MICROBIAL GROWTH IN A MIXTURE OF HYPERBARIC BUPIVACAINE AND FENTANYL PREPARED IN A MULTI-DOSE SYRINGE IN THE OPERATING THEATRE ENVIRONMENT

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I, Dr Gwen Morgan, declare that this Research Report is my own work. It is being submitted for the Degree of Master of Medicine in the branch of Anaesthetics in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Signed on the 20th day of July 2010

PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS STUDY

Publications

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ABSTRACT

Introduction

A protocol has been devised in which a 20ml mixture of hyperbaric bupivacaine and fentanyl is prepared in a multi-dose syringe, from which aliquots are withdrawn into individual sterile syringes for use in spinal anaesthesia. The risk of microbial contamination of these multi-dose syringes is unknown and this study was designed to assess such risk.

Methodology

In this pilot study, each syringe was prepared using non-aseptic technique to contain a mixture comprising Fentanyl 10 $\mu g.ml^{-1}$, Bupivacaine $4mg.ml^{-1}$and Dextrose $64mg.ml^{-1}$, with a total volume of 20ml. Syringes were then allocated to pairs. Aliquots were withdrawn hourly from one syringe of each pair for a twelve-hour study period, whilst the other syringe was sampled only at the beginning and end of the same period. All aliquots were withdrawn using standard aseptic technique in an operating theatre environment. For each syringe pair, both samples from the control syringe and four of the samples from the multi-dose syringe were submitted for microbiological culture.

Results

Of the 120 samples taken, one sample was excluded. Of the remaining 119 samples submitted for microbiological investigation, only one yielded growth. This sample had been taken from a multi-dose syringe at the beginning of the study period. Subsequent samples withdrawn from the same syringe were found to be sterile. The

organism which had been cultured from this sample was *Staphylococcus aureus* (*S. aureus*).

Conclusion

It is possible that the culture medium which yielded the microbial growth was contaminated, which would explain why subsequent samples from the same syringe were sterile. Alternatively, bupivacaine is known to be strongly antimicrobial against some pathogens and it is conceivable that there may have been initial contamination of the syringe by *S. aureus*, which was inhibited by the bupivacaine to produce subsequent sterile samples. Whilst this may suggest that the use of multi-dose syringes for spinal anaesthesia could be safe, in light of the inconclusive result, further investigation is warranted.

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NOMENCLATURE

Aerobic organisms: organisms which require oxygen in order to survive

Anaerobic organisms: organisms which require a lack of oxygen in order to survive

Arachnoiditis: inflammation of one of the meninges called the arachnoid mata

Cephalad: towards the head

Dermatome: an area of the body which is supplied by a single spinal nerve root

Dura (also dura mata): outermost covering of the brain and spinal cord

Epidural abscess: collection of pus in the space outside of the meninges caused by infection

Hypotension: low blood pressure

Lumbar lordosis: the curvature of the spinal column convex anterior (forwards) at the level of the lower back

Meningitis: inflammation of the meninges, which are the layers of tissue covering the brain and spinal cord

Minimum bactericidal concentration (MBC): minimum concentration of a drug or substance which will kill a specific type of bacterium

Minimum inhibitory concentration (MIC): minimum concentration of a drug or substance which will prevent growth of a specific type of bacterium

Opioids: group of synthetic drugs related to morphine

Pathogen: microorganism which causes disease

Supine: the position of lying on the back facing upwards

Thoracic kyphosis: the curvature of the spinal column convex posterior (backwards) at the level of the chest

CHAPTER 1: INTRODUCTION

This Introduction will first present the background to the research question at hand and then will give an overview of the subsequent research report.

1.1 Background

1.1.1 Neuraxial blockade and obstetric anaesthesia

The term "neuraxial drug administration" describes techniques that deliver drugs into the vicinity of the spinal cord (1) with "neuraxial blockade" referring to the introduction of local anaesthetics and adjuvant drugs in order to create blockade of transmission through motor and sensory nerves.

In the 1960s, maternal mortality in association with anaesthesia was estimated to be around 36 per 100 000 caesarean sections. The majority of these deaths resulted from difficulty in airway management. In more recent years, the maternal death rate has decreased substantially to 1 death per 100 000 caesarean sections, correlating with the increasing use of neuraxial blockade for caesarean section. The use of neuraxial blockade allows the patient to maintain control of her own airway, decreases the risk of loss of airway control and thus decreases the maternal mortality associated with anaesthesia (2). For this reason, evidence increasingly suggests that neuraxial blockade is safer than general anaesthesia for pregnant women. The most recent recommendations by the American Society of Anesthesiologists state that

"neuraxial techniques are preferred to general anesthesia for most caesarean deliveries"(1). It is the author's experience that the commonest type of neuraxial blockade used for caesarean section in South African hospitals is spinal anaesthesia, where local anaesthetics and adjuvant drugs are introduced into the intrathecal space. Here, they act by blocking sodium channels and preventing electrical conduction through the spinal nerve roots and spinal cord (3).

1.1.2 Agents used to produce spinal anaesthesia

Various combinations of medications have been used to achieve spinal anaesthesia. The most widely used combination at Chris Hani Baragwanath Hospital (CHBH) is hyperbaric bupivacaine and an opioid, fentanyl. Bupivacaine is an amino-amide local anaesthetic with relatively slow onset and prolonged duration of action (4). When bupivacaine is mixed with dextrose, the resulting combination is denser than the cerebral-spinal fluid into which is it injected, hence making it settle according to gravity. The use of this "hyperbaric" bupivacaine allows for the local anaesthetic to sink to the most dependent part of the thoracic kyphosis in the supine patient, which provides a T6 dermatomal level of anaesthesia (Figure 1.1). This level is sufficiently high to allow for some abdominal surgery, such as caesarean section. Fentanyl is a potent, lipophilic opioid (1). When added to the mixture for spinal anaesthesia, fentanyl decreases dose requirement of bupivacaine, decreases the incidence of hypotension associated with spinal anaesthesia, and prolongs the duration of postoperative analgesia, as compared with bupivacaine alone (5).

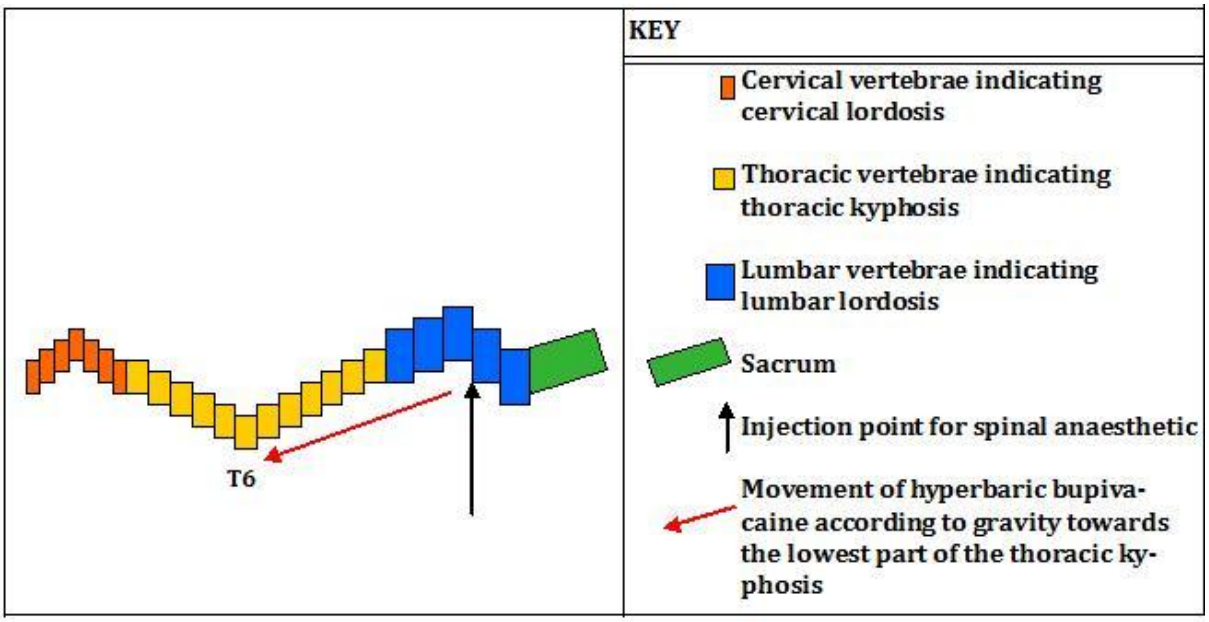

Figure 1.1: Schematic diagram indicating cephallad spread of hyperbaric bupivacaine in the supine patient

1.1.3 Protocol for preparing drug mixture in a multi-dose syringe

There are various methods of preparing this mixture of local anaesthetic, dextrose and opioid in the proportions required for spinal anaesthesia. A protocol has been devised at CHBH in which a 20ml syringe is used to prepare an appropriate mixture of proprietary hyperbaric bupivacaine and fentanyl (Appendix A). Small aliquots of this mixture are then drawn out of this 20ml syringe into smaller, sterile syringes, each of which is used to produce spinal anaesthesia in a different patient.

1.1.4 Current opinion on the use of multi-dose syringes

Guidelines published by the American Society of Anaesthesiologists (ASA) regarding preparation of medications for parenteral administration recommend that single dose items be used for one patient only and that medications be drawn up into a

syringe immediately before administration (6). The position of the South African Society of Anaesthesiologists (SASA) is to discourage ampoule-sharing between patients as a method to limit costs (7). However, these and other similar guidelines have largely been established based upon assumption of good practice, rather than broad-based evidence. Further, the practice of using multi-dose vials of local anaesthetic for neuraxial blockade has been noted in several developing countries owing to limited resource availability (8), despite international recommendations to the contrary.

1.1.5 Concern over microbial contamination of multi-dose syringes

Whilst it appears that the use of such multi-dose syringes is common, there are concerns over the possibility of microbial contamination of the drug mixture during the period between preparation and administration. This is especially relevant if this period is prolonged. Of particular concern are several points:

1. Pathogens which are inoculated directly into the intrathecal space bypass various immune barriers which would otherwise need to be evaded before the pathogen could cause meningitis. These barriers are the mucosal epithelium, intravascular cellular and humoral immunity and the blood-brain barrier (9). Since the cerebrospinal fluid (CSF) provides an excellent culture medium for certain pathogens and with a paucity of immune-mediating cells in the intrathecal space, organisms deposited directly into the CSF multiply rapidly and produce a fulminant meningitis within hours of inoculation (9). Therefore, *any* microbial contamination of a drug intended for intrathecal administration is potentially clinically significant.

2. The drug mixture described above necessarily contains dextrose in order to make it hyperbaric. There have been contradictory findings as to whether dextrose-containing solutions of varying concentrations support or inhibit bacterial growth (10). It has been known since as early as 1909, and more recently re-examined, that many local anaesthetics, in fact, exert an *antimicrobial* effect (11). This knowledge has been used by some clinicians to justify the safety of using a multi-dose syringe technique.
3. Drug preparations used for intrathecal injection must be preservative-free in order to avoid the chemical arachnoiditis which many preservatives have been shown to cause (12). This raises further concern for the possibility of microbial contamination of these drugs if left drawn up in syringes in the operating theatre environment.
4. By necessity, the use of a multi-dose syringe demands that needles, albeit sterile ones, make multiple entrances into the multi-dose syringe in order to withdraw aliquots for clinical use. It is the author's concern that these multiple entries and the turbulence which they cause in the drug mixture in the multi-dose syringe may exacerbate the risk of microbial contamination of the contents of the syringe.

