the gel. The pH of the gel was adjusted to 5.0 to 6.0 by adding 200mg 1N sodium hydroxide (NaOH) followed by mixing with a spatula and then with a high-shear mixer (IKA-12) at high speed. Before use, the BT gel was allowed to stand for 24 to 48 hours at room temperature to eliminate any additional air bubbles; gels were not sterilized before use as the very broad-spectrum antimicrobial activity of the BTs was thought to prevent any microbial growth in the gels.

5.3.2 Bismuth Thiols: Study Groups and Methods

The model described in **Chapter Three** and above in Section 5.1.1 was used both with, and without, the 7-doses of 5mg/Kg of Cefazolin described in **Section 3.6**. As previously described, the outcome measure was the presence of bacteria in bone or on implants retrieved from animals 14 days after 'injury' and treatment.

There were two phases of the study. In the first phase, three different BT compounds at a single concentration were evaluated both in isolation and in combination with

systemic antibiotics. The second phase evaluated one of the compounds at three different concentrations.

For Phase One of the study, each of the study groups of ten animals were inoculated with 1×10^5 CFUs of the Xen 36 strain of *S. aureus*. Without treatment this would result in a 100% infection rate.

Six hours after 'injury' and 'contamination', the animals were treated with surgical excision of contamination and irrigation with 60mls of saline. After this, 1ml of BT-hydrogel (containing 5mg of BT) was placed into the wound. Each compound was tested for efficacy against bacteria both individually and in combination with systemic antibiotics.

Based on the results of Phase One, a single compound was selected for further investigation. The study groups are detailed below in **Table 5.5**:

	Group	Number of animals	Antibiotics	Bismuth Thiol
Phase 1	1	10	Y	Nil
	2	10	N	5 mg Bis-EDT
	3	10	N	5 mg MB-8-2
	4	10	N	5 mg MB-11
	5	10	Y	5 mg Bis-EDT
	6	10	Y	5 mg MB-8-2
	7	10	Y	5 mg MB-11
Phase 2	8	10	Y	0.5 mg MB-8-2
	9	10	Y	0.05 mg MB-8-2
	10	10	Y	0.005 mg MB-8-2

Table 5.5: Study groups for Phase 1 and 2 of the study. 'Antibiotics' comprised of a regime of 5 mg/kg of cefazolin subcutaneously with the initial dose of antibiotic six hours after injury and continued every 12 hours thereafter for three days (seven identical doses in total).

5.3.3 Bismuth Thiols: Results
Across all groups treated with BTs at the initial concentration (5 mg/wound = 12.5 mg/kg); local toxicity with wound breakdown was observed with MB-8-2 and MB-11 and was present to a notably lesser extent with BisEDT.

Though apparently more locally toxic, MB-8-2 and MB-11 both demonstrated higher antibacterial activity than did BisEDT at this dose. Bacteria were detectable in all animals treated with BT alone and 60% of those treated with only systemic antibiotics. Animals treated with both systemic antibiotics and BTs did not have a significantly lower infection rate compared to those treated with systemic antibiotics alone as shown in **Figure 5.5**.
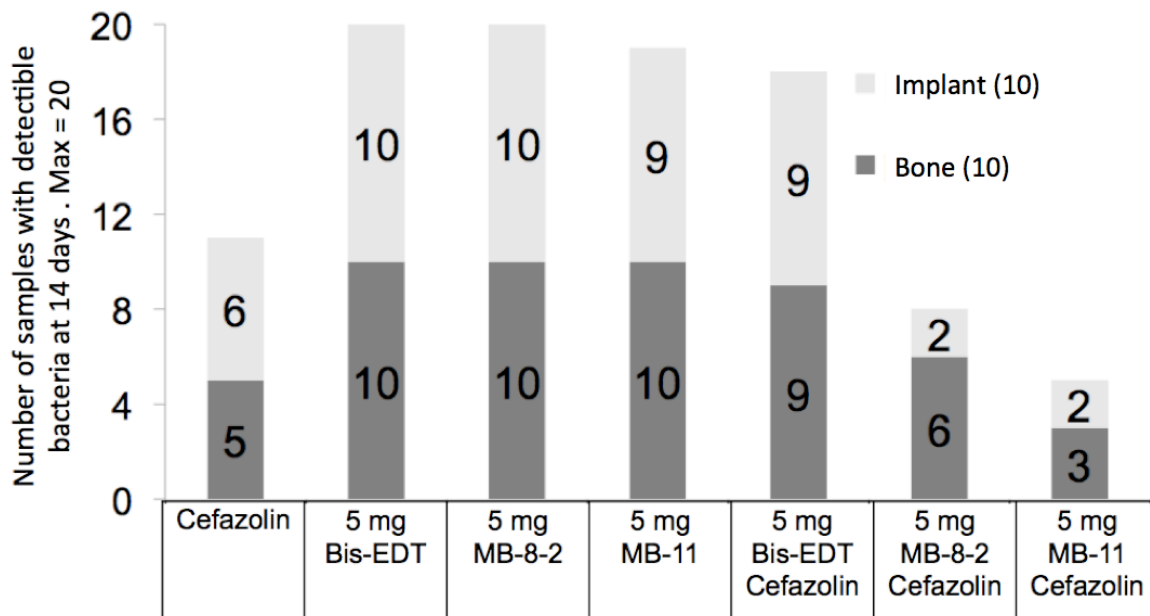
Figure 5.5: Phase 1 results showing the proportion of 20 samples from each treatment group of ten animals with detectible bacteria at 14 days after treatment with three different BT formulations with and without cefazolin. No significant difference between groups was noted by Fishers exact test.

However, animals treated with antibiotics alone had significantly more bacteria in their wounds than those treated with systemic antibiotics plus MB-8-2 (p = 0.031) or MB-11 (p = 0.008) as shown in **Figure 5.6** below:

	Cefazolin	5 mg Bis-EDT	5 mg MB-8-2	5 mg MB-11	5 mg Bis-EDT Cefazolin	5 mg MB-8-2 Cefazolin	5 mg MB-11 Cefazolin
Bone	1.46×10^6	9.80×10^5	9.1×10^5	8.75×10^5	3.70×10^5	9.30×10^3	2.90×10^3
Bone SEM	1.03×10^6	1.27×10^5	1.98×10^5	2.31×10^5	1.76×10^5	8.67×10^3	$2.64E \times 10^3$
Implant	1.60×10^5	3.50×10^5	5.50×10^5	2.20×10^5	4.13×10^5	9.00×10^3	2.60×10^2
Implant SEM	9.65×10^4	9.14×10^4	2.84×10^5	5.53×10^4	2.03×10^5	8.52×10^3	1.37×10^2
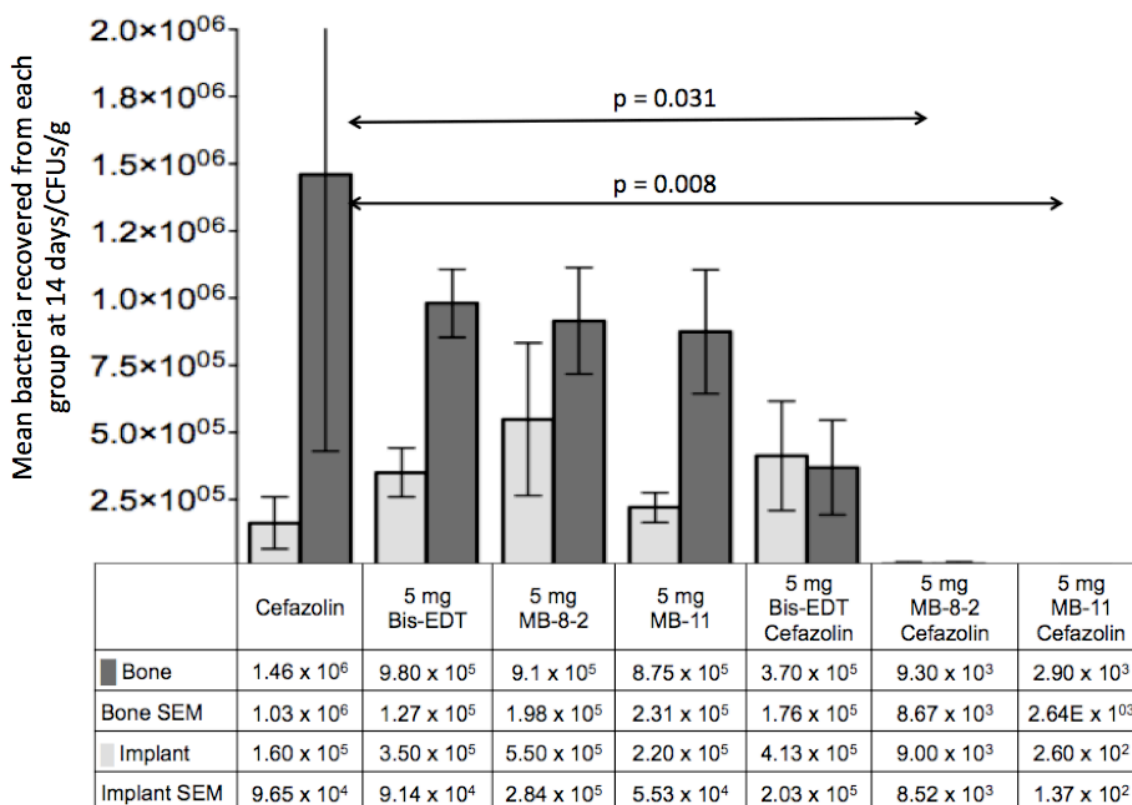
Figure 5.6: Phase 1 results showing mean bacterial quantification results from each treatment group of 10-animals treated with three different BT formulations with and without cefazolin in Colony Forming Units per gram (CFUs/g). Error bars show standard error of the mean (SEM). Differences between the control group and the MB-8-2 and MB-11 according to Mann-Whitney analysis are shown.