1.1.6 Implications of potential microbial contamination

The injection of anaesthetic agent which has been contaminated by pathogens can lead to meningitis or abscess formation. Whilst the incidence of these appears to be low (9,13), long term neurological sequelae are potentially catastrophic. Therefore, every reasonable precaution should be taken to prevent these complications. Studies

which have been conducted on drugs intended for epidural administration have suggested that microbial growth is inhibited by local anaesthetics. However, only one of these studies examined mixtures containing dextrose (14), and none were conducted in the clinically-applicable context of the operating theatre environment.

1.2 Definition of research question

1.2.1 Problem Statement

Multi-dose syringes containing drugs intended for intrathecal injection may be subject to microbial contamination, which may be exacerbated by frequent sampling from the syringe over the course of time. The consequences of microbial contamination may be considerable, and therefore there is a need to determine the risk of this contamination.

1.2.2 Aim

A pilot study was designed to explore the risk of microbial contamination of syringes containing a mixture of hyperbaric bupivacaine and fentanyl in the operating theatre environment. The aim of this study was to establish whether microbial contamination of drugs prepared in a 20ml syringe according to the protocol occurs.

1.2.3 Objectives

1.2.3.1 Primary objectives

a) To determine whether there is microbiological growth in the drug mixture of hyperbaric bupivacaine and fentanyl prepared in multi-dose syringes from which aliquots are withdrawn hourly.
b) To compare the microbiological growth in samples from the multi-dose syringes with any growth in the mixture prepared in a control syringe from which no aliquots are withdrawn during the same 12 hour period.

1.2.3.2 Secondary objectives

a) To determine whether microbial growth in the syringes is influenced by ambient theatre temperature.
b) To determine whether number of cases done in the theatre during the study period influences microbial contamination of the syringes.

1.2.4 Methodology

A prospective longitudinal pilot study was designed to give an indication of whether the use of a multi-dose syringe poses a clear risk to the patient. The methodology of this initial study was kept as simple as possible so as to avoid confounding factors. The intention was to use the data collected during the pilot study to inform a future

definitive study if the pilot did not indicate that the use of the multi-dose syringe protocol was clearly hazardous.

1.3 Summary of introduction

A protocol has been devised in which a 20ml mixture of hyperbaric bupivacaine and fentanyl is prepared in a multi-dose syringe, from which aliquots are withdrawn into individual sterile syringes for use in spinal anaesthesia. The risk of microbial contamination of these multi-dose syringes is unknown and this study was designed to assess such risk.

First, a systematic literature review was performed in order to gain an understanding of current knowledge (Chapter 2). Then a pilot study was designed in order to establish whether contamination occurs as a rule (Chapter 3). Results are presented (Chapter 4) and discussed (Chapter 5) in the latter part of the report. Conclusions and insights for future studies are presented in Chapter 6.

CHAPTER 2: LITERATURE REVIEW

The current study examines the risk of occurrence of microbial contamination of multi-dose syringes containing a mixture of hyperbaric bupivacaine and fentanyl in the operating theatre environment. Thorough literature review revealed that this specific subject has never before been investigated. However, valuable work has been done in related areas which may offer some insight into various contributory factors. These various factors are to be discussed in the following Literature Review.

2.1 Methodology for Literature Review

2.1.1 Design of literature review

This literature review was designed to examine various aspects of the topic. First, the literature was reviewed to establish whether microbial contamination of a solution intended for intrathecal use may be of clinical concern. Then the literature was reviewed on the subjects of microbial contamination of:

1) bupivacaine
2) dextrose
3) fentanyl
4) multi-dose containers

2.1.2 Detailed methodology used for literature review

The electronic database, Pubmed (*http://www.ncbi.nlm.nih.gov/pmc*), was employed for on-line literature searches. Pubmed is a free digital archive of biomedical and life sciences journal literature administered by the United States' National Institutes of Health. The search parameters listed in Table 2.1 were used to conduct the searches on each subject. English language journal articles from all dates were considered, and titles were scanned in order to compile a list of potential candidate studies. Full text articles were then obtained for all of these candidate studies and assessed for applicability. All references made in these candidate studies were also scanned for further potentially applicable studies.

Table 2.1: Search parameters used for literature reviews

Infectious Complications **AND** Spinal Anaesthesia
[Bupivacaine **OR** Local Anaesthetic **OR** Local Anesthetic] **AND** [Microbial Contamination **OR** Antimicrobial]
[Intravenous **OR** Dextrose] **AND** Microbial Contamination
Opioids **AND** Microbial Contamination
Multi-dose **AND** Microbial Contamination

2.2 Implications of microbiological contamination of agents intended for intrathecal administration

2.2.1 Definition of the problem

Introduction of microbes into the intrathecal space, as would occur if a contaminated agent was used for spinal anaesthesia, could potentially result in post dural puncture

infections, which usually take the form of either meningitis (PDPM) or epidural abscess (15). It appears, however, that iatrogenic meningitis associated with neuraxial blockade occurs almost exclusively after spinal anaesthesia. Epidural abscesses are more common following epidural catheter insertion, although do very rarely occur after spinal anaesthetic (16).

2.2.2 Incidence

Whilst most authors agree that the occurrence of infective complications following dural puncture is very rare (13,15,17), the actual incidence remains unknown (9,18). Previous work has estimated the incidence of post dural puncture meningitis at between 0 – 7.2/1 000 000 (15-17,19-22), whilst the incidence of epidural abscess following spinal anaesthesia is probably far lower (16). The range of incidences estimated almost certainly reflects different patient populations, surveillance methods and sample sizes. Under-reporting is probably also a major factor contributing to the difficulty in establishing the true incidence of these complications (15). A large retrospective study carried out in Sweden noted that most complications of neuraxial blockade occurred in the orthopaedic population, with very few complications occurring in the obstetric population (16). Presumably this population is generally young and healthy with intact immunity and are more resistant to infections in general.

2.2.3 The pathogens involved

A review of 179 cases of PDPM revealed that 49% of these to have been caused by bacteria from the Viridans group of Streptococci (9). This group of Gram-positive bacteria generally have a low virulence and are normal commensals of the upper respiratory tract, the female genital tract, the gastrointestinal tract and are most abundant in the oral cavity (17). Meningitis caused by the Viridians group is unusual when not associated with dural puncture (17). Other pathogens which have caused post dural puncture meningitis include Gram-negative bacteria and Staphylococci (20). Epidural abscesses which are either sporadic or associated with neuraxial blockade are primarily associated with *Staphylococcus aureus* (13).

2.2.4 Pathogenesis

Ordinarily, for a pathogen to be able to cause meningitis, it needs to invade the host epithelium, gain access to the intravascular space, cross the blood-brain barrier and then survive in the cerebrospinal fluid (CSF) (9). However, with dural puncture, pathogens may gain direct access to the CSF, thereby avoiding major host defences (23).

During neuraxial blockade, microbes may be either intrinsic or extrinsic in origin (18) and may gain access to the cerebrospinal fluid in one of four ways:

1. By haemotogenous spread from distant infectious foci
2. By translocation of the patient's skin flora along the puncture tract or catheter

3. By inoculation of pathogens spread by droplet infection from the respiratory tract of the person performing the procedure or
4. By contamination of anaesthetic agents (21) or equipment (24) through failure of infection control methods (25).

Whilst the first is exceedingly rare (25), it would appear from the common pathogens involved that most cases of post-dural puncture meningitis result from droplet infection originating from the respiratory tract of the person performing the procedure (15,16,18,20) or from needle contamination following incomplete sterilisation of the skin (9,20).

Cerebrospinal fluid provides an excellent culture medium for mouth and skin commensals. Once these pathogens gain access to the CSF, they may multiply rapidly (9). Because of this, the introduction of bacteria directly into the CSF probably causes meningitis in any patient, however healthy (19). Within hours a fulminant meningitis may result (9), the complications of which may be severe and are discussed below. The literature reviewed made no estimate of bacterial load required to initiate an infection. However, in light of the preceding points, it is expected that a very small inoculum may lead to clinical infection.

In the case of epidural abscesses, it is assumed that direct trauma is cause by insertion of the spinal needle, particularly when technical difficulty is encountered in performing the dural puncture. The resulting haematoma then provides an excellent nidus for growth of any organisms which may reach the haematoma by the same routes mentioned previously (13).

2.2.5 Risk factors

2.2.5.1 Procedural risk factors

The intrathecal space is breached during various diagnostic and therapeutic procedures. Of 179 cases of post-dural puncture meningitis reviewed by Baer, only 17 of them were associated with diagnostic lumbar puncture, with the rest being associated with introduction of agents into the intrathecal space for various reasons (9). In fact, most cases of dural puncture-associated infections have been reported in association with administration of spinal anaesthesia (18,26). It would therefore appear that the introduction of foreign substance into the intrathecal space increases the risk of infection. However, it is yet to be established whether intrathecal injection of local anaesthetics, as opposed to injection of opioids or clonidine alone, may be protective owing to the antimicrobial properties of these agents (21).

2.2.5.2 Breach in aseptic technique

Although epidemiological studies are lacking, breach of strict aseptic technique during the procedure may play a role (17). Currently, there is little consensus as to what constitutes strict aseptic technique for neuraxial blockade, however most authors conclude that the factors listed in Table 2.2 are important (27). The use of opioids drawn up from ampoules which have been opened in a non-sterile fashion (22) and the potential contamination of multi-dose vials (9,21) have been raised as possible points of concern.

Table 2.2: Important components of aseptic technique (after Hebl) (27)

Major components
Removing watches and jewellery
Pre-procedural hand washing with antiseptic solution
Protective barriers
Surgical mask
Surgical hat
Sterile gloves
Appropriate selection and application of skin disinfectant
Proper sterile draping techniques
Maintenance of a sterile field
Appropriate dressing techniques
Minor components
Proper use of bacterial filters during long term catheterisation
Prevention of catheter, hub and site violations

2.2.5.3 Patient risk factors

Risk factors for epidural abscess have been listed as compromised immunity (including that caused by infection with the Human Immunodeficiency Virus), disruption of the spinal column (for example, by trauma) and distant sources of infection (13). However, in the retrospective study done in Sweden, of the 29 patients with PDPM, only two were found to have co-morbidity (one had diabetes mellitus and the other was on corticosteroid replacement therapy for Addison's disease) (16).

2.2.6 Sequelae of post-dural puncture infections

The potentially devastating sequelae of post-dural puncture infections include paralysis and even death (15,27). The literature is not clear on the incidence of specific sequelae of post dural puncture infections. However, the Swedish study found that 21% of patients presenting with meningitis and 57% of those presenting with epidural abscesses associated with neuraxial blockade had undefined "permanent neurological damage" (16). Halaby notes that meningitis caused by low virulence organisms such as the Viridians group Streptococci usually has a good prognosis. However, he and his colleagues present a case where a young patient with no known immune-compromise died as a result of iatrogenic meningitis caused by *Streptococcus salivarius*, which is one of the Viridans Group of Streptococci (17).

Baer reports that survivors of community acquired meningitis suffer from cranial nerve palsies in 19%, hemiparesis in 4%, quadraparesis in 1% and aphasia in 2%, with other long term sequelae known to be seizures and impaired cognition (9). Whilst the pathogenesis and pathogens involved in community acquired meningitis are different from PDPM, a warning is still clear that the long term sequelae from PDPM may be catastrophic.

Grewal et al report that epidural abscesses of all causes carry a mortality of up to 16%, with death being due to systemic sepsis or prolonged immobility. They caution that there are many other neurological sequelae which depend upon site and severity of the abscess (13).

2.2.7 Minimizing the risk

In view of the potentially devastating discussed above, it is essential that all possible precautions be taken to prevent the initial infection. Most authors advocate the use of strict aseptic technique when performing dural puncture, including the use of face masks (9,13,15-24,26,27). Of particular interest to the current study is that several authors have specifically cautioned against the use of multi-dose vials (9,21,28). Instead, they recommend the use of single-dose ampoules contained in individual sterile packs which avoid contamination upon opening (22). The Deutshe Gesellshaft fur Anästhesiologie und Intensivmedizin (DGAI) recommends that single dose vials be used instead of multi-dose vials wherever possible, particularly when being used for multiple patients (29). The America Society for Regional Anesthesia and Pain Medicine have published guidelines for hand washing, mask wearing and skin preparation (15). The Centre for Disease Control (CDC) has published guidelines for infection control measures in central venous catheter placement, however there is no equivalent at this time for neuraxial blockade. There is a need for various risk factors to be studied to allow for appropriate, evidence-based guidelines to be established (21). It is hoped that the current study will contribute to this knowledge.

2.3 Antimicrobial effects of bupivacaine

2.3.1 Basic pharmacology

As with other local anaesthetics, bupivacaine exerts its action by inhibiting the passage of sodium ions through ion-selective sodium channels in nerve membranes

(4). This blockage of sodium ion channels slows the rate of depolarisation of the nerve membrane, which prevents an action potential from being propagated along it. Bupivacaine is an amide local anaesthetic with a slow onset (5 – 8 minutes) and long duration of action (1,5 – 2 hours) (5). This long duration and a differential moto-sensory blockade make bupivacaine a popular choice for use in obstetric anaesthesia (30).

2.3.2 Past investigations into the antimicrobial activity of bupivacaine

As early as 1909, Jonnesco suggested that "The substances [local anaesthetic agents] need not be sterilized since they are themselves antiseptic"(11). Unfortunately, this statement was not further substantiated in the original publication. From the 1950s, further research was prompted by a growing concern that, in some clinical situations, the antimicrobial properties of local anaesthetics may be disadvantageous (31,32). These situations would include those when local anaesthetics administered at the time of a procedure for collecting microbiological specimens may decrease microbial yield from specimens obtained, such as in diagnostic broncholavage. Since then, interest in the antimicrobial activities of local anaesthetics have spread to clinical disciplines as diverse as dentistry (33), anaesthetics (34-42), otorhinolaryngology (43), pulmonology (44) and ophthalmology (45,46).

2.3.3 Antibacterial actions of bupivacaine

Mechanisms of bactericidal activity of local anaesthetics have been proposed to include changes to cell membrane fluidity and expansion caused by disturbance in

membrane molecular organisation. This leads to fracturing and solubilisation of the bacterial cell membranes. Changes also occur at the nuclear level, with alterations in the DNA and ribosomal configuration noted. The nett result of local anaesthetics on bacteria include inhibition of growth, reduction in numbers of viable cells, lysis of protoplasts, permeability changes, ultra structural alterations and inhibition of the activity of membrane-bound enzymes (47).

2.3.4 Findings of previous investigations into the antibacterial activity of bupivacaine

Several studies have examined the antimicrobial properties of bupivacaine (Table 2.3). All have suggested that this activity is concentration-dependent (14,33,34,39,48-54), however there is conflicting evidence as to the specifics of bupivacaine's antimicrobial activity. Some suggest that bupivacaine has good action against a variety of pathogens at clinically significant concentrations (14,50,51,54). However, several studies indicated that, whilst bupivacaine inhibits some organisms at clinically-significant concentrations, others appear to be resistant to its antimicrobial effects. Some authors suggest that bupivacaine 0.5% is ineffective against *Pseudomonas aeruginosa* (*P. aeruginosa*) (33,39,52,53), an observation which has been refuted by others (34,49,51,54). *Candida albicans* (*C. albicans*) was also found to be largely resistant to bupivacaine in several studies (33,34,52) and one study noted Klebsiella species to be less susceptible to the antimicrobial effects of bupivacaine than other organisms (49). Another study found bupivacaine to have no effect on the growth of *Enterococcus faecalis* (*E. faecalis*) (48), however the

Table 2.3: Summary of investigations into the antimicrobial properties of bupivacaine (*continues over*)

AUTHOR	CONCENTRATIONS OF BUPIVACAINE	MICROBES STUDIES	INCUBATION TEMP	ADDITIVES TO BUPIVACAINE	STUDY SETTING	CONCLUSIONS
Coghlan (2009) (39)	0.06 – 1%	*E. coli, E. faecalis, P. aeruginosa, S. aureus*	35°C	Not stated	Laboratory	Bupivacaine was active against *S. aureus* (MIC = 0.125 – 0.2%), *E. faecalis* (MIC = 0.25%) and *E. coli* (MIC = 0/25%)but not against *P. aeruginosa*
Pelz (2008) (33)	0.0125 – 0.2%	311 bacterial strains & 14 strains of *C. albicans*	35°C	Preservative-free	Laboratory	36 bacterial & 14 Candidal species were completely or partly resistant to bupivacaine. *P. aeruginosa & E. faecalis* were the most resistant strains. MBC for bupivacaine was 76 – 100%+ of that routinely used in clinical dental practice
Tamanai-Shacoori, (2004) (48)	0.08%	*E. coli, E. faecalis, S. aureus*	37°C	Not stated	Laboratory	Bupivacaine had no effect on the growth of *E. faecalis*, but inhibited the growth of *E. coli and S. aureus*
Goodman, (2002) (14)	0.008 – 0.38%	*S. aureus*	37°C	Dextrose (0.11 – 4.15%)	Laboratory	MIC for bupivacaine at 48 hours was 0.25%
Aydin (2001) (34)	0.625 – 0.5%	*C. albicans, E. coli, P. aeruginosa, S. aureus*	Room temperature	Not stated	Laboratory	Bupivacaine 0.25% & 0.5% reduced the viable cells of *P. aeruginosa*. The results for the other microbes were not presented, but bupivacaine was noted to have poor antimicrobial effectiveness.
Pere (1999) (49)	0.0938%, 0.1875%, 0.375%	*E. coli, K.pneumoniae P. aeruginosa, S. aureus, S. epidermidis S. pyogenes*	37°C	Preservative-free	Laboratory	Bupivacaine 0.375% fully inhibited the growth of *E. coli, P. aeruginosa & S. epidermidis.* Even the lowest concentration had a significant inhibitory effect on the growth of *P. aeruginosa, S. epidermidis & S. pyogenes. K. pneumonia*e was less well inhibited.
Hodson (1999) (50)	0.125%, 0.25%, 0.5%	*E. faecalis, S. aureus, S. epidermidis*	37°C	Preservative-free	Laboratory	No growth of any species was noted in bupivacaine 0.5% or 0.25%. All three species multiplied in bupivacaine 0.125%.

Table 2.3: Summary of investigations into the antimicrobial properties of bupivacaine (*continued*)

AUTHOR	CONCENTRATIONS OF BUPIVACAINE	MICROBES STUDIES	INCUBATION TEMP	ADDITIVES TO BUPIVACAINE	STUDY SETTING	CONCLUSIONS
Cook (1998) (51)	0.1%, 0.5%	*E. coli,* *E. faecalis,* *P. aeruginosa*, CNS	30°C	Diamorphine	Laboratory	Viable counts for all organisms decreased over a 7 day period for all formulations tested, but bupivacaine 0.5% had the greatest activity.
Grimmond (1986) (52)	0.05%, 0.125%, 0.25%, 0.5%	*E. coli,* *C. albicans* *P. aeruginosa,* *S. aureus*, MRSA *S. epidermidis* *S. pyogenes.* *S. pneumoniae*	35°C	Preservative-free	Laboratory	Bupivacaine was ineffective against *C. albicans & P. aeruginosa*. MICs for the remaining species studied were 0.25 – 0.5%.
Rosenberg (1985) (53)	0.05%, 0.125%, 0.25%, 0.5%	*B. cereus,* *E. coli,* *C. albicans,* *P. aeruginosa,* *S. aureus,* *S. epidermidis,* *S. faecalis,* *S. pneumonia,* *S. pyogenes,*	35°C	Preservative-free	Laboratory	Bupivacaine 0.5% inhibited all organisms except *P. aeruginosa*. Bupivacaine 0.25% inhibited *S. epidermidis, S. pneumonia & S. pyogenes*. Bupivacaine 0.125% had little effect.
Zaidi (1977) (54)	0.5% with serial dilutions	*E. coli,* *P.aeruginosa,* *S. aureus,* *S. pyogenes*	37°C	Preservative-free	Laboratory	MBC for bupivacaine for all organisms tested was 0.5%

B. cereus = Bacillus cereus, C. albicans = Candida albicans, CNS = Coagulase Negative Staphylococcus, E.coli = Escherichia coli, E. faecalis = Enterococcus faecalis, K. pneumoniae = Klebsiella pneumonia, MRSA = Methicillin-Resistant Staphylococcus Aureus, P. aeruginosa = Pseudomonas aeruginosa, S. aureus = Staphylococcus aureus, S. epidermidis = Staphylococcus epidermidis, S. pyogenes = Streptococcus pyogenes, S. pneumoniae = Streptococcus pneumonia,

MIC = minimum inhibitory concentration, MBC = mean bactericidal concentration

concentration of bupivacaine used in their study (0.08%) was far below that which is used clinically in our setting.

2.3.5 Effect of temperature on the antibacterial activity of bupivacaine

It has been suggested that the temperature of local anaesthetics may also affect their antimicrobial activity. The antimicrobial activity of lignocaine is considerably higher at 40 °C than at 37°C (55) and a clinical study has suggested that *Staphylococcus aureus* survives for a far longer time in lignocaine stored in a refrigerator than at room temperature (56). This finding has been repeated in investigations of microbial survival in other types of medication (57). Many of the candidate studies from our literature review examined the antimicrobial activity of bupivacaine at temperatures which were substantially higher than room temperature (14,33,39,48-54). In the only study which did observe the antimicrobial activity of bupivacaine at room temperature, the local anaesthetic was found to have generally poor antimicrobial effectiveness (34). Different ambient temperatures during the studies could, in part, explain the diverse results.

2.3.6 Effect of preservatives

Drug preparations used in intrathecal injections must be preservative-free to avoid causing a chemical arachnoiditis (12). Whilst it seems unlikely that many preservatives commonly added to local anaesthetics exert an appreciable antimicrobial affect alone, there is some evidence that there may be a synergistic antimicrobial effect between preservatives and local anaesthetics (33). Therefore, it

is important in examining previous studies to know whether drug preparations which were used contained preservative or not. For some studies reviewed, this was information was not stated (34,39,48) and therefore extrapolation of their results to preservative-free drug mixtures should be approached with caution.

2.3.7 The clinical environment vs. the laboratory environment

The clinical environment is very different from the laboratory environment, with far greater opportunity for bacterial contamination by direct hand contact with syringes and airborne contamination in the former (58). All studies found in the literature examined the fate of microbes inoculated into a preparation of bupivacaine in the laboratory environment, and documented any decrease in microbial numbers in order to establish the antimicrobial effect of bupivacaine (14,33,34,39,48-54).

2.4 Microbiological impact of the addition of dextrose

2.4.1 Dextrose and bupivacaine

Some authors of studies examining the antimicrobial effects of bupivacaine specifically excluded the use of nutritional substances to prevent confounding of their results (34). However, dextrose is added to the bupivacaine used for spinal anaesthesia in caesarean sections. The resultant mixture is denser than the CSF into which it is injected, allowing for the bupivacaine to gravitate to the thoracic kyphosis, and so achieve a dermatomal level sufficiently high for the surgery (Figure 1.1).