MB-8-2 was subjectively assessed to cause less local tissue toxicity than MB-11 while demonstrating more potent antibacterial activity than BisEDT. Accordingly, it was selected for further study in Phase 2. As shown in **Table 5.5** above, MB-8-2 was tested in a further three groups at reduced dosages: 0.5mg, 0.05mg and 0.005 mg: all groups also received systemic antibiotics.

A dose of 0.05 mg of MB-8-2 combined with systemic antibiotics resulted in a significantly lower infection rate ($p = 0.0057$) and bacterial quantification load ($p < 0.001$) compared to systemic antibiotics alone as shown in **Figures 5.7 and 5.8**.
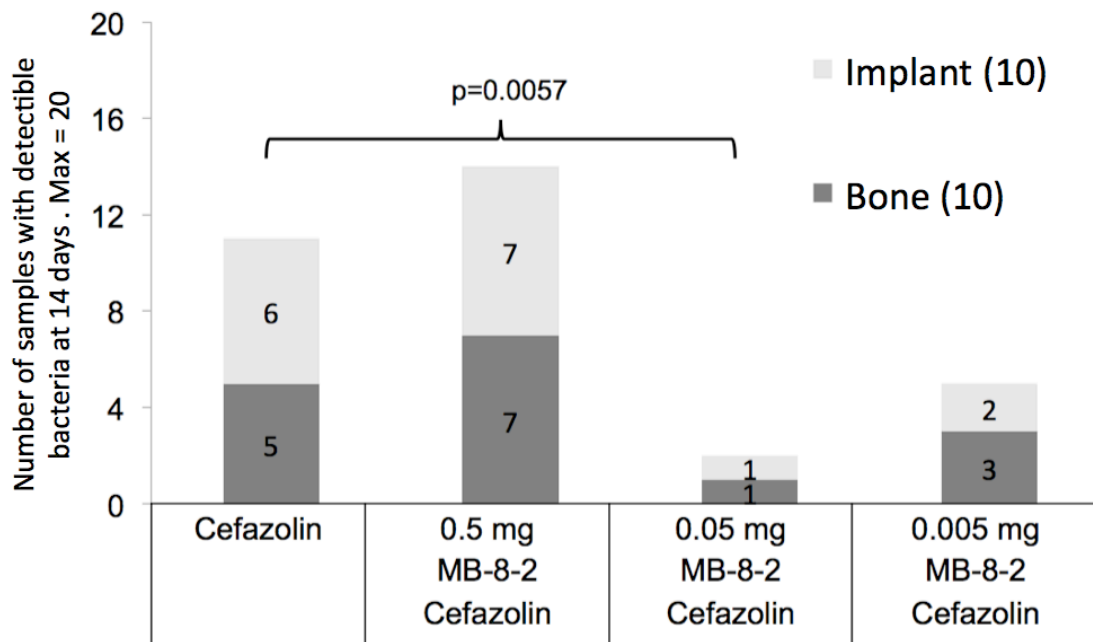
Figure 5.7: Phase 2 results showing the proportion of sample types from each treatment group of ten animals treated with reducing doses of MB-8-2 combined with a cefazolin treatment. Twenty samples were recovered from each group: 10 bone and 10 implants, thus giving a maximum of 20 positive samples for each group. Significance by Fisher's exact test is shown.

	Cefazolin	0.5 mg MB-8-2 Cefazolin	0.05 mg MB-8-2 Cefazolin	0.005 mg MB-8-2 Cefazolin
■ Bone mean	1.46×10^6	2.19×10^5	8.02	2.93×10^5
Bone SEM	1.03×10^6	6.16×10^4	7.60	2.16×10^5
▨ Implant mean	1.60×10^5	9.0×10^3	2.6×10^2	1.60×10^5
Implant SEM	9.65×10^4	2.7×10^4	36.2	7.11×10^4
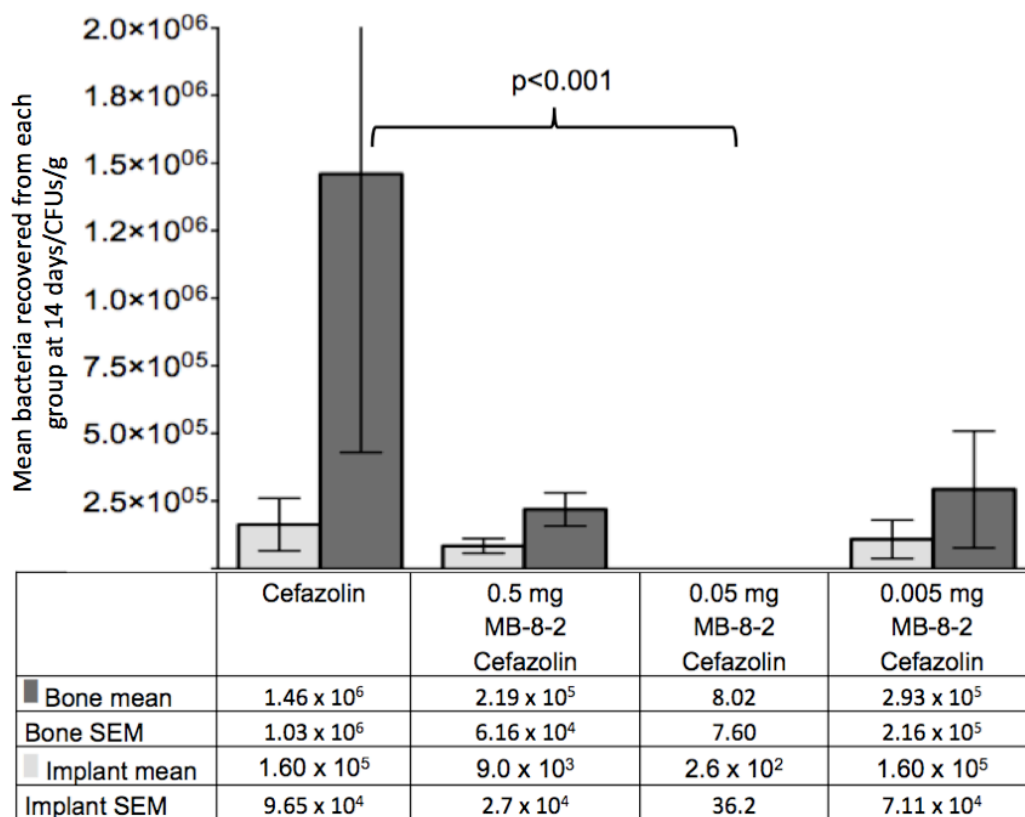
Figure 5.8: Phase 2 results showing the proportion of sample types from each treatment group of 10 animals treated with reducing doses of MB-8-2 combined with a cefazolin treatment. Twenty samples were recovered from each group: 10 bone and 10 implants, thus giving a maximum of 20 positive samples for each group. Significance by Fisher's exact test is shown.

No systemic or local toxic effects were observed at this concentration and there was no gel residue. The higher and lower doses (0.5 and 0.005 mg) were not as effective at reducing bacteria.

5.4 Conclusions

The results of the three studies presented in this chapter allow certain conclusions to be drawn. Firstly, these results are consistent with previous clinical and animal studies that have failed to demonstrate that any antiseptic solutions are superior to saline for reducing infection in open fractures. Secondly, there are more effective vehicles for delivering local antibiotics into open fracture wounds than the current clinical standard of PMMA beads. Finally, the findings presented demonstrate the proof of the concept that bismuth thiols exert a synergistic effect with antibiotics, potentially identifying a new class of treatment of infection after open fracture.

5.5 References

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Chapter Six: Discussion

6.0 Introduction

In **Chapter One** at the start of this thesis I set out the gaps in our current understanding of the nature of combat casualties, and particularly those who sustain open fractures. Specifically, the following four questions were identified as outstanding:

1. *Has survival after combat injury improved and are we measuring it accurately?*
2. *How common is infection after open combat fractures, and what are the consequences?*
3. *How does the timing of surgical and antibiotic treatment of open fractures affect infection?*
4. *Are there novel treatments that might reduce infection in open fractures?*

In **Chapters Two** to **Five** a series of studies were described that aimed to answer each of these questions. In this final chapter, the findings of these studies will be reviewed and examined further in the context of both the published literature and the strengths and limitations of the approaches I have used.

6.1 Has survival after combat injury improved, and are we measuring it accurately?

The original rationale for exploring this question was to examine the premise that improvements in trauma care have led to a cohort of casualties surviving with more complex extremity wounds than have previously been encountered[1].

The second part of the question: i.e. '*Are we measuring it* [survival] *accurately?*' has wider significance beyond this work. To improve the performance of a complex system such as combat casualty care, the ability to accurately measure survival and therefore the effect of any new intervention, permits the identification of more successful techniques and practices as they are introduced.

6.1.1 The probability of survival

In **Chapter One** I described how registries of traumatically injured patients evolved in both the civilian and military sectors in an attempt to measure system performance. In order to do this, for each patient included in the registry, a Probability of Survival (*Ps*) is determined. If the *Ps* is greater than 0.5 that individual is expected to survive: if it is below 0.5, they are expected to die from their injuries. A patient who survives with a *Ps*<0.5 is termed an *unexpected survivor,* while a patient who dies with a *Ps*>0.5 is referred to as an *unexpected fatality*. The greater the ratio of unexpected survivors to unexpected fatalities, the better the performance of the trauma system.