It has been noted that certain bacteria, especially Klebsiella, Enterobacter and Serratia species, possess the enzyme aldolase. This enzyme splits dextrose into pyruvic acid and thus allows this organisms to metabolise dextrose (59). Therefore, it is important to consider the implications of the additional of dextrose to the drug mixture under investigation. One of the studies examining bupivacaine did use hyperbaric bupivacaine with dextrose concentrations of 0.11- 4.15% (14). In this study, bupivacaine 0.25% with dextrose 4.15% was found to be inhibitory to *S. aureus.* However, this study investigated the effects of hyperbaric bupivacaine on *S. aureus* only, and other studies have found bupivacaine to be particularly active against this species and less active against other species (33,39,48,49,52,53). Therefore, global conclusion on the antimicrobial properties of hyperbaric bupivacaine cannot be drawn from this one study.

2.4.2 Studies of other dextrose-containing fluids

Studies which have investigated microbial growth in dextrose containing solutions were also reviewed and summarised in Table 2.4. There is an indication that higher concentrations (i.e. above 10%) of dextrose decrease the risk of microbial growth through an osmotic effect (60). However, Crighton found both Dextrose 5% and 10% in water to sustain the growth of various organisms, noteably Klebsiella and Serratia (61). Mendie et al found that various microorganisms in fact metabolise dextrose, so making the environment more favourable for themselves (62). The literature therefore suggests that certain pathogens are able to survive in concentrations of dextrose which are similar to those which will contribute to the drug mixture under investigation in our study.

Table 2.4: Summary of studies investigating microbial survival in dextrose

AUTHOR	CONCENTRATIONS OF DEXTROSE	ORGANISMS STUDIED	CONCLUSIONS
Hugbo (1998) (10)	2.5%, 5.0%, 10%, 20%	*B. subtilis,* *C. albicans* *E. coli,* *K. pneumoniae,* *P. aeruginosa,* *S. aureus,*	When starved organisms were added to dextrose, there was a decrease in generation rates with higher concentrations of dextrose. Different organisms behaved differently and *C. albicans* showed neither increase nor decrease in viable number over the study period in any dextrose concentration. *P. aeruginosa and E. coli* were unable to survive in >5% dextrose.
Blech (1986) (59)	10%	*Acineto. calcoaceticus,* *Aerom. hydrophila,* *C. freundii,* *E. coli,* *E. cloacae,* *Flavobacterium* *K. pneumoniae,* *P. aeruginosa,* *Ser. marcescens,* *S. aureus*	Dextrose 10% was found to be inhibitory for all species studies except for Klebsiella, Enterobacter and Serratia species.
Gacia-Caballero (1985) (60)	5%, 10%, 20%, 30%, 50%, 70%	*C albicans,* *P aeruginosa,* *S. epidermidis,* *Ser. marcescens*	Dextrose 10% allowed growth of *C. albicans & Ser. marcescens*. Dextrose 5% allowed growth of *S. epidermidis, C. albicans, P. aeruginosa, and Ser. marcescens*
Crighton (1973) (61)	3.3%, 5%, 10%, 20%, 50% (in various media)	Klebsiella species, *P. aeruginosa,* *P. cepacia* *Serratia,* *S. aureus,* *S. albus,*	Serratia, Klebsiella and to a lesser extent Pseudomonas species grew in many fluids containing 5% and 10% dextrose

Acineto. calcoaceticus = Acinetobacter calcoaceticus, Aerom. hydrophila = Aeromonas hydrophila , B. subtilis = Bacillus subtilis, C. albicans = Candida albicans, C. freundii = Citrobacter freundii , E. cloacae = Enterobacter cloacae, E.coli = Escherichia coli, K. pneumonia = Klebsiella pneumoniae, P. aeruginosa = Pseudomonas aeruginosa, P. cepacia = Pseudomonas cepacia, S. albus = Staphylococcus albus, S. aureus = Staphylococcus aureus, S. epidermidis = Staphylococcus epidermidis, Ser. marcescens = Serratia marcescens

2.5 Implications of the addition of fentanyl

2.5.1 Background to investigations into microbial contamination of opioids

Several studies have examined the microbiological implication of the addition of opioids (of which fentanyl is an example) to local anaesthetics (40,48,50-53,63) or the risk of microbial contamination of opioids alone (64) (Table 2.5). The reason for this area interest in existing literature is that epidural infusions often contain plain (non-dextrose containing) local anaesthetics and opioids, as well as other adjuvant drugs which are mixed in large-volumes with the view to infusion over a period of hours. This has led to investigations into the risk of microbiological contamination of these particular drug mixtures.

2.5.2 Microbial contamination of opioids in general

Pethidine had some antimicrobial activity at clinically significant concentrations (52,63) which may be related to its local anaesthetic-like properties (52). In contrast to this, however, an in-use clinical study found that morphine and pethidine prepared for titration for individual patients have a high rate of contamination during clinical use (64). There is some evidence that sufentanil may have some synergistic antimicrobial activity when used in combination with bupivacaine (48), although another study found sufentanil to neither inhibit nor encourage microbial growth (40). Tamanai-Shacoori suggests that this non-specific antimicrobial activity of

Table 2.5: Summary of studies of the microbial survival in opioids

AUTHOR	OPIOID STUDIED	ORGANISMS STUDIED	CONCLUSIONS
Hodson (2009) (50)	Fentanyl 2µg/ml & 4 µg/ml	*E. faecalis* *E. coli* *P. aeruginosa* *S. aureus*	Fentanyl had no antibacterial effect in isolation or any synergistic effect when added to local anaesthetics.
Guillier (2007) (40)	Sufentanil 0.5 - 0.75 µg/ml	*E. coli* *S. aureus* *S. epidermidis*	Sufentanil neither encouraged nor inhibited growth of thebacteria studied.
Tamanai-Shacoori (2004) (48)	Sufentanil 0.38 µg/ml (with bupivacaine) & 0.5µg/ml (with ropivacaine)	*E. faecalis* *E. coli* *S. aureus*	Sufentanil alone inhibited only the growth of S. aureus but increased the inhibitory effect of bupivacaine on the growth of all bacteria studied.
Cook (1998) (51)	Diamorphine 0.01% , 0.1%, 1% (with bupivacaine)	CNS *E. faecalis* *E. coli,* *P. aeruginosa*	Formulations with 1% diamorphine had greater antimicrobial activity than those with lower concentrations of the opioid.
Rota (1997) (63)	Morphine: 2.5, 5, 10 & 20 mg/ml Pethidine: 6.25, 12.5, 25 & 50mg/ml Fentanyl: 6, 12,5 & 50 µg/ml	CNS *E. coli* *K. enterobacter* *P. aeruginosa* *Proteus species*	MIC of Morphine was 10mg/ml for P. aeruginosa and 20mg/ml for others except for the Proteus species, which were not inhibited Pethidine had the most antibacterial effect with an MIC starting at 6.25mg/ml (specifics not reported). Fentanyl showed no antibacterial effect.
Taylor (1997) (64)	Morphine & pethidine	Opioids in clinical use sampled & cultured	12% yielded microbiological growth, but no distinction wasmade between morphine and pethidine.
Grimmond (1986) (52)	Pethidine 0.5 - 50mg/ml	*C. albicans* *E. coli* MRSA *P. aeruginosa* *S. aureus* *S. epidermidis* *S. faecalis* *S. pneumonia* *S. pyogenes*	At concentrations of 5mg/ml, pethidine inhibited 6 of 10 organisms. Resistant organisms were P. aeruginosa, S. aureus and C. albicans (MIC for these was 12,5mg/ml) and S. faecalis (MIC was 25mg/ml).
Rosenberg (1985) (53)	Morphine 0.2 & 2mg/ml (with bupivacaine)	*B.cereus* *C.albicans* *E.coli* *P.aeruginosa* *S.aureus* *S.epidermidis* *S.faecalis* *S. pneumoniae* *S.pyogenes*	Morphine alone had no antimicrobial effects at the concentrations tested and had no synergistic antimicrobial effect with bupivacaine when tested in combination.

B. cereus = Bacillus cereus, C. albicans = Candida albicans, CNS = Coagulase-Negative Staphylococcus aureus, E. faecalis = Enterococcus faecalis, E. coli = Escherichia coli, K. enterobacter = Klebsiella enterobacter, MRSA = Methicillin-Resistant Staphylococcus sureus , P. aeruginosa = Pseudomonas aeruginosa, S. aureus = Staphylococcus aureus, S. epidermidis = Staphylococcus epidermidis ,S. faecalis = Streptococcus faecalis, S. pneumoniae = Streptococcus pneumoniae, S. pyogenes = Streptococcus pyogenes, MIC = Minimum Inhibitory Concentration

certain opioids may be related to their molecular weight, thermodynamic activity and pH rather than their chemical structure (48).

2.5.3 Microbial contamination of fentanyl in particular

Evidence suggests that fentanyl itself has no discernable antimicrobial properties(50,63).

2.6 Microbial contamination of multi-dose containers

2.6.1. Clinical use of multi-dose containers

A true multi-dose vial is one which contains preservatives and, according to manufacturer's specifications, may be used more than once (65). However, at a time when cost containment in health care is increasingly important, many centres are looking to using single dose products for multiple patients (66). The reasons for this are increased convenience, decreased environmental waste and presumed cost-reduction (56,67), although some authors dispute whether this is indeed a cost-saving practice (68). Concern over microbial contamination of such products has prompted extensive investigation in the literature.

2.6.2. Risk of microbial contamination of multi-dose vials in clinical practice

Many studies have investigated the risk of microbial contamination of multi-dose vials containing various different medications in clinical use. In 1984, Longfield reviewed the subject and showed that previous studies had found 0 – 27% of multi-dose vials in clinical use to be contaminated (67). However, the authors of that review noted 90% of culture positive results had occurred before 1973 and suggested reasons for this being an evolution in preservatives used in multi-dose vials as well as a shift in drugs which were available in multi-dose formulations. They also mentioned that the practice of using multi-dose diluents has largely been replaced by using single-unit substitutes. In their synopsis of the eleven studies, only 24 of 4036 vials tested were found to be contaminated. When excluding multi-dose antibiotics or diluents, the authors estimated that the incidence of contamination of multiple dose vials is 0.28%. A positive correlation was noted between contamination and number needle entries into the multi-dose vials (67). In their own study, Longfield et al investigated the risk of contamination of various drugs used in clinical practice and found the incidence of contamination to be exceedingly small. None of the 1223 samples taken from various drugs in clinical use yielded positive microbial culture (67).

Since Longfield's review, several authors have examined the occurrence of microbial contamination of multi-dose vials containing various medications in clinical practice (57,64,66,69-74). Contamination rates in these studies were found to be 0 – 12% (Table 2.6). This considerable range can be accounted for in part by methodology and by the different agents investigated. The study which showed the highest

contamination rate examined the sterility of opioids in syringes prepared and used for titration in a single patient (64). In this study, the syringes came into direct contact with patients, which could account for the higher contamination rate. In one study, no specific aseptic technique was used in withdrawal of medications from multi-dose vials and the contamination rate was found to be 5.6% (70). However, in the studies which used multi-dose containers from which aliquots were withdrawn using aseptic technique and standard infection control precautions, the contamination rate was found to be very low at 0 – 0.88% (57,64,66,69,71-74).