In the civilian setting the UK Trauma Audit and Research Network (TARN) and the US National Trauma Data Bank (NTDB) use probability of survival methodology to compare survival at individual hospitals to that of the overall system. This allows the performance of the hospital relative to all others in the system to be established.

The US NTDB uses the TRISS methodology to calculate a patient's *Ps* (described in detail in **Chapter Two**). The UK TARN registry initially used TRISS then transitioned to a methodology devised specifically for the UK to account for the more elderly population, and the preponderance for blunt mechanisms of injury of traumatically injured patients in the UK[2].

The military trauma registries in the UK and the US both use TRISS methodology. In the military combat casualty care system, a single casualty is likely to receive treatment in several facilities as they are sequentially moved away from their point of wounding and back to their home country. It is therefore not possible to isolate the performance of a single 'hospital' or facility as described for civilian trauma healthcare. Instead the number of *unexpected survivors* has been cited as a measure of the performance of the UK Defence Medical Service's Combat Casualty Care system. The 2010 National Audit Office report on this subject stated:

'The Department's and the NHS' methodology for calculating unexpected survivors differs and so a direct comparison is not easy, but ostensibly its unexpected survivor rate compares favourably with that achieved by the best NHS hospitals.'

It is important to note that the numbers of *unexpected fatalities* were not presented in this report; arguably one figure without the other is meaningless. This omission is not necessarily for nefarious reasons. It would cause huge distress to bereaved families for the term *unexpected fatality* to be used as it implies 'unnecessary' or 'avoidable' death, which is inaccurate. While the TRISS methodology is accurate at predicting survival across a population of trauma patients, it is an imperfect tool for individuals, failing to take into account specific circumstances e.g. pre-existing co-morbidities or medications.

However, in the course of this work I determined that the UK military trauma registry, the Joint Theatre Trauma Registry (JTTR), was using the original TRISS coefficients, meaning that the standard of survival was based on data from the US gathered in the 1970s and 1980s as part of the Major Trauma Outcomes Study. Therefore, not only was the probability of survival not being used as intended as a ratio of unexpected survivors to unexpected fatalities, but the expectation of survival was decades out of date.

As a result of this I proceeded to determine whether combat casualty survival improved over the 14-years of the conflicts using the New Injury Severity Score (NISS), with the intention to return to the TRISS methodology to see whether this could be improved.

6.1.2 Using New Injury Severity Score to measure survival
As described in **Chapter Two**, each of the cases in the JTTR has been coded with the New Injury Severity Score (NISS) giving a quantification of injury severity according to anatomic scoring. For each calendar year, there was a different number of heterogeneous cases precluding direct comparison of survival rates. In order to establish the relationship between a non-parametrically distributed independent variable (NISS) and the dichotomous dependent variable (mortality), the use of logistic regression modelling was the preferred technique.

In order to ensure that the model was expertly constructed and tested, a specialist biostatistician at the Birmingham University Clinical Trials unit, Dr Jon Bishop, was approached. Dr Bishop confirmed that the use of a logistic regression model was an appropriate technique and developed a model using the R statistical suite. Within

each year it was clear that there would not be a NISS 'threshold' of survival i.e. a level above which all casualties survived and below which they all died. Therefore the relationship between the NISS and survival was not linear and restricted cubic splines were used in the development of models to 'smooth' the data[3].

In order to select the most appropriate model, the choice of methodology was between the use of the Akaike Information Criterion (AIC)[4] or a Bayesian information criterion (BIC)[5]. Both techniques would likely produce similar result, however given that none of the postulated models could be absolutely 'true', the use of AIC was judged to be theoretically most appropriate[6]. The goodness of fit of the selected model was assessed by using the le Cessie-Van Houwelingen-Copas-Hosmer goodness-of-fit test[7].

As described in **Chapter Two** the model finally selected demonstrated that the NISS associated with an observed Ps of 0.5 increased every year from 32 in 2003 to 60 in 2012, and that this improvement was highly significant.

We believe that this analysis was the first time that survival improvement over the course of a conflict has been reliably demonstrated. This analysis was reported in the lay-media as shown in **Figure 6.1** below:

Medical advances save 38 troops with 'unsurvivable' injuries

By Ben Farmer

ADVANCES in battlefield medicine during years of fighting in Iraq and Afghanistan helped 38 British troops recover from wounds previously judged unsurvivable, a landmark study has found.

Improvements in care meant that since 2003, an estimated 265 casualties survived injuries that would have been judged likely to be fatal at the start of the conflicts.

The findings are the most comprehensive attempt to measure how much lifesaving battlefield techniques improved survival rates over the two campaigns.

The skill of surgeons and nurses, well-equipped field hospitals such as the one at Camp Bastion, and the speed of helicopter evacuation crews have long been credited with giving levels of battlefield care unseen in earlier conflicts.

Surg Lt Cdr Jowan Penn-Barwell of the Royal Navy, said that while experts believed survival rates had improved, there was no scientific evidence until now. In the study by Navy surgeons and an academic at the University of Birmingham, published in the *Journal of Trauma*, every casualty was given a New Injury Severity Score, ranging from 1 for a minor wound, to 75 for unsurvivable injuries.

In 2003 the level of severity where half of casualties would be expected to survive was 33. By 2013, that had climbed to 60.

Improvements in rehabilitation also meant that many of those who were at one point thought certain to die not only survived, but have made remarkable recoveries.

Figure 6.1: Article from *Daily Telegraph* 4th June 2015 regarding the publication of the NISS survival analysis earlier that year in the *Journal of Trauma.*

It is important to acknowledge that although all of the techniques used in our analysis have been used extensively in other fields of medicine, this appeared to be the first time they had been applied to combat casualty data. The use of sophisticated logistic modelling is considerably more complex than traditional attempts to demonstrate improved survival e.g. Case-Fatality Rate, as detailed in **Chapter One**.

A disadvantage of this approach is that it requires a reasonably sized data set, i.e. a large number of casualties. In military operations with low numbers of casualties e.g. peace-keeping roles, a similar analysis is unlikely to be possible. Furthermore, the NISS of fatalities as well as survivors needs to be recorded for the analysis described to be possible. This means that comparing survival rates across other military systems is challenging (e.g. the US JTTR has incomplete NISS data on fatalities), and similar issues exist with records of historic conflicts. This last point was illustrated when, in unrelated work, I tried to compare survival during the Falklands conflict with that in Afghanistan 35 years later[8]. Due to the lack of NISS data on fatalities in the historic group, the only way to indicate likely differences in survival between the two conflicts was to identify the survival of more casualties with more severe injuries in the later conflict.

However, in the case of the recent UK military experience in Iraq and Afghanistan the JTTR does provide sufficient data for this analysis. The thorough examination of this analysis, described above and in **Chapter Two**, gives me a high level of confidence that this methodological approach has been proved to accurately describe the relationship between NISS and survival over the study period.

6.1.3 Developing Trauma Injury Severity Score coefficients to predict survival after contemporary combat injury

As previously stated, NISS is based on the Abbreviated Injury Scale (AIS)[9], an anatomic description of injuries which fails to account for differences in physiology. Combat casualties are typically young men with a median age of 25[10]. By definition, individuals deploying on military service are fit, free from active co-morbidities and have completed a rigorous pre-deployment training process. It is feasible that measuring military trauma system performance using a scoring system based purely

on an anatomic description of injuries, and established from civilian registry data with older, less healthy individuals, is likely to exaggerate performance.

As mentioned previously, it was my initial intention to use the TRISS methodology to generate a Ps for each patient based on their injuries and physiological status and taking into account the age of the casualties. This would also be consistent with the civilian UK and US approach to measuring trauma system performance. However, as stated in **Chapter Two** the TRISS values in the JTTR were based on out-dated coefficients and without any indication of whether casualties injured by explosive weapons were coded as 'penetrating' or 'blunt'.

By the stage that the NISS based phase of the analysis had been completed, a further 2-years of JTTR data was available bringing the study period to 12-years (2003-14). As detailed in **Chapter Two**, only those casualties injured by gunshot wounds (GSW) or from explosive weapons were included in this re-analysis.

In order to 'reverse-engineer' two sets of TRISS coefficients that reflect contemporary survival rates, multi-variant regression would be required, in the same way that this method was used to derive the original TRISS coefficients. I once again requested that Dr Jon Bishop assist me in constructing the model, and the more complicated task of validating the coefficients.

New coefficients were derived as described in **Chapter Two**, however validating them was a challenge. Unsurprisingly the Receiver-Operator Curve (ROC) analysis of the performance of the revised coefficients in predicting outcome in the data set from which they were derived was excellent. However, this performance with the original data set does not necessarily assure similar performance in a different but comparable dataset e.g. from a peer-military such as the US, or from a future UK conflict.

There were a number of solutions to the problem of validation. Firstly, given that the US had been involved in the same conflicts as the UK military over a similar period, using their Joint Theatre Trauma Registry as a comparable dataset presented an option for possible external validation. In order to access this, in 2015 I instigated a discussion which included the UK Defence Professor of Military Surgery, Surg Capt

Rory Rickard, and the then head of the US JTTR. My aim was to agree a memorandum of understanding between the UK Ministry of Defence and the US Department of Defence for a collaborative team to be established in order to jointly evaluate the newly developed TRISS coefficients using a sample dataset from the US JTTR.

This goal was never achieved: it became apparent during discussions that the US JTTR data on fatalities was far less complete than the UK's and that a majority of US fatalities did not have NISS data recorded. A further cause of sensitivity was the possibility that this process would lead to a direct comparison between the two countries' combat casualty care systems' performance. Discussions failed to achieve agreement. For obvious reasons a dataset from a civilian trauma registry would not be suitable to validate combat TRISS coefficients. I therefore concluded that validation with an external data set was not possible.

Having decided that the new TRISS Coefficients would need to be internally validated, I approached Dr Jon Bishop for expert advice on this area.

The main techniques used for internal validation (with respect to discriminative ability, calibration and overall accuracy) for logistic regression models are split-sampling (10-fold/cross validation) and bootstrapping. Split-sampling was considered, but given the limited size of the original data set, dividing this into a derivation set and a validation set would likely significantly reduce the accuracy of the coefficients that could be derived originally. Bootstrapping is a development of the split sampling approach where the sample is 'replaced' within the original dataset. Bootstrapping has been demonstrated as a superior technique for assessing internal validation, and was therefore selected for this purpose in this study[11].

While external validation with a second, comparable data set would be ideal, the internal validation and the performance of the new TRISS coefficients leads me to be highly confident that the new coefficients are precise and accurate in predicting the probability of survival after combat injury.

6.1.4 Using contemporary Trauma Injury Severity Score coefficients to predict survival after contemporary combat injury

When the new TRISS coefficients were compared to the original ones, there was only a very modest difference in the Receiver-Operator Curves as shown in **Figures 2.4** and **2.5**. This might lead to the conclusion that developing newer TRISS coefficients is unnecessary as the improvements in accuracy are extremely marginal. However, it is worth noting that when the new TRISS coefficients are used to model trends in survival using the same modelling techniques previously described in conjunction with NISS, the results are very different.

The plot in **Figure 2.6** shows that over the course of the conflict survival improves to the point where the original TRISS coefficients are *unable* to accurately predict survival. However, the new TRISS coefficient allows the model to work as one would anticipate: an improved performance over the initial years of the conflict until a plateau of performance is reached. This plateau occurs around the level at which a Ps of 0.5 is associated with 50% survival equipoise i.e. exactly how TRISS should perform.

Even if the performance of the old and new TRISS coefficients was not as stark in practice, it would still be important to use them. The performance of the UK Defence Medical Service's combat casualty care systems is measured based on statistics gathered by the Ministry of Defence (MOD) administrative agency, *Defence Statistics*. In effect, the MoD is measuring its own performance, and under such circumstances it is important to be able to demonstrate that every effort is made to ensure that that this process is rigorous and accurate. For that reason, it would be preferable for the MOD to use updated and mechanism-specific TRISS coefficients to measure the performance of its combat casualty care system.

From this I draw two conclusions, firstly, using two different measures of injury severity, NISS and TRISS, survival after combat injury improved over the course of the conflict. Secondly, that the use of a modified TRISS will allow accurate measurement of combat casualty care, even at performance levels at which the original TRISS coefficient failed.

6.1.5 Future work in measuring survival after combat injury

Other investigators have attempted to improve the methodology for measuring survival in the area, by adapting purely anatomic injury scoring systems for military casualties[12]. However, systems that incorporate both the anatomical injury and the physiological response to that injury have theoretical and practical advantages over purely anatomic based systems, and are favoured by the UK MoD[13], TARN[2] and the US National Trauma Data Bank[14]. In practice the modified military TRISS model developed in this study significantly outperforms recently described military anatomic systems[12].

Some researchers have attempted to use Bayesian networks to improve the ability to model survival after combat injury. This is a very attractive approach particularly for handling data from registries, as Bayesian networks are capable at handling missing data, an inherent feature of registries. However, so far efforts to use Bayesian networks for this purpose have been less precise than the improved TRISS methodology described above[15].

It is worth considering whether 'survival' is the only standard by which the performance of combat casualty care systems should be measured. Mortality is clearly important, and is an objective and simple outcome, however other metrics are important and feasible.

Patient Reported Outcome Measures (PROMs) and military specific outcomes such as Return to Duty (RTD) and completion of fitness tests, could be used as measures of recovery.

PROMs are increasingly recognised as an important approach for judging the effectiveness of healthcare interventions[16]. In separate studies unrelated to this thesis I have used PROMs (Short Form-36[17]) to determine whether patients have a better quality of life with amputation or limb reconstruction following combat injury[18,19]. It would be feasible to incorporate follow-up with PROMs into the UK JTTR, providing a measure of generalised *rehabilitation* rather than just *survival*. Given that many cases in JTTR have very minor injuries, for practical purposes it would be appropriate only to gather PROMs on those with injuries more severe than

a given threshold e.g. NISS≥15, and at specific points following injury e.g. twelve and 24 months.

In addition to PROMs it is also possible to gather outcome measures unique to the military. After traumatic injury, service personnel will be placed into a medical category: fully fit with no restrictions; medically downgraded with specific restrictions e.g. no load carrying; or medically discharged. An individual's return to duty following injury is recorded on the Joint Personnel Administration (JPA) system, and could be incorporated into the JTTR as an outcome measure. Return to duty status is related to whether an individual passed their annual fitness test after their injury and is likewise recorded on their JPA record.

As survival reaches the limits of human physiology measuring improvements in combat casualty care system performance becomes more challenging. The addition of these outcome measures for quantifying the recovery would allow future changes in performance to be detected.

6.2 How common is infection after open combat fractures, and what are the consequences?

Having established that survival had improved over the course of the Afghanistan and Iraq conflicts, this phase of study was focused on *defining the problem* of open combat fractures in these survivors.

In order to address this question, it was divided into components which were examined separately:

1. How common are open fractures after combat injury?
2. How many combat injuries are complicated by infection?
3. What organisms are responsible for infections?
4. Is infection associated with a poorer outcome?

This phase of the study relied on identifying cases through the JTTR registry and then correlating them with clinical records. With any research based on registry data errors and inaccuracies in initial coding will be reflected in the results and

conclusions. It is believed that the quality of coding may have been weaker in the first three years (2003-2006), and coding of lesser injuries may have been incomplete. Similarly, it is reasonable to speculate that in extremely severely injured casualties some minor injuries might be overlooked.

6.2.1 How common are open fractures after combat injury?

As detailed in **Chapter Two** around 75% of injured survivors have an extremity injury: approximately 5% of these involved an open tibia fracture, the most frequently open fractured bone.

The relative frequency of open tibia fractures represents a particular challenge as it is regarded by orthopaedic surgeons as particularly prone to infection and healing problems due to its poor soft tissue envelope[20].

6.2.2 How many combat injuries are complicated by infection?

Having defined the number of open fractures in general and the frequency of open tibia fractures in particular, the next question was how many of them are complicated by infection?

Defining surgical site infection after elective surgery can be challenging[21]: this is certainly true after open fractures and especially true of those sustained on the battlefield. Combat open fractures are, by definition, contaminated to some degree with microorganisms. There is likely to be a spectrum from universal contamination to colonisation and then infection.

All combat open fractures were treated with antibiotics, which were typically continued beyond the 72 hours recommended by the BAPRAS-BOA national standards[22]. Prolonged antibiotic therapy was often used prophylactically because of the heavy contamination frequently seen in these injuries, despite the recognised lack of evidence for this strategy[23,24].

Given this potentially confusing clinical picture of contaminated, complex open fracture wounds already receiving prolonged antibiotic treatment, determining which were infected was challenging, particularly as this was to be done from a retrospective review of clinical notes. To avoid ambiguity, I decided that the standard

of infection in this study should be that the treating consultant surgeon decides that there is a clinical diagnosis of infection requiring surgical treatment.

While this is still a subjective definition, much of clinical healthcare is subjective and based on judgment. I believed that using a definition with a 'threshold' i.e. surgical treatment, makes it more specific, but at the risk of reducing the sensitivity. This would mean that wound infections that could be successfully treated by resuming antibiotic treatment were not included as 'infections'. However, this also avoids Type I error of over-diagnosis of infection in the presence of e.g. inflammation or haematoma.

This definition of infection was used by Burns *et al.* in their case series of US military personnel injured on operations in Iraq an Afghanistan. The findings of their study were strikingly similar to mine; they reported an infection rate of 27% in 213 open tibia fractures compared to 23% in 93 fractures in this work[25].

The largest prospective study of lower extremity trauma in the civilian literature is the Lower Extremity Assessment Project (LEAP). This work did not clearly define infection either in the protocol[26] or published results[27]. However, they do report that 22% of 541 cases had wound infections (undefined) that required surgical treatment. It is important to note that the LEAP study included a range of severe lower-limb threatening injuries, and not just open tibia fractures.

Some researchers have sought to define infection as the presence of detectable bacteria in microbiological samples taken from the wound. In their series of 35 open tibia fractures in US service personnel injured in Iraq and Afghanistan, Johnson *et al.* reported an infection rate of 77%[28].

As stated previously, the presence of detectable bacteria may not necessarily be pathological, as all of these wounds are contaminated and all open wounds are likely to be colonised to some degree with bacteria. It is interesting to note that the two studies (LEAP and Burns *et al.*) that used a similar clinical definition of infection report an infection rate of around 20%, consistent with my findings, substantially lower than when a microbiological definition of infection is used.

6.2.3 What organisms are responsible for infections?

In the clinical care of the patients with open tibia fractures described in this work, multiple tissue samples rather than wound swabs were taken at each surgical episode. In cases meeting the definition of infection, Dr Debbie Mortiboy, the clinical consultant microbiologist at University Hospitals Birmingham responsible for processing these samples, was contacted and asked to confirm the causative organisms in each case, based on contemporaneous results.

While I would readily acknowledge that multiple species were detectable in many of the infected wounds, in the experience of the clinicians treating these injuries a single microorganism typically predominated in clinically significant infection, possibly due to the background of extensive antibiotic use in these patients[29]. It is arguably an over-simplification to focus on a single organism in every case, but I regard this as a pragmatic approach in the clinical setting.