In one study, the investigators matched 265 vials of medication in clinical use with vials containing culture broth (71). These matched vials were submitted to the same clinical handling and storage as the vials in clinical practice over a period of time. None of the 265 vials of broth yielded any microbial growth on culture. Since broth would allow growth of any pathogen which had been introduced into the vial during clinical practice, this study gives staunch support to the notion that microbial contamination of multi-dose containers in clinical practice is exceedingly rare. A logical extension would be that the risk of contamination would be further decreased if the agent contained in the multi-dose vial possessed inherent antimicrobial properties (66), such as in the case of bupivacaine. It has, however, been noted that the risk of microbial contamination depends not only on the nature of the medication being prepared, but also on the aseptic technique of the health practitioner carrying out the preparation (70,72).

Table 2.6: Summary of investigations into microbial contamination of multi-dose vials in clinical use

AUTHOR	DRUGS INVESTIGATED	SAMPLE SIZE	METHODOLOGY	OUTCOME
Chen (2009) (69)	Bevacizumab (recombinant anti-VEGF antibody)	12	4 – 5 aliquots were drawn out of a multi-dose vials for clinical use using aseptic technique. The remaining contents were left in vials & refrigerated for 1, 2 & 6 months, at which times microbiological samples were taken.	No microbial contamination was detected.
Motamedifar (2009) (70)	Medications of various types in clinical use	637	All multi-dose vials used clinically in a hospital over a 4-month period were collected at tested for aerobic bacteria.	5.6% (38 samples) were found to be contaminated
Murray (2005) (71)	Sterile radio-pharmaceuticals with long shelf lives	265	Vials of drug were matched with vials of culture broth. The broth accompanied the clinical vial throughout its time in use. When an aliquot was withdrawn for clinical use, a sample of broth was withdrawn & sent for microbiological investigation.	No microbial contamination was detected.
Mattner (2004) (72)	Medications of various types in clinical use	227	On a single day, all opened vials and syringes intended for multiple dosing were collected from all wards in a hospital. Samples were withdrawn under aseptic conditions and cultured on blood agar and thioglycollate broth.	0.88% (2 samples) were found to be contaminated.
Taylor (1997) (64)	Pethidine & Morphine	100	Opioids which had been prepared in syringes, each used to titrate opioids in the same patient, were submitted for microbiological investigation. Half of the syringes were prepared in the Emergency Room and half under aseptic conditions in the pharmacy.	12% of the syringes were found to be contaminated, with equal numbers contaminated from those prepared in the Emergency Room and Pharmacy.
Green (1995) (66)	Gadolinium MR contrast medium	15	The residual contrast medium of single-dose vials that had been used for multiple patients were submitted for microbiological investigation.	No microbial contamination was detected.
Christensen (1992) (73)	Lyophylized virus vaccine	200	Empty vials which had been used in clinics were tested for microbial contamination.	No microbial contamination was detected.
Longfield (1985) (57)	Various medications in clinical use	1223	1223 weekly samples were cultured from 864 multi-dose vials in clinical use.	No microbial contamination was detected.
Rathod (1985) (74)	Insulin	69	Vials of insulin in clinical use were submitted for microbial investigation.	8 (11.6%) samples showed microbial contamination. 5 were re-cultured after 24 hours yielded no microbial growth.

2.6.3. Clinical implications of contamination

Despite this very low risk of contamination, there have been case reports in the literature of outbreaks of various infections which can be traced to contamination of multi-dose vials (56,75). Mattner quotes 17 such reports, which include accounts of bacterial and protozoan contamination (72). The same authors quote newspaper reports of two deaths caused by intrathecal administration of contrast media contaminated by *P. aeruginosa* (72). On the other hand, one investigation into the clinical relevance of microbial contamination of opioids found that, although 12% syringes prepared for titration of opioids showed microbial contamination, this resulted in no contribution to patient morbidity or mortality (64). This study was investigating the risk of intravenous rather than intrathecal administration of contaminated agents, and the patients were all immunocompetent. The findings may have been different had the administration been via the intrathecal route or to immunocompromised patients.

Whilst the literature presents a wide range of evidence, it seems that the overall risk of contamination of multi-dose vials is exceedingly low when aseptic technique is employed in handling of these vials and when the vials are stored in the correct manner. However, there have been cases of microbial contamination reported and some of these have had severe clinical sequelae. Some authors have indicated that, although the risk of microbial contamination of multi-dose vials may be rare, it would still seem prudent to use single-dose containers for each patient (70,76).

2.7 Overall conclusions from the literature

Never before has there been a study conducted to ascertain the extent of risk of microbial contamination associated with the drug mixture described in Appendix A. Related previous studies have suggested that infectious complications following spinal anaesthesia are rare. In order to minimise even this small risk, previous authors have cautioned against the use of multi-dose vials for intrathecal agents. However, it would appear that the risk of contamination of multi-dose vials in general is exceedingly low. This, combined with some antimicrobial effect of bupivacaine, makes the risk of microbial contamination, survival and proliferation in the drug mixture to be studied seem to be very small indeed.

However, the addition of dextrose as a source of nutrition and the fact that bupivacaine has not been shown to have universal antimicrobial properties is concerning. Of prime concern are the potentially catastrophic sequelae in the event of infections related to spinal anaesthesia.

CHAPTER 3: MATERIALS AND METHODS

This chapter details the materials and methods used for the current pilot study.

3.1 Location

The study was carried out in one of the obstetric theatres at Chris Hani Baragwanath Hospital (CHBH), Johannesburg. These operating theatres service the labour ward at CHBH, which functions as the tertiary referral centre for the greater population of Soweto, Johannesburg and surrounding areas. Patients are referred from surrounding primary and secondary health facilities for complicated pregnancies and labours. There are approximately 1800 deliveries per month and a 30% caesarean section rate in this Department (77). The average number of caesarean sections carried out in the Obstetric Theatres over an 18 month period starting from January 2008 was 608 per month (77).

3.2 Permissions

The protocol for this study was submitted to the Human Research Ethics Committee (Medical) at the University of the Witwatersrand and was granted an Ethics Waiver (Appendix B). It was also submitted to the Faculty of Health Sciences Post Graduate Committee at the University of Witwatersrand, which granted permission to continue with the study (Appendix C), as well as to the Medical Superindendent of Chris Hani

Baragwanath Hospital (CHBH), who likewise gave permission for the study to be carried out on the premises of CHBH (Appendix D).

3.3 Research Methodology

3.3.1 Research Design

This investigation is a prospective and longitudinal observational pilot study.

Prospective: *Data which are to be studied are yet to be generated*

Longitudinal: *The study will be carried out over time and chronological time will have the opportunity to exert an effect on the variables*

Pilot study: *A small-scale study conducted in order to check feasibility or improve on study design prior to initiation of the main study*

3.3.2 Study population and study sample

3.3.2.1 Study Population

The study population was a mixture of hyperbaric bupivacaine and fentanyl prepared by the investigator at Chris Hani Baragwaneth Hospital, according to a protocol at that hospital (Appendix A). Syringes prepared according to this protocol were allocated to pairs. Each pair had one syringe from which only two samples were drawn, one at the beginning and one at the end of the study period, referred to from here on as the control syringe. The other syringe in the pair was sampled each hour during the study period and is referred to as the multi-dose syringe.

3.3.2.2 Study sample

As an investigation of this nature has never been done before, it was decided to first perform a pilot study in order to assess methodology and final sample numbers needed to adequately power a future study. It was decided that submission of 100 samples for microbiological investigation would give sufficient opportunity for this assessment. Since each syringe pair yields six samples for microbiological investigation, it was decided that the pilot study should utilise twenty syringe pairs. It was anticipated that there would be very few, if any, contaminated syringes, and therefore it is unlikely that this sample size would be sufficient for a final study with sufficient power to generate significant results. However, the results from the pilot study would be able to inform the sample size required for such a future study.

3.4 Procedure for Data Collection

3.4.1 General Procedure

It has been previously shown that anaesthetic work areas are quickly contaminated by microbes (78). It is therefore important to assess possible microbial contamination in the theatre setting, as opposed to a laboratory setting. Therefore, during the study period, the investigator worked in an obstetric theatre whilst cases were being done, in an attempt to carry out this study in a situation which mirrors the clinical setting as closely as possible. During the study period, however, the investigator was in no way responsible for the anaesthetic care of any of the patients in the theatre. This was so as to ensure that the study had no negative impact upon

patient care in the obstetric theatres. Furthermore, the drug mixture in the syringes under investigation was not administered to any patients during the study period.

3.4.1.1 Preparations prior to commencement of the study period

The literature has an account of a local anaesthetic similar to bupivacaine (ropivacaine) causing turbidity of a growth medium similar to thioglycollate (Mueller-Hinton broth) as a result of precipitation rather than microbial contamination (14). In order to ensure that this was not the case using bupivacaine with thioglycollate broth, one tube of thioglycollate was inoculated with 1,5ml of the drug mixture under investigation and observed for turbidity. None was noted after 10 days at room temperature and it was concluded that any turbidity of the broth could be attributed to microbial contamination and not precipitation due to chemical reaction.

Prior to the commencement of each study period, the technicians at the National Health Laboratory Service (NHLS) laboratory at CHBH placed the required number of tubes containing thioglycollate broth into an aerobic incubator for 48 hours. This was to ensure that none of the tubes containing this growth medium were contaminated by microbial growth prior to inoculation with study samples. It was agreed that, should any of the broth become turbid during this overnight incubation, it should be discarded.

3.4.1.2 Control of fentanyl

Fentanyl is a Schedule Six drug and therefore it is required by law that this drug be controlled (79). After delivery by Fresenius-Kabi to the investigator, the fentanyl for this study was counted and signed off by the investigator and a senior consultant in the Department of Anaesthesia at CHBH. The fentanyl stock was then kept in the locked obstetric theatre drug cupboard separate from that in clinical use. A Schedule Six drug register was kept against the invoice from Fresenius-Kabi, with the Sister-in-Charge of obstetric theatre signing for the initial receipt from the investigator of eighty ampoules of fentanyl 100 $\mu g.2ml^{-1}$. Thereafter, sufficient ampoules for each study period were issued to the investigator upon signature. At each issuing, one of the anaesthetic nurses signed as the "controller" and the investigator co-signed as the "administrator" of the drugs. The study numbers which were assigned to each syringe were used instead of the patient's name in the register, and two ampoules were signed off for each syringe as per the protocol below. On three occasions during the study, the stock of fentanyl was counted and signed off by a Chief Professional Nurse. At the end of the study period there was no remaining fentanyl. Certified copies of the Scheduled Substance register were made, with the original being kept in the Department of Anaesthesia and copies being held by the investigator and supervisor.

3.4.1.3 General procedure during data collection

At the beginning of the twelve-hour study period, the investigator prepared two syringes containing the same mixture of hyperbaric bupivacaine and fentanyl, in accordance with the Departmental Protocol, Option 2 using fentanyl 200µg

(Appendix A). Both these syringes remained in the theatre for the full twelve-hour study period. When not being sampled, the syringes were capped using a sheathed hypodermic needle. Each pair of syringes was assigned a study number (MIC 001-MIC 020), with one syringe from each pair being designated as the control syringe ("CS") and the other the multi-dose syringe ("MS").

For purposes of clarity, volumes withdrawn from the syringes which were sent to the laboratory as specimens are referred to as "samples" whilst those discarded are referred to as "aliquots."

A 1,5ml sample from the drug mixture in the control syringe was withdrawn at *Time = 0 hours*, which was within 15 minutes of the preparation of the drug mixture in the syringe. A second 1,5ml sample was withdrawn twelve hours later (*T = 12 hours*). These two samples were each transferred immediately to thioglycollate broth using standard aseptic technique, labelled CS-00 and CS-12 respectively, and taken to the microbiology laboratory for investigation in accordance with the protocol outlined below.