The findings detailed in **Chapter Two** show that the causative pathogens in UK service personnel with open tibia fractures were overwhelmingly *S. aureus* species, responsible for 60% of infections. In the series described by Burns *et al*., *S. aureus* was responsible for only 35% of infections, but with another Staphylococcus species, *S. epidermidis,* responsible for a further 20%. This is in stark difference to the series reported by Johnson *et al*., which reported that *S. aureus* species were responsible for only 9% of open tibia infections, with gram-negative species responsible for the majority.

My interpretation of the disparity between the studies based on a clinical diagnosis i.e. my study and the Burns series[25] and Johnson's microbiological defined series[28] is that gram-negative bacteria may colonise many wounds without necessarily causing clinically important infection. Furthermore, it appears that gram-positive Staphylococcus species, specifically *S. aureus,* cause the majority of clinically relevant infection following open fracture.

6.2.4 Is infection associated with a poorer outcome?

In **Chapter Two**, the question of whether infection was associated with a poor outcome was examined in a series of 57 open tibia fractures[30]. The premise of this

phase of the work was to determine whether infection detrimentally affected efforts at surgical reconstruction. Therefore, the outcome measures and study period were selected accordingly.

Twelve-month follow-up was used in this study, as although the acute care of casualties occurs at one institution, this military population live in all regions of the UK. At their request, ongoing care may be transferred to hospitals closer to their home. As a result, there is a significant drop in the proportion of patients being followed up at this institution beyond the twelve month point. It is likely that if the follow-up period was longer, a greater proportion of poor outcomes would be detected. However, the authors regard the 91% follow-up of all fractures as sufficient for conclusions to be drawn. It was also reasonable to predict that clinically relevant problems such as infection would manifest within this twelve month period

The choice of outcome measures also reflected the premise of this phase of the study. A surgical outcome i.e. revision surgery (including amputation) was used. This was in preference to choosing a patient reported outcome measure (PROM), as the effect of infection would likely be confounded by coexisting injuries.

However, while PROMS were not directly measured in my study, they were used by the LEAP study, which demonstrated the detrimental effect of unplanned revision surgery on the Sickness Injury Profile PROM[31].

The statistical analysis approach used was simplistic-the relationship between each of the dependant variables and the independent variable was examined separately. An alternative approach would have been developing a logistic regression model, this would have provided greater detail about the relationship between the variables and the outcome. However, for simplicity a direct comparison was used together with Bonferonni correction to allow for multiple comparisons.

It is important to acknowledge that a retrospective examination of this type can only measure association without establishing causation.

6.3 How does the timing of surgical and antibiotic treatment of open fractures affect infection?

The studies in **Chapter Two** defined and characterised the nature of the problem of infection in open fractures, thereby answering the first two questions laid out in **Chapter One**.

In order to answer the second two questions laid out in **Chapter One**, I decided that an animal model would be necessary to examine the issues involved. The rationale behind the choice of rodent model was outlined in **Chapter Three**, as was the need to refine and evolve the model to better address the specified questions.

6.3.1. Model development: bacterial inoculation

The early experience of this model in the US Army Institute for Surgical Research used an inoculant of 1×10^5 Colony Forming Units (CFUs). When this was treated with irrigation and debridement at six hours post-inoculation, robust infection was detectable in all animals necessitating differentiation in the degree of contamination using bacterial quantification[32]. When I joined the laboratory, I identified the dangers of this approach: when the bacterial contamination is this stringent, the effect of treatments that produce anything less than a massive effect on bacterial levels will not be detected, leading to a Type II error. Additionally, a treatment that actually exerted a negative influence on infection i.e. increased infection rates, might not be detected. Finally, in an under-contaminated model where none of the wounds would go on to infection, a positive treatment effect might not be detected. These examples show the importance of avoiding a floor or ceiling effect.

The results of this study establish the bacterial inoculation associated with a 50% infection rate, therefore refining this model for the follow-on studies described below[33]. It also established a dichotomous 'infected or not' measure to be used alongside quantification of detectable bacteria as the principal outcome measure for these studies.

6.3.2 Model development: systemic antibiotics

In clinical practice, antibiotics may be delivered locally as well as systemically[34]. In fact this combined delivery approach may represent the area of treatment with the

most potential for development. The most common local antibiotic vehicle remains antibiotic-loaded polymethylmethacrylate (PMMA) 'bone cement'[35]. Prior to my arrival, the US Army Institute of Surgical Research had previously published work on the introduction of antibiotic PMMA beads into their open-fracture model[32].

This model-development study established that animals inoculated with 10^5 CFU of bacteria and then surgically treated at six hours post-inoculation had an infection rate of approximately 50% when treated with 5mg/kg of systemic cephazolin for three days. This enabled the model of infection to more closely mimic the regular clinical practice of concurrent treatment with systemic and local antibiotics. This then permitted the model to be used to investigate the relationship between infection and antibiotics delivered in a variety of different combinations and forms[36]. Furthermore, it allowed surgical treatment and antibiotic treatment to be initiated at different time points. This was an essential step in the model development in order for it to be used to accurately examine the relationship between the timing of treatment and infection[37].

Use of systemic antibiotics at a dosage that still results in 50% infection rates allows treatments that work synergistically with antibiotics to be tested, and avoids floor or ceiling effects in this modification of the basic model.

6.3.3 Model development: interpretation of results

The results of these model development studies had some common features. Specifically, there were groups in both studies in which either all or none of the animals were infected: when all of the animals were infected, bacterial levels were similarly high. Furthermore, in both studies there was a single group where the balance of antibiotic dose or bacterial inoculation resulted in an infection rate of approximately 50%.

I interpreted these findings as representing a balanced 'tipping point' between pro- and anti-infective factors, which I represent graphically in the idealised sigmoid curve in **Figure 6.2** below:
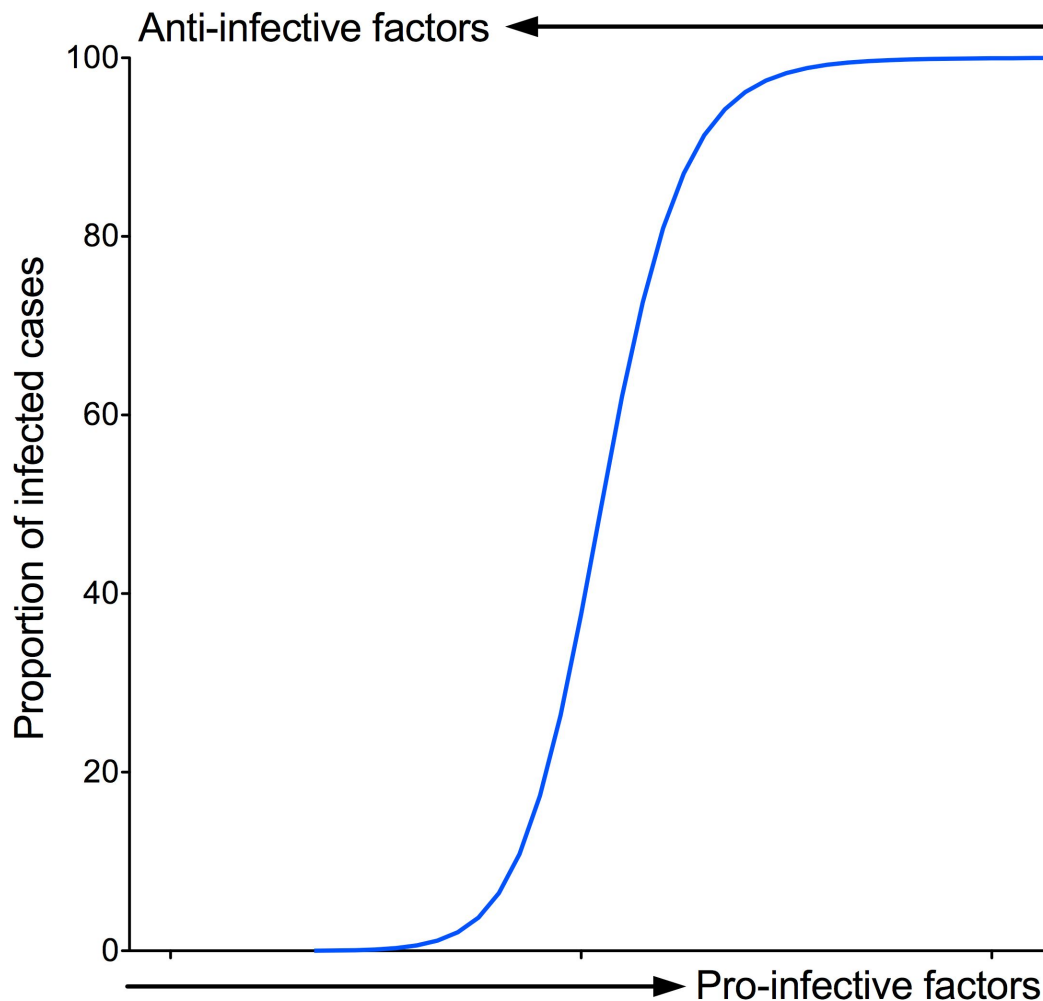
Figure 6.2: Idealised curve representing the 'tipping point', at which a host's immune system is unable to eradicate infection and a group of animals in a study group will move from 0% infection to 100% infection with a small increase in pro-infective factors or a small decrease in anti-infective factors.

The sigmoid curve as shown above in **Figure 6.2** is a common treatment effect across dose response studies. In a clinical scenario, a low level of contamination is easily managed by the casualty with an open fracture with no resultant infection; however, at a certain level of contamination (or other pre-infective factor e.g. tissue necrosis), a tipping point is reached above which bacterial multiplication overwhelms this host immunity and an infection occurs.

Clinically, we may influence this outcome with a therapy that translates this situation to the left of the curve. If it moves the contamination off the steepest part of the

curve, it may mean that even a modest treatment effect creates a sufficiently different infection rate to reach significance. In the model, defining this 'tipping point' for initial contamination or bacterial dosage allows the maximum potential for demonstrating an effect, whether positive or negative, of an intervention group.

The results of the model development studies in **Chapter Three** demonstrate the effect when the pro- and anti-infective variables are changed subtly to result in a significant effect on the proportion of animals with bacteria recoverable from their wounds. These results demonstrate this discrete 'tipping point' in the balance between pro- and anti-infective factors across multiple studies testing novel products and treatment strategies. They also demonstrate the importance of this to avoid floor or ceiling effects of tested interventions[33,38].

6.3.4 Model strengths and weaknesses

The development of this model allowed it to be adapted to examine the questions described in **Chapters Four** and **Five,** and to be published in international journals e.g. Bone and Joint Journal[38] and the Journal of Orthopaedic Trauma[33,39,40].

However, there are limits to the extent that findings from animal studies can be extrapolated to clinical practice or even trials due to obvious differences in scale, anatomy and physiology. The model used in this study mimics clinical practice but does not recreate the clinical reality of open fractures. Some key differences include the absence of significant soft tissue damage, a surgically created defect rather than fracture and a single surgical treatment. Furthermore, the immediate fixation and primary closure between injury with contamination and surgical treatment including debridement and irrigation clearly differs from clinical practice.

I have previously argued in this chapter that the presence of bacteria is a measure of contamination or colonisation and is not necessarily analogous to infection. However in the animal studies I did equate the presence of detectable bacteria as equivalent to infection. This apparent contradiction was primarily to allow an objective and un-ambiguous outcome measure for this animal study. Other studies have relied on clinical, histological or radiological definitions of infection, all of which rely on subjective assessment[41,42].

Cephazolin was used in this model because cephalosporins are the recommended antibiotic option in open fractures[20], and previous experience with cephazolin in similar animal models reduced the model development work required to design this study. I believe that since this model involves an organism known to be sensitive to the antibiotic used, the effects observed should be similar with different combinations of bacteria and antibiotic provided there is microbiological sensitivity.

The choice of a *S. aureus* species is appropriate given this species was identified in **Chapter Two** as being responsible for the majority of tibia open fractures in UK service personnel.

It is possible that the effects observed might be more pronounced with the intravenous route of administration of antibiotics typical in clinical practice rather than with oral ingestion or the subcutaneous route that was used in this model. These latter routes were chosen in this animal study to avoid the morbidity associated with repeated venepuncture in a small mammal.

Within the limits of animal welfare and practicality of a cost-effective model use, I believe my results demonstrate the appropriateness of this model in answering the research questions for which it was used.

6.3.5 The timing of surgical and antibiotic treatment of open fractures

The first study examining the timing of wound debridement was originally based on an animal study involving 21 guinea pigs performed by Friedrich in 1898[43]. The study found that animals with wounds debrided within six hours had no infection; this finding became the basis of the orthopaedic doctrine known as the 'six hour rule'[44].

Further animal based research repeated these findings: Dhingra *et al.* in 1976 demonstrated that a delay of debridement from two to four hours led to a significantly greater infection rate in soft tissue wounds[45]. Recently, Brown *et al.* used a rat model of contaminated open fracture to show that the quantity of bacteria in subsequent infection is proportional to the initial delay until surgical debridement[32].

Most of the clinical evidence on the timing of initial surgery in open fractures comes from observational cohort studies. Results in these studies were analysed with either regression analysis or by directly comparing infection rates between 'early' and 'delayed' cohorts.

Studies using this methodology have reached divergent conclusions with a majority concluding that early surgery is of little benefit. Only three studies have identified a link between delayed surgery and infection: Kindsfater and Jonassen examined all Gustillo-Anderson[46] (GA) Grade II and III open fractures of the tibia and found greater infection rates in fractures debrided more than five hours after injury compared to those debrided less than five hours after injury[47]. Kreder and Armstrong examined open tibia fractures in children and similarly found that surgery beyond six hours correlated to increased infection, but their results did not reach significance[48]. Jacob *et al*. examined US military casualties of the 1989 invasion of Panama and found a higher rate of infection in GA III fractures whose surgery was delayed until return to the US compared with those surgically treated rapidly in Panama[49].

Using a similar observational approach, Patzakis and Wilkins; Dellinger *et al*.; Bednar and Parikh, Skaggs *et a.l,* Harley *et al.,* Khatod *et al.*, Ashford *et al.,* Spencer *et al.*, Charalamous *et al.*, Al Arabi *et al.*, Reuss and Cole; and Al-Hilli and Salih, all found conversely that the risk of infection or non-union *did not* increase despite delayed debridement in patients who had received early systemic antibiotics[22,50-61].

A component of the LEAP study included a prospective observational study of 315 patients with GA III open fractures of the tibia, foot and ankle, who received standard treatment including antibiotics. In a multi-variant regression analysis they also found that delay between injury and surgical debridement was not related to infection rate[62].

More recently in 2014 Hull et al., published a clinical study which used retrospective registry data on 459 open fractures[63]. These researchers regarded delay to surgical treatment as a continuous variable and performed their analysis with a multi-variant regression model similar to the LEAP approach. Their results however were different, as they found that a significantly increased risk of infection for every hour of delay (odds ratio = 1.033: 95% confidence interval 1.01 to 1.057).

The opposing conclusions drawn by the animal studies and the majority of observational case series can be explained by their respective methodologies. Previous animal studies examining the timing of surgical debridement do not involve systemic antibiotic administration which may well make delaying surgery 'safer' with respect to infection.

For obvious ethical reasons all clinical studies to date have been observational in nature and this means that the likely tendency of clinicians to prioritise the most heavily contaminated injuries for earlier surgical debridement potentially 'balances' late and early treatment arms. Early surgery groups in these studies may well contain a greater proportion of cases performed 'out of hours' by less experienced, on-call staff and consequently may have received sub-optimal treatment relative to those patients treated on scheduled trauma operating lists by consultant surgeons.

Altemier *et al.* published the first experimental work on the effect of the timing of the antibiotics on wound infection in 1947. Their study considered the impact of timing of antibiotic administration on the survival of guinea pigs with wounds infected with *C. perfringens*. They demonstrated a significant deterioration in survival times when a regular intramuscular penicillin regime was initiated six-hours post injury compared to immediate post-injury administration[64].

Owen-Smith and Metheson showed that even with wound debridement at six hours, delaying antibiotics worsened survival in sheep with penetrating soft-tissue wounds contaminated with *C. perfringens*[65]. Reaching similar conclusions Mellor *et al.* used a porcine penetrating injury model to demonstrate that when the start of a three day course of benzylpenicillin was delayed from one to six hours post-injury, it was rendered ineffective in preventing infection[66].

Despite the early animal studies, clinical opinion remained divided on the benefit of 'prophylactic' antibiotics. Two case series of open fractures published in the 1960s did not support the use of antibiotics at all until infection was suspected[67,68]. The issue of the use of prophylactic antibiotics was settled definitively in Patzakis, Harvey and Ilver*'s* 1974 randomized control trial of antibiotics in all types of open fracture. They demonstrated that those not treated with antibiotics had significantly greater

rates of infection compared to the group treated with a cephalosporin[50]. Unfortunately, the effect of timing of antibiotic administration was not evaluated.

Two case series of military patients injured in separate conflicts provided a natural experiment on antibiotic timing: British servicemen in Borneo in 1963-65 were issued oral oxytetracylcine to take immediately if injured. Wound infection rates were lower than in casualties from the 1982 Falklands conflict with similar injuries who did not receive antibiotics until evacuated to a medical aid post. This difference was only observed in patients reaching surgery within six hours, and numbers were too small to reach significance[69,70].

In the case of the Falklands conflict, seven of the nine cases of wound infection did not have initial antibiotics administered in the first six hours after wounding: there was no infection when antibiotics were administered within three hours.

Two similarly designed observational studies have provided conflicting data on the significance of antibiotic timing. Petzakis and Wilkins reported that a delay in antibiotic administration of greater than three hours was associated with increased risk of wound infection[24]. Conversely, Al-Arabi *et al.* found that whilst a delay of greater than 24 hours until systemic antibiotic administration was associated with a higher risk of infection, delays of up to 24 hours were not[58]. However, neither study was able to control for the effect of different timing of surgery.

While there remained a lack of definitive clinical evidence on the question of the timing of systemic antibiotics, the compelling data from animal studies supporting early administration and principal clinical guidelines currently advocate this position[20,71].

The findings of this study regarding the importance of initiating *both* antibiotic and surgical treatment early is consistent with previous work describing the progress of bacteria from its colonizing planktonic form to adherence to tissue and eventually the formation of biofilm, the so-called 'race to the surface'[72]. As bacteria progress through these stages, the vulnerability to conventional treatments of debridement, irrigation and antibiotics decreases; the six hour time point appears to be significant[73].

It is simpler to influence the timing of antibiotic initiation than surgery. This study, together with the existing literature, indicates that the earlier systemic antibiotics are administered the greater the effect on infection. It is reasonable to conclude that civilian Emergency Medical Services should follow their military counterparts and regard antibiotics as a key component of pre-hospital care of the casualty with an open fracture. Indeed, in instances where antibiotic administration has been delayed several hours, this study indicates that delaying surgery for up to 24 hours is likely to result in significantly greater infection rates compared to emergent surgery.