This study required a growth medium which would be non-selective and would yield growth even with a very small initial inoculum of microbes. For these reasons, we selected thioglycollate broth as the growth medium. This is an all-purpose growth medium which is useful in isolating a wide range of organisms, including aerobic and anaerobic bacteria and yeasts, and is good a yielding recovery from low numbers of microbes in the initial inoculum (68,80).

A 1,5ml sample from the drug mixture in the multi-dose syringe was withdrawn at *Time = 0 hours*, which was within 15 minutes of the preparation of the drug mixture in the syringe. Further 1,5ml samples were withdrawn at *Time = 4, 8, and 12 hours.* Each of these samples were immediately transferred into thioglycollate broth, labelled MS-00, MS-04, MS-08 and MS-12 respectively, and taken to the microbiology laboratory for investigation in accordance with the protocol outlined below. In addition to these samples, 1,0ml aliquots were withdrawn each hour where a sample was not being withdrawn (i.e.: *Time = 1,2,3,5,6,7,9,10* and *11 hours).* These 1,0ml aliquots were discarded immediately after withdrawal. The intention of the withdrawal of these aliquots was to approximate the clinical situation in the obstetric theatres at CHBH, where caesarean sections performed approximately hourly.

During the study period, theatre temperatures were measured hourly using a digital thermometer. A record was kept of the following parameters on a Data Sheet (Appendix E).

a) Sample numbers
b) Corresponding NHLS bar code and NHLS laboratory numbers
c) Time syringes were prepared
d) Time the samples reached the laboratory
e) Times the samples were placed in the incubator
f) Theatre temperatures at the time that the samples were taken
g) Number of cases done during the study period

3.4.2 Labelling of samples

All samples in thioglycollate broth tubes were labelled with a unique study number printed on a self-adhesive label. This number allowed identification of a) the syringe pair (001 in the example below), b) whether it was withdrawn from the control or the multi-dose syringe (CS for control syringe in the example below) and c) the time during the study period at which the sample was taken (*Time = 0 hours* in the example below).

Example of a study number:

MIC 001 – CS – 00

In addition to the unique study number, the detachable barcode from the National Health Laboratory Service (NHLS) request form was affixed to the sample bottle. A record of this barcode was kept along with the unique sample number for ease of recovery of results from the laboratory system.

3.4.3 Preparation of drug mixtures

The method for preparation of drug mixtures was designed so as to mimic the clinical situation as closely as possible. Drug mixtures were prepared by the investigator only in the obstetric theatre at CHBH. Hands were washed using chlorhexidine hand scrub prior to preparation of the drug mixtures, but no gloves or facemask were worn. In accordance with the protocol used at CHBH (Appendix A), four ampoules of Micro Bupivacaine Spinal with Dextrose Injection (Micro Healthcare, Pty, Ltd), each containing 4ml volume, were drawn up into a newly-opened sterile 20ml syringe

(BBraun Medical, Pty, Ltd) using a newly-opened sterile 22 gauge hypodermic needle (BBraun Medical, Pty, Ltd). Two ampoules of Fentanyl-Fresenius (Fresenius Kabi South Africa, Pty, Ltd), each containing 100 μg, were then drawn into the same 20ml syringe using the same hypodermic needle. The resulting drug mixture comprised fentanyl 10 $\mu g.ml^{-1}$, bupivacaine $4mg.ml^{-1}$ and dextrose $64mg.ml^{-1}$ with a total volume of 20ml.

The syringes were then left on a trolley in the obstetric theatre for the following twelve hours and remained capped with the original 22 gauge needles at all times except for when samples and other aliquots were being withdrawn. This was applied regardless of other activity in the theatre (i.e.: theatre being empty, cases being done or theatre being scrubbed down between cases).

3.4.4 Withdrawal of samples and other aliquots

In clinical practice, according to the protocol in Appendix A, once a syringe has been prepared with the drug mixture described above, aliquots are withdrawn using newly opened sterile syringes for administration in obstetric spinal anaesthesia. With an average of over 600 caesarean sections per monthat CHBH (77), hourly sampling seemed a reasonable estimate of clinical sampling frequency.

Prior to the withdrawal of samples intended for microbiological processing, the investigator labelled a tube of thioglycollate broth with a unique sample number previously described, as well as a barcode which linked the sample to the laboratory

request form. The thioglycollate tube was then laid upon the corresponding laboratory request form.

In preparation for the withdrawal of all aliquots, the investigator donned a standard surgical mask and opened a sterile pack containing a lotion cloth and hand towels on a stainless steel trolley in the obstetric theatre. Onto the sterile lotion cloth, a pack of sterile gloves (Biogel Skinsense ®, Mölnlycke Healthcare, Sweden), a sterile 5ml syringe (Omnifix®, BBraun Medical, Pty, Ltd) and a sterile 22 gauge hypodermic needle (Sterican®, BBraun Medical, Pty, Ltd) was opened for each sample to be taken. All packs were opened in an aseptic manner. The control and multi-dose syringes from which samples were to be taken were then shaken. The capping hypodermic needle on each syringe was removed and laid next to the syringe. The syringes were positioned under the edge of the lotion cloth to enable the investigator to with draw the sample whilst maintaining asepsis.

Whilst still wearing the surgical mask, the investigator performed a surgical hand scrub using a 4% chlorhexidine gluconate skin cleanser (Bioscrub™, Dismed Pharma, Pty, Ltd). Forearms were scrubbed up to the elbows and hands were washed according to the hand washing protocol used at CHBH (81). This procedure was carried out three times. Hands were then dried on the sterile hand towels and the sterile gloves were donned. If, for any reason the gloved hands touched a non-sterile object (e.g.: when samples were being withdrawn from control and multi-dose syringes during the same sampling period), the potentially contaminated gloves were discarded, hands were re-scrubbed as per the above procedure and a new pair of sterile gloves were donned.

The hypodermic needle which had been opened onto the sterile cloth was then attached to the 5ml syringe. Whilst holding the 20ml control or multi-dose syringe through the sterile lotion cloth, the investigator withdrew a 1,5ml sample for microbiological processing or a 1,0ml aliquot to be discarded, as dictated by the abovementioned protocol.

3.4.5 Transfer of sample to growth medium

The syringe containing the sample was held in the investigator's right hand. The thioglycollate tube into which the sample was to be transferred was then picked up in the left hand. The little finger of the right hand was used to remove the lid of the thioglycollate tube as per standard aseptic technique, taking care not to touch the under surface of the lid. Then, without letting the hypodermic needle touch the sides of the tube, the sample was expressed into the thioglycollate broth. The lid was immediately replaced using only the little finger, and care was taken to ensure that it fitted snugly.

3.4.6 Disposal of sampling materials

After each sample was withdrawn, the used gloves and syringes were discarded into the appropriate medical waste bin. The 5ml syringes containing the aliquots from the multi-dose syringes which were not intended for microbiological investigation were also discarded in this manner. All needles were discarded in the sharps containers available.

3.4.7 Transport of the samples to the microbiological laboratory

Once the labelling of the samples had been carefully checked, the investigator personally transferred both the tubes of thioglycollate broth containing the samples together with the corresponding NHLS request forms to the NHLS laboratory at CHBH. Upon arrival at the laboratory, the request forms were franked to indicate time of arrival at the laboratory. The staff in the reception of the laboratory then logged the samples onto the NHLS computer system and made two copies of the request forms. The original request form was kept at the laboratory reception, with one of the copy being sent to the Department of Microbiology and the other remaining with the investigator for study records. The investigator then personally placed the samples on a rack in a walk-in aerobic incubator within thirty minutes of the sample being withdrawn.

3.4.8 Microbiological processing of samples

Processing of the samples in the laboratory was according to NHLS Standard Operating Procedures (80). The tubes of inoculated thioglycollate broth were incubated aerobically at 37 °C and examined daily for turbidity by the microbiology technicians. Turbid samples were sub-cultured onto 5% sheep blood agar using a sterile wire loop. The agar plates were then incubated for a further 24 hours at 37 °C and examined for any microbial growth by qualified laboratory technicians using standard techniques. For logistical purposes, the technicians knew that the samples

were being investigated as part of a sterility study. However, they were not informed of the significance of sample labels.

The sample was considered to be positive for microbial growth as defined by turbidity of the thioglycollate broth with subsequent growth of any microorganism demonstrated on the blood agar. In the event of any microbial growth on the sheep blood agar, the organism(s) were further identified by a microbiologist. If a Viridans group Streptococcus or a Coagulase-negative Staphylococcus was identified, then further speciation was carried out using an automated process. When the inoculated thioglycollate broth remained clear after 48 hours in the incubator, the sample was be considered to be sterile and discarded according to laboratory protocol.

3.4.9 Recording of temperature

The effect of differences in temperature is important to note since we are examining the potential contamination of syringes kept in the theatre environment at room temperature, and it may be inappropriate to assume that previous investigations into the antimicrobial properties of bupivacaine at higher temperatures may be extrapolated to the environment of the current study (see 2.3.6). Therefore, a record was kept of theatre temperatures during the study period. Each hour during the study period, the theatre air temperature was recorded using a digital thermometer (Flash Check Pocket Probe Thermometer, DeltaTRAK ®, Inc, USA). The thermometer was strapped to the leg of the trolley on which the test syringes were kept with hourly recordings made in degrees Celsius. According to the manufacturers, this

thermometer has an accuracy of ± 0,5°C in the temperature range of -20°C to 90°C, which suited the purposes of this study.

3.4.10 Recording the of number of cases

The number of cases done during each study period was recorded as per the theatre register in an attempt to estimate theatre traffic, which was hypothesised may contribute to contamination of the syringes. All types of operation were included in the count of operations. Any case which was in progress during the study period was included, even if it had begun prior to the start of the study period or continued after the end of the study period.

3.5 Inclusion & exclusion criteria

3.5.1 Inclusion criteria

Samples taken in the prescribed manner from drug mixtures prepared by the investigator according to the existing protocol (Appendix A, Option 2 using 200µg fentanyl) for the purpose of this study were included.

3.5.2 Exclusion criteria

Prior to commencement of the study, it was decided that samples would be excluded from the study under the following circumstances:

3.5.2.1 Samples which reached the aerobic incubator in the NHLS laboratory greater than 30 minutes after withdrawal from the multi-dose or control syringe

3.5.2.2 Samples in containers which were damaged during transport

3.5.2.3 Samples from syringes which were inadvertently left uncapped between samplings

3.5.2.4 Samples from syringes which were inadvertently removed from the operating theatre during the study period

3.5.2.5 Any breach of aseptic technique during the withdrawal of aliquots

3.6 General procedure for obtaining of results

According to the protocol for microbiological processing, results of the microbiological investigation were available within 72 hours of the sample reaching the laboratory. The staff of the results office printed hard copies of the laboratory reports, which were collected by the investigator. Results were then recorded on the data sheet (Appendix E). Microbial contamination was recorded as categorical data with 0 indicating no growth and 1 indicating growth. Quantitative data collected during the study periods was entered and note was made of species identification of samples which yielded microbial growth.

3.7 Data analysis

3.7.1 Analysis of primary outcome

Data for the primary outcome was considered as categorical data, i.e. the presence or absence of contamination of the samples. Descriptive statistics were presented as percentages of samples found to be contaminated. The comparison between multi-dose and control syringes was then used to calculate the sample size which will be required for statistical significance using a two-tailed calculation.

3.7.2 Analysis of secondary outcomes

Data for secondary outcomes, namely temperature variation in the theatres and number of cases done, were presented as quantitative data with respect to time. The means and standard deviations of these data were presented for each day and for each study period. Statistical analysis was performed using Microsoft Excel 2007.

3.8 Funding

Funding for this project was obtained from the Faculty of Health Sciences.

3.9 Summary of materials and methodology used

This chapter has detailed where this pilot study took place, the nature of the study design, and the materials and methodology which were used. The protocols for data collection, inclusion and analysis have also been presented.

CHAPTER 4: RESULTS

In this chapter, results are presented for both Primary and Secondary outcomes for this study.

4.1 Inclusion of samples

4.1.1 Number of samples taken

Twenty syringe pairs were studied. Four samples were taken from each multi-dose syringe and two from each control syringe.

4.1.2 Time period

The study was carried out over a period of five days, on 23 November, 30 November and 1 – 3 December 2009. Samples from all twenty syringe pairs were collected on these days. Two syringe pairs were prepared simultaneously with the initial samples drawn and taken to the incubator in the NHLS laboratory. The next two syringe pairs were then prepared an hour later. A maximum of six syringe pairs were processes in any single day. Table 4.1 summarises the dates and times of the twelve-hour study period for each syringe pair.

Table 4.1: Temporal relationships of study periods for each syringe pair

SYRINGE PAIR	DAY NUMBER OF STUDY	DATE OF DATA COLLECTION	STARTING TIME OF DATA COLLECTION	END TIME OF DATA COLLECTION
MIC-001	1	23 November 2009	06h30	18h30
MIC-002	1	23 November 2009	06h30	18h30
MIC-003	2	30 November 2009	05h30	17h30
MIC-004	2	30 November 2009	05h30	17h30
MIC-005	2	30 November 2009	06h30	18h30
MIC-006	2	30 November 2009	06h30	18h30
MIC-007	3	1 December 2009	04h30	16h30
MIC-008	3	1 December 2009	04h30	16h30
MIC-009	3	1 December 2009	05h30	17h30
MIC-010	3	1 December 2009	05h30	17h30
MIC-011	3	1 December 2009	06h30	18h30
MIC-012	3	1 December 2009	06h30	18h30
MIC-013	4	2 December 2009	04h30	16h30
MIC-014	4	2 December 2009	04h30	16h30
MIC-015	4	2 December 2009	05h30	17h30
MIC-016	4	2 December 2009	05h30	17h30
MIC-017	5	3 December 2009	04h30	16h30
MIC-018	5	3 December 2009	04h30	16h30
MIC-019	5	3 December 2009	05h30	17h30
MIC-020	5	3 December 2009	05h30	17h30

4.1.3 Exclusion of sample

During transfer of one sample (MIC-006-CS-12) to the thioglycollate broth, the inside of the receiving tube was inadvertently touched with the needle being used for inoculation. Aseptic technique was deemed to have been breached, and this sample was excluded. Since it was one of the samples taken at *Time = 12 hours* from a control syringe, the remainder of the samples drawn from that particular syringe pair were included in the study. This left 119 eligible samples belonging to twenty syringe pairs.

4.1.4 Adherence to protocol

Table 4.2 indicates the time taken from withdrawal of each sample until the sample was placed in the incubator. As per the protocol, all samples which were presented for microbiological investigation reached the incubator in the microbiological laboratory

Table 4.2: Time in minutes from sample withdrawal to placement in incubator

	CS-00	MS-00	MS-04	MS-08	MS-12	CS-12
MIC-001	24	21	16	21	30	27
MIC-002	19	17	14	19	24	21
MIC-003	18	14	19	14	18	15
MIC-004	7	10	22	18	11	8
MIC-005	16	14	19	17	15	12
MIC-006	11	8	17	13	9	*Excluded*
MIC-007	19	16	20	20	18	22
MIC-008	12	8	16	17	11	14
MIC-009	19	16	26	23	24	21
MIC-010	12	9	22	19	18	15
MIC-011	20	17	12	17	24	21
MIC-012	13	10	17	14	17	14
MIC-013	19	15	19	12	24	20
MIC-014	12	8	10	8	16	16
MIC-015	29	24	19	19	28	25
MIC-016	20	16	15	15	19	16
MIC-017	26	20	11	12	24	27
MIC-018	15	8	8	9	21	17
MIC-019	26	23	20	19	21	19
MIC-020	19	16	16	16	28	24

CS-00 & CS-12 were samples taken from the control syringes at 0 and 12 hours respectively
MS-00, MS-04, MS-08 and MS-12 were samples taken from the multi-dose syringes at 0, 4, 8 and 12 hours respectively
MIC-001 through MIC- 020 correspond to the 20 syringe pairs investigated
All times are presented in minutes

within 30 minutes (mean = 17 minutes, range = 7 to 30 minutes) and no further specimen had to be excluded under this criterion. Likewise, no sample containers were damaged during transport and no syringes were inadvertently left uncapped or removed from the theatre.

4.2 Primary Outcome Results: Microbiology

4.2.1 Processing of microbiological results

Hardcopy of results for all samples presented to the laboratory were obtained. All samples presented to the laboratory had been processed as per the previously detailed protocol.

4.2.2 Microbiological findings

One sample of 119 presented for microbiological investigation yielded microbial growth after 48 hours of observation. The sample (MIC-007-MS-00), which was a sample from a multi-dose syringe withdrawn at the beginning of a study period, yielded growth of *Staphylococcus aureus*.

4.2.3 Data analysis in the microbiological investigation

4.2.3.1 Description of data

When considering all samples presented for microbiological investigation, 1 of 119 samples (0.84%) yielded a positive culture. However, in order to facilitate further calculations, the same data may be considered in terms of two groups, namely the multi-dose and the control syringes. In this case, 1 of the 20 multi-dose syringes (5%) yielded a contaminated sample, whilst none of the 20 control syringes (0%) yielded a contaminated sample.

4.2.3.2 Calculation of sample size required

In order to prove that contamination *never* occurs, an infinite sample would be required. However, to prove statistically that there is a difference in contamination rates of at least 5% between the two groups, a sample size of n = 581 syringes in each group would be required using a two-tailed test ($\alpha = 0.05$ and $\beta = 0.10$).

4.3 Secondary Outcome Results: Theatre temperatures

4.3.1 Recording of theatre temperatures

Theatre temperatures were measured using a digital thermometer every hour during the daily study periods listed in Table 4.1. A record was kept of these measurements and presented in Table 4.3 according to the times of each day of the study period, as

Table 4.3: Temperatures recorded on each day of investigation in ° C, showing mean and standard deviation (SD) for the temperatures on each day

TIME	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
04:30			22,6	22,9	22,6
05:30		23,2	22,4	22,9	22,4
06:30	23,0	23,2	22,6	22,6	22,7
07:30	23,0	23,4	23,2	22,9	22,4
08:30	23,3	23,5	23,6	23,1	23,2
09:30	24,0	24,0	23,9	23,2	23,4
10:30	24,2	24,4	24,1	23,2	23,6
11:30	25,2	25,0	23,5	23,3	23,7
12:30	25,2	25,2	23,9	23,6	23,7
13:30	26,2	25,2	23,9	24,2	23,7
14:30	26,0	25,7	23,5	23,7	24,2
15:30	26,2	26,2	24,2	24,1	24,3
16:30	25,9	26,4	24,2	23,2	24,2
17:30	25,7	26,2	24,1	23,2	23,7
18:30	25,4	24,9	23,8		
Mean (SD)	24,9 (1,2)	24,8 (1,2)	23,6 (0,6)	23,3 (0,5)	23,4 (0,7)

referred to in Table 4.1. In order to facilitate the comparison of temperatures between corresponding study periods for each syringe pair, the same data is presented in Table 4.4 according to syringe pair.

4.3.2 Data analysis of theatre temperatures

Temperatures varied according to time of day, with temperatures generally being lowest in the morning, reaching a peak in late afternoon, and then subsiding. Since the study periods were all commenced between 04h30 and 06h30 and extended for twelve hours (Table 4.1), the temperature profiles of the study periods closely resembled those of the diurnal variation. However, the range of temperatures within corresponding sample times for each syringe pair was as much as 3,0° C (Table 4.4).