Even when casualties receive very early antibiotics, this study supports the position that emergency surgical debridement can still reduce rates of infection. However, beyond the first few hours the advantage offered by urgent surgery appears to be negated.

6.4 Are there novel treatments that might reduce infection in open fractures?

This section of work aimed to test therapies with the potential to improve the treatment of open fractures and therefore reduce subsequent infection. The model development described in **Chapter Three** and evaluated earlier in this chapter ensured that the model was optimised to be sensitive to any effect in reducing rates of infection.

6.4.1   Irrigation with chlorhexidine solution versus saline

In this study a commonly used antiseptic, chlorhexidine, was evaluated as an irrigation fluid for reducing infection in open fractures. The study group with the lowest infection rate or quantity of bacteria in the wound was irrigated with 0.05% chlorhexidine followed by removal of antiseptic residue by rinsing with saline: this was superior than the control group but this difference did not reach significance.

I conducted a *post-hoc* power analysis to determine whether this study was under-powered. It indicated that study groups of 69 animals would have an 80% chance of demonstrating a statistically significant difference between them. I regarded this as an unacceptably large number of animals to use in an attempt to identify a very subtle difference. Being aware of the typical loss of effect as therapies are translated

from laboratory to clinical practice I did not think repeating the study with groups of animals of this size could be justified.

The concentration of 0.05% chlorhexidine was selected for the "irrigate and rinse" group as this is the concentration that has been most thoroughly studied in the literature[74-77].

Lister's practice of irrigating open fracture wounds with carbolic acid resulted in an unprecedented reduction in infections compared with the dire rates typical of the 1860s[78]. In hindsight, much of his improved results might be attributed to the application of carbolic acid by the surgeon and to his instruments, and the development of antiseptic *practice* rather than the application of antiseptic directly into the wound.

"Listerism," as it became known, was the clinical standard until Fleming's first great contribution to the management of open fractures: the recognition that the use of antiseptics in open fracture wounds actually increased bacterial loads. He ascribed this counter-intuitive observation to the toxicity of chemical antiseptics to the host immune system, which he thought was the most important factor in wound infection.

The reason that many seemingly innocuous antiseptics are toxic in traumatic wounds can be explained by using the model proposed by Jackson in 1953 when he was working at the Birmingham Accident Hospital. This model divides the wounds into the inner zone of coagulation (necrotic tissue) and the peripheral zone of hyperaemia (inflamed tissue) divided by the zone of stasis, which is potentially viable but vulnerable to secondary insult[79]. Branemark later expanded on this work and demonstrated that the tissue in this zone of stasis is very sensitive to damage by antiseptics[80].

This conceptual model also explains the rebound phenomenon of bacterial load in a wound after irrigation with solutions other than saline. This was described in a paper by Owens et al. in a goat model of contaminated complex wounding[81]. Although the antiseptics and soap solutions removed more bacteria from the wound than saline initially, the wounds irrigated with solutions other than saline had higher levels of bacteria two days after debridement and irrigation. It is believed that tissue damage

caused by the irrigation solutions created an environment within the wound that allowed the bacteria to thrive.

Because of the continuing challenge of infection in open fractures, investigators have continued to evaluate potential irrigation solutions. The benefit of inert fluids in physically rinsing bacteria from the wound should ideally be combined with a fluid with active antimicrobial properties that does not damage host tissue.

Chlorhexadine is believed to offer this combination of bactericidal effect with low cytotoxicity and has been evaluated by a small number of studies. In a soft-tissue wound study, Platt et al. found that irrigation with a 0.05% Chlorhexidine solution was superior to 1% povidine iodine, 0.1% benzalkonium, and 0.9% saline at removing bacteria in a guinea pig contaminated dorsal wound model[75].

Various studies have evaluated the potential for chlorhexidine to have a negative effect on wound healing despite its beneficial effect in reducing bacterial loads. Brennan *et al*. found that there was no difference in rat wound healing or collagen production in a rat wound model exposed to saline or 0.05% chlorhexidine, whereas an adverse effect on both of these was associated with exposure to hypochlorite antiseptic [76]. This study also found that 0.05% chlorhexidine and saline exerted a similarly negligible effect on microvascular flow to the wound[77]. However, Salami *et al*. found that rats with an uncontaminated full-thickness dorsal wound healed significantly faster when irrigated with saline than with chlorhexidine[82]. Conversely, in a recent *in vitro* study, Thomas *et al*. concluded that the negative effect on healing may only be significant when higher concentrations of chlorhexidine are used[83].

Of additional concern to orthopaedic surgeons is the chondrolytic effect of chlorhexidine that has been reported after the accidental use of it as an irrigation fluid during arthroscopy at both high[84] and low concentrations[85]. A 2007 *in vitro* study using non-arthritic human cartilage suggests that there is no significant effect on cartilage health of a one minute exposure to 0.05% chlorhexidine[86].

Possibly because of these concerns about the effect on cartilage and wound healing, only one very limited clinical trial of chlorhexidine irrigation in orthopedic trauma has been performed. This trial compared irrigation with 0.05% chlorhexidine in closed hip

fractures with no irrigation and used bacterial quantification of intraoperative wound swabs as the outcome measure[74]. This very limited study demonstrated a small reduction in recovered bacteria, but statistical analysis was not provided. Small numbers of surgeons do use chlorhexidine to irrigate open fractures in their current clinical practice[87].

Anglen compared castile soap solution with bacitracin solution for the irrigation of lower extremity open fractures in 400 patients[88]. He found an insignificantly lower infection rate in the castile soap group and a significantly higher rate of wound breakdown in the bacitracin group. This study did not include irrigation with saline as a control.

Most surgeons currently irrigate open fractures with low pressure saline[87], a practice proved to be superior to irrigation with soap solution by a large multicenter randomised trial, the Fluid Lavage of Open Wounds (FLOW) study[89].

My results indicate that chlorhexidine at concentrations of 0.05% is the most superior preparation but is neither worse nor superior to saline as a solution for irrigating open fractures.

6.4.2 *Local antibiotic gel versus antibiotic polymethylmethacrylate 'beads'*
The results of this study demonstrate that local delivery of antibiotic by the bioabsorbable gel was more effective at reducing bacteria within a contaminated rat bone defect than the commonly used antibiotic polymethylmethacrylate (PMMA) bead method.

Direct application of antimicrobial drugs into open fracture wounds is not a novel concept. In 1939, Jenson *et al.* presented their experience of treating open fractures with sulfanilamide powder poured directly into the wound prior to closure and credited introduction of this technique with a reduction in their infection rate from 30% to 5% [90]. The attraction of local delivery is that high concentrations of antibiotic can be achieved in the wound even in avascular areas, without the cost or toxic effects associated with systemically administered antibiotics.

Buchholz and colleagues in Germany first developed the use of PMMA cement blended with antibiotics in order to treat infected joint prosthesis[91,92]. This approach was then adapted to treat chronic osteomyelitis by Klemm who published his experiences in 1974[93]. However, the only prospective randomized clinical trial of antibiotic PMMA beads in osteomyelitis did not find that they were superior in isolation or in combination with systemic antibiotics compared to systemic antibiotics alone in the treatment of osteomyelitis[94].

Despite this, there is some evidence that local antibiotics are effective at combating infection. In a 1990 case-series Henry *et al.* described the prophylactic use of antibiotic PMMA beads in addition to systemic antibiotics to reduce the infection rate in open fractures[95]. There were significantly less infections in the Gustillo-Anderson[46] II and III fractures treated with antibiotic-PMMA beads in comibination with systemic antibiotics compared to those who just received systemic antibiotics. In 1995 Ostermann *et al.* published a similar comparison of 240 patients with open limb fractures who received intravenous (IV) antibiotics and 845 patients who received both IV antibiotics and antibiotic-PMMA beads at the fracture site. He found an infection rate of 12% in the IV only group compared to 3.7% in the IV/antibiotic-PMMA bead group (p=0.001)[96]. It should be noted that neither of these studies randomised the treatment groups.

Moehring *et al.* published the results of a prospective randomised trial designed to compare antibiotic PMMA beads with IV antibiotics in the prevention of infection in open fractures. This study described a trend toward superiority of antibiotic beads but it did not reach significance in the 67 patients studied[97].

Interestingly animal studies have also failed to convincingly establish a significant benefit of augmenting systemic antibiotics with antibiotic-PMMA beads in musculoskeletal infection[98,99].

Despite their widespread use, there is recognition that PMMA beads do not represent the ideal delivery vehicle for local antibiotics. They are bulky and not bioabsorbable, which potentially complicates wound closure and necessitates subsequent removal[35,100]. This prevents their use during definitive closure of a wound.

In complex high-energy wounds, there is concern that the antibiotic eluting from the discrete depots of a PMMA bead will not diffuse sufficiently to reach all the recesses of a wound. This effect is potentially exacerbated by the concurrent use of negative pressure wound therapy[101], which is well supported as a beneficial technique to reduce infection in open fractures[102].

There is also concern that self-manufactured PMMA beads have a varied and unpredictable antibiotic elution rate[103]. The commercially available antibiotic-PMMA beads Septopal® (Biomet, Bridgend, UK) contains gentamicin alone. However, when antibiotic-PMMA beads are manufactured de novo in the operating room, they are frequently formulated with both an aminoglycoside and vancomycin in order to ensure coverage of both gram positive and negative organisms[35]. This study mimicked this clinical practice and used both an aminoglycoside and vancomycin.

Other local antibiotic delivery vehicles have been used: gentamicin-impregnated collagen sponges have been tested as a bioabsorbable vehicle. However, in a recent non-orthopaedic randomised clinical trial of CollaRx (Innocoll, Gallowston, Ireland), the sponge group had a higher rate of surgical site infection than the control group (30% versus 20%, p=0.01). It was speculated that the antibiotics eluted faster than the sponge degraded, leaving foreign material in the wounds without antibiotics[104].

Recent development work has focused on other absorbable antibiotic vehicles, including a range of synthetic bone grafts impregnated with antibiotics[100]. Osteoset 'T' (Wright Medical, Arlington, TN, USA) are calcium sulphate pellets with 10% Tobramycin by weight. These have been used clinically to treat osteomyelitis, and have been found to be as effective at treating osteomyelitis as antibiotic delivery via PMMA beads, with a requirement for less surgery[105],[106]. Other investigators have examined gel-based vehicles for delivering antibiotics in pre-clinical *in vitro* models of orthopaedic infection[107].

The ideal release profile for a local antibiotic delivery vehicle used to prevent infection in open fractures is not known. It is speculated that eluted local antibiotics should quickly rise above the Minimum Inhibitory Concentration (MIC) of relevant bacteria, be sustained above this level for several days, then rapidly drop to avoid bacteria being exposed to sub-inhibitory antibiotic concentration, thus promoting

resistance[108]. It is entirely possible that the ideal release profile of local antibiotic vehicles used for treating established osteomyelitis will be different and may require a more sustained release.

It is possible that the observed differences between groups in my study is due to the alternative aminoglycocides used. It was decided to use tobramycin in the bead preparation as this study was testing the preparation against the current clinical standard. The initial work in Europe on delivery of antibiotics via PMMA beads in osteomyelitis involved gentamicin[93]. However, when this work was translated to infection prevention in open fractures in the US, tobramycin rather than gentamicin was used[109] as until recently, this was the only aminogylcoside available in the US in powdered form[35,110]. Since this work was conducted in a US laboratory in collaboration with the US Department of Defence, I wanted to ensure my work was maximally relevant to my host's clinical practice. The difference between the preparations was accepted in this study as it represented the clinical standard, and because the efficacy of gentamicin and tobramycin against gram positive bacteria in general is very similar[111].

A group was treated with both gel and beads as it was speculated that since antibiotic-PMMA cement is used by surgeons as a spacer to maintain soft tissues and eliminate dead space, there might be future utility in evaluating the compatibility of simultaneous antibiotic delivery by both gel and cement. The results from this group suggest that this is possible, but that it confers no additive antimicrobial effect.

The differences in efficacy between the gel and beads is marked and may be due to the active amount of antibiotics released from each vehicle. A study that examined beads made using the same technique as this study found that only 20% of the total antibiotic was released within 60 days, but half of this was released in the first day[112]. Conversely, a bioabsorbable vehicle, such as the gel used in the study, will release 100% of the carried antibiotics as it is degraded.

The maximum antibiotic content of the beads used in these studies is limited by the dilution of the MMA co-polymer powder negatively affecting cement integrity[113]. In practice, this is a further advantage on the gel vehicle in that it enables greater quantities of antibiotic to be delivered, as shown in this study, where there was much

higher dosages of antibiotics in the animals treated with the gel. It is also possible that the superior performance of the gel is due to the improved distribution of antibiotic throughout the wound, rather than in discrete 'pockets' around the beads. This is due to the need for the active drug to elute and diffuse throughout the wound bed, compared to the gel's immediate drug delivery to the entire wound contact area.

I think that these results prove the concept of a bioabsorbable gel as a local antibiotic delivery vehicle capable of preventing infection in open fractures. Further evaluation is warranted to determine potential efficacy.

6.4.3 *Local Bismuth thiol gels in combination with systemic antibiotics*

Bacteria in biofilm can persist despite exposure to concentrations of antibiotics 100 to 1,000 times greater than that which would inhibit planktonic bacteria[114]. For example, ampicillin demonstrated a minimum inhibitory concentration against *Klebsiella pneumoniae* of 2ug/ml in the standard planktonic conditions for this test. However, when grown as a biofilm, treatment of the identical strain with 5000ug/ml of ampicillin for four hours had very little effect on bacterial levels (much less than 1 log reduction). Conversely, the planktonically grown bacteria were completely eradicated at this dose[115].

This increased resistance of biofilms to antibiotics has also been observed *in vivo,* where experiments have demonstrated robust survival characteristics of adherent bacteria in wounds. Colonisation of experimental wounds challenged with clinical burn-isolated *Pseudomonas aeruginosa* resulted in populations of wound-adherent bacteria 500-fold more resistant to antibiotics than the non-adherent bacteria of the same strain in the same wound[116].

Unfortunately these biofilms can form very quickly[117]. *In vitro* experiments with clinical strains of *Pseudomonas aeruginosa* from human wounds have been shown to rapidly form adherent biofilms. These bacteria demonstrated adherence to surfaces within three hours, formation of visually recognizable biofilms within five hours, and mature biofilms within ten hours[118].

The increased understanding of biofilms has explained three previously observed discrepancies related to infection and antibiotics. Firstly, there is a disparity between preclinical and clinical antibiotic efficacy. Secondly, if tissue levels of systemically administered antibiotics do not reach a critical threshold within the first four hours following injury, the ability to prevent bacterial adherence to the wound is impaired[119]. Thirdly, microbiological sampling techniques that rely on planktonic bacteria (e.g. swabs and joint aspirations) significantly under-detect the presence of bacteria.

With this improved understanding of the true *in vivo* behavior of bacteria, novel strategies for eradication can be developed. One such approach is the combination of traditional systemic antibiotics (at a typical dosage sufficient to inhibit planktonic bacteria) with a novel local treatment to inhibit bacterial adherence and formation of microbial biofilms in wounds.

In a recent study, MB-8-2 (bismuth-2,3-butanedithiol/Bismuth-2-mercaptopyridine N-oxide) achieved a >4.9 log reduction in methicillin-resistant *Staphylococcus aureus* (MRSA) biofilm and a 6.2 log reduction in *Pseudomonas aeruginosa* biofilms when tested in a drip-flow biofilm system[120].

Other Bismuth Thiols (BTs) have also been shown to inhibit and/or disrupt biofilm. Against staphylococci, bismuth dimercaptotoluene (BisTOL) reduces slime production by >90% at 1.25µM (0.25µg/ml), without appreciably inhibiting bacterial growth[121]. Biofilms and exopolysaccharides produced by Pseudomonas species are also inhibited at sub-growth concentrations of BT[122,123]. Indeed, several major virulence factors in *P. aeruginosa* were mitigated by low concentrations of BisEDT, including resistance to serum bactericidal activity, resistance to phagocytosis, and adherence to collagen matrix[122]. By dismantling the protective biofilm matrix, BTs, including MB-8-2, may augment therapy both by enhancing immune defenses and by promoting antibiotic activity though inhibition and dismantling of biofilm.

The study for this thesis screened three BT formulations known to inhibit or eradicate bacterial biofilms for *in vivo* effectiveness when used with systemic antibiotics. One BT in particular (MB-8-2) was selected for further characterisation based on my judgement of it exhibiting the most promising balance of toxicity and anti-bacterial

effect. An optimum dose of this BT formulation was identified, and a marked enhancement of the effect of systemic antibiotics was demonstrated.

I think that these results echo the findings of other investigators who have examined the effect of local antiseptics on wound infection[33,124]. Specifically, there is a balance between the concentration required to be toxic to bacteria, and the concentration that becomes toxic to wound tissue and therefore impairs the host's ability to respond to infection. This study defined this "therapeutic window" for this model.

These findings indicate that local BTs can increase the effectiveness of systemic antibiotics on bacteria in an open fracture wound. Preventing biofilm formation, disrupting existing biofilm, and sensitising bacteria to attack by antimicrobial agents and immune defences may mean that BTs are a potential technique for fighting biofilm-related infections, including wound infections. The use of BTs in combination with other agents (including those that have lost their effectiveness) has the potential to offer a therapy against more established musculoskeletal infection.

6.5 Conclusion

This thesis describes a range of studies conducted in order to answer a series of questions. Initially broad concepts of survival and how this is measured were examined, before the work moved on to focus on a specific clinical problem and the development of potential treatments for infection associated with open fractures.

The initial work confirmed the belief that survival had improved over the course of the conflicts in Iraq and Afghanistan. As part of these efforts, the methodology for measuring survival after combat injury was refined. Registry data was used to quantify how common open fractures were, and the most frequently occurring of these, open tibia fractures, were examined in more detail. The infection rate for these injuries was established at 23%, with 60% of these infections due to *S. aureus*.

A rodent femur fracture inoculated with *S. aureus* was used to model contaminated open fractures. This model was refined to incorporate systemic antibiotics and a bacterial inoculation that resulted in a 50% infection rate. Using this refined model,

the relationship between the timing of antibiotic and surgical treatment was characterised and the importance of early antibiotic administration established.

Wound irrigation with the antiseptic solution chlorhexidine was evaluated, and was found not to be superior to saline. Antibiotic gel was found to be a more effective vehicle for delivery of local antibiotics than polymethylmethacrylate beads. Finally, a novel anti-bacterial biofilm treatment in the form of topical Bismuth-Thiol preparation was shown to potentiate systemic antibiotics. These last two studies lay the foundation for translational and clinical studies of local antibiotics and Bismuth Thiols.

This thesis has demonstrated that there is still much work to be done to improve the reconstruction and rehabilitation of those recovering from combat injury. However, this is a problem resulting from the significant improvements in survival and therefore in it of itself, demonstrates the potential for improving care.

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