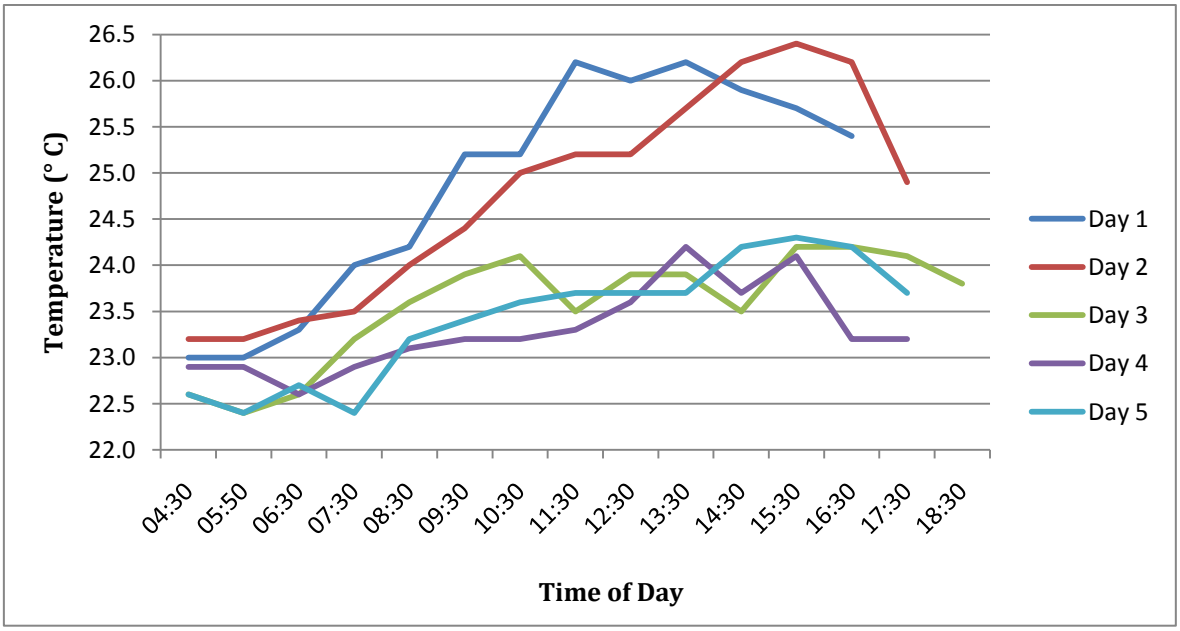

Figure 4.1: Line graph of daily theatre temperatures

Table 4.4: Theatre temperatures (°C) according to study periods

	t = 0	t = 1	t = 2	t = 3	t = 4	t = 5	t = 6	t = 7	t = 8	t = 9	t = 10	t = 11	t = 12
MIC-001	23,0	23,0	23,3	24,0	24,2	25,2	25,2	26,2	26,0	26,2	25,9	25,7	25,4
MIC-002	23,0	23,0	23,3	24,0	24,2	25,2	25,2	26,2	26,0	26,2	25,9	25,7	25,4
MIC-003	23,2	23,2	23,4	23,5	24,0	24,4	25,0	25,2	25,2	25,7	26,2	26,4	26,2
MIC-004	23,2	23,2	23,4	23,5	24,0	24,4	25,0	25,2	25,2	25,7	26,2	26,4	26,2
MIC-005	23,2	23,4	23,5	24,0	24,4	25,0	25,2	25,2	25,7	26,2	26,4	26,2	24,9
MIC-006	23,2	23,4	23,5	24,0	24,4	25,0	25,2	25,2	25,7	26,2	26,4	26,2	24,9
MIC-007	22,6	22,4	22,6	23,2	23,6	23,9	24,1	23,5	23,9	23,9	23,5	24,2	24,2
MIC-008	22,6	22,4	22,6	23,2	23,6	23,9	24,1	23,5	23,9	23,9	23,5	24,2	24,2
MIC-009	22,4	22,6	23,2	23,6	23,9	24,1	23,5	23,9	23,9	23,5	24,2	24,2	24,1
MIC-010	22,4	22,6	23,2	23,6	23,9	24,1	23,5	23,9	23,9	23,5	24,2	24,2	24,1
MIC-011	22,6	23,2	23,6	23,9	24,1	23,5	23,9	23,9	23,5	24,2	24,2	24,1	23,8
MIC-012	22,6	23,2	23,6	23,9	24,1	23,5	23,9	23,9	23,5	24,2	24,2	24,1	23,8
MIC-013	22,9	22,9	22,6	22,9	23,1	23,2	23,2	23,3	23,6	24,2	23,7	24,1	23,2
MIC-014	22,9	22,9	22,6	22,9	23,1	23,2	23,2	23,3	23,6	24,2	23,7	24,1	23,2
MIC-015	22,9	22,6	229	23,1	23,2	23,2	23,3	23,6	24,2	23,7	24,1	23,2	23,2
MIC-016	22,9	22,6	22.9	23,1	23,2	23,2	23,3	23,6	24,2	23,7	24,1	23,2	23,2
MIC-017	22,6	22,4	22,7	22,4	23,2	23,4	23,6	23,7	23,7	23,7	24,2	24,3	24,2
MIC-018	22,6	22,4	22,7	22,4	23,2	23,4	23,6	23,7	23,7	23,7	24,2	24,3	24,2
MIC-019	22,4	22,7	22,4	23,2	23,4	23,6	23,7	23,7	23,7	24,2	24,3	24,2	23,7
MIC-020	22,4	22,7	22,4	23,2	23,4	23,6	23,7	23,7	23,7	24,2	24,3	24,2	23,7
Range	0,8	1,0	1,2	1,6	1,3	2,0	2,0	2,9	2,5	2,7	2,9	3,2	3,0
Mean	22,8	22,8	23,0	23,4	23,7	24,0	24,1	24,2	24,3	24,6	24,7	24,7	24,3
SD	0,29	0,34	0,42	0,51	0,46	0,70	0,76	0,94	0,91	1,03	1,04	1,03	0,93

T = 0 through T = 12 indicate time at 0 through 12 hours respectively
MIC-001 through MIC- 020 correspond to the 20 syringe pairs investigated
Range = range of temperatures for corresponding times across study periods
SD = standard deviation
All temperatures quoted in degrees Celsius

The sample which yielded microbial growth was taken at *Time = 0 hours*, when the theatre temperature was 22,6° C. This is one of the lowest temperatures recorded (z score = -1,29).

4.4 Secondary Outcome Results: Numbers of cases done in theatre

4.4.1 Recording of number of cases done in theatre during the study period

Official case start and end times were extracted from the theatre register in the obstetric theatre on the days over which the study ran. Start and end times for cases which were in progress during the study periods on each day referred to in Table 4.1 were noted (Table 4.5). These times were then used to determine the number of cases which had been commenced between when each syringe pair was prepared and the corresponding samples were withdrawn (Table 4.6).

Table 4.5: Start and end times of all cases in progress during study periods on each day

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
1ST CASE	09h15 – 09h50	09h20 – 10h00	04h10 – 05h00	09h10 – 10h20	05h05 – 05h30
2ND CASE	10h10 – 11h15	10h40 – 11h20	05h30 – 06h30	10h30 – 11h20	Not recorded
3RD CASE	11h30 – 12h10	12h15 – 12h50	08h15 – 09h10	11h30 – 12h15	09h30 – 10h10
4TH CASE	12h30 – 13h50	13h15 – 14h15	09h25 – 10h00	13h00 – 13h50	10h25 – 11h25
5TH CASE	14h45 – 15h45	14h35 – 15h25	11h15 – 12h15	14h35 – 15h05	11h50 – 12h55
6TH CASE		16h45 – 17h45	12h40 – 13h50	16h15 – 17h20	13h50 – 14h40
7TH CASE		18h00 – 20h20	17h00 – 17h50		15h35 – 16h10
8TH CASE			18h15 – 19h45		

4.4.2 Findings of investigation into number of cases done during the study period

Within each corresponding sampling period, the number of cases which had been commenced was very similar. In fact, the greatest range for any sampling period was 3 cases (Table 4.6).

Table 4.6: Number of cases performed between preparation of syringe pair and withdrawal of samples with the range for corresponding samples

	CS-00	MS-00	MS-04	MS-08	MS-12	CS-12
MIC-001	0	0	1	4	4	5
MIC-002	0	0	1	4	4	5
MIC-003	0	0	1	4	6	6
MIC-004	0	0	1	4	6	6
MIC-005	0	0	1	5	7	7
MIC-006	0	0	1	5	7	7
MIC-007	1	1	2	5	6	6
MIC-008	1	1	2	5	6	6
MIC-009	0	0	1	4	5	5
MIC-010	0	0	1	4	5	5
MIC-011	1	1	2	4	5	5
MIC-012	1	1	2	4	5	5
MIC-013	0	0	0	3	6	6
MIC-014	0	0	0	3	6	6
MIC-015	0	0	1	4	6	6
MIC-016	0	0	1	4	6	6
MIC-017	0	0	2	5	7	7
MIC-018	0	0	2	5	7	7
MIC-019	1	1	2	5	7	7
MIC-020	1	1	2	5	7	7
Range	1	1	2	2	3	2

CS-00 & CS-12 were samples taken from the control syringes at 0 and 12 hours respectively
MS-00, MS-04, MS-08 and MS-12 were samples taken from the multi-dose syringes at 0, 4, 8 and 12 hours respectively
MIC-001 through MIC- 020 correspond to the 20 syringe pairs investigated

4.5 Summary of findings

One sample from one of the twenty multi-dose syringes yielded microbiological growth, which was identified as *Staphylococcus aureus*. None of the control syringes

yielded growth. In a future study, the sample size needed to confirm a difference in contamination rates between the two groups is 581 syringes in each group.

Measurement of theatre temperatures indicated a consistent diurnal variation. However, there was considerable discrepancy between temperatures recorded for correlating times between the twelve-hour study periods. The only sample which yielded microbial growth, however, was taken when the theatre was almost at the coldest temperature recorded during the study.

The number of theatre cases commenced at each sampling time was recorded. It was found that there was not a great variation in number of cases commenced for corresponding sampling times between twelve-hour study periods.

CHAPTER 5: DISCUSSION

Various difficulties and limitations were encountered during the execution of this study and these will be discussed in this chapter. Further, the legal implications of using the controlled substance, fentanyl, in a multi-dose syringe will be considered. The results of this pilot study will then be examined and, finally, suggestions will be made for future work in this area.

5.1 Difficulties encountered

Some unforeseen practical difficulties were encountered during the implementation of this pilot study. It will be useful to make note of these difficulties for future research.

5.1.1 Exclusion of one sample

Whilst the greatest of care was taken to maintain asepsis at all times, a needle used to inoculate one sample was accidentally brushed against the inside of the thioglycollate tube as the sample was being transferred. This was considered to constitute a breach in asepsis and the sample was discarded.

5.1.2 Difficulty in getting the samples into the incubator within 30 minutes

Even though the NHLS laboratory at CHBH is within walking distance from the obstetric theatres, there were difficulties in negotiating the practicalities of transporting the sample to the laboratory, ensuring that the sample was properly logged onto the computerised system and then placed in the incubator within 30 minutes of collection. In order to mitigate this, an arrangement was made with the laboratory staff that the investigator placed the samples in the incubator before logging them onto the system.

5.1.3 Time constraints and processing of multiple syringe pairs each day

Due to external time constraints, it was necessary for more than one syringe pair to be investigated each day. An arrangement was made to stagger the sampling intervals (as discussed in 4.1.2), which allowed all samples to be properly handled according to the protocol. However, because of this, some of the secondary outcome data was lost as the time periods were overlapping. Ideally, only one syringe pair should be processed each day.

5.2 Limitations of the study

Since research of this nature has not been done before, this pilot study was designed in order to inform both study methodology and sample size needed for a future study. The design of the pilot study intentionally removed as many confounders as possible

in order to examine the question at hand. However, in removing these variables, the clinical context in which the results of this study are applicable have been narrowed.

5.2.1 This study was carried out by a single investigator

Since anaesthetic personnel differ in their performance of aseptic technique (15), it is possible that the findings of this study were dependent upon the person carrying out the procedure. Therefore results may not necessarily be able to be extrapolated to all anaesthetic personnel using this protocol.

5.2.2 This study was carried out in a single theatre

Microbial contamination of the syringes under investigation may depend upon general microbial colonisation of the theatre environment. Therefore, the results obtained in this particular theatre may not be able to be extrapolated to other theatres.

5.2.3 Each drug studied was supplied by a single manufacturer

For practical purposes, the hyperbaric bupivacaine and fentanyl were acquired from MicroHealth Products and Fresenius-Kabi respectively. Further, the ampoules of each of these drugs came from a single batch, respectively. In order to be able to extrapolate the results further, the investigation should be carried out using the ampoules from multiple batches produced by different manufacturers.

5.2.4 Only one type of culture medium was used

Thioglycollate broth is an all-purpose growth medium which is useful in isolating a wide range of organisms, including aerobic and anaerobic bacteria and yeasts, and is good a yielding recovery from low numbers of microbes in the initial inoculum (68,80). This is the broth which is used in sterility testing under the NHLS laboratory protocols (80). However, there are fastidious organisms which may require other speciality broths in order to grow. It is possible that the extent of our positive results was limited by the decision to use only one broth type.

5.2.5 Samples were sent to one laboratory only

For practical purposes, the NHLS laboratory at Chris Hani Baragwanath Hospital was used to carry out microbiological investigation on the samples obtained during the study. In order to avoid ambiguity in positive results, however, it would be beneficial to draw two samples at a time and send each to a different laboratory for investigation under the same protocol. This would avoid the situation which occurred in this pilot study whereby conclusive interpretation of an ambiguous positive result became impossible.

5.2.6 The samples were incubated in an aerobic incubator only

Samples were processed according to the NHLS Standard Operating Procedures, which specifies incubation in an aerobic incubator (80). There is a possibility that anaerobic organisms may not have grown because of this.

5.3 Discussion of results of investigation of microbiological contamination

Of 119 samples submitted for microbiological investigation, one yielded growth of *Staphylococcus aurues (S. aureus)*. The sample which yielded this grown was taken at the beginning of a study period from a multi-dose syringe (*Time = 0 hours*). However, subsequent samples taken from the same syringe at T*ime = 4, 8 and 12 hours* yielded no growth, either of *S. aureus* or any other microbe. There are several possible explanations for these results. These explanations will be considered in two categories: 1) why it was that one sample was found to be contaminated and 2) if one sample was contaminated, why it was that more samples were not found to be contaminated.

5.3.1 Possible explanations of why one sample was found to be contaminated

5.3.1.1 The possibility that this was true contamination of the syringe

The first explanation for one sample having yielded microbial growth is that there was microbial contamination of the syringe under investigation at the time that the sample was taken. It is assumed that if contamination was sufficient to yield a positive culture result, that this could be clinically significant. Our literature review has suggested that bupivacaine is a potent antimicrobial, which is particularly effective against *S. aureus* (14,33,34,39,48-51,53,54). If the *S. aureus* present in the first sample was subsequently killed by the bupivacaine, this would explain why subsequent samples from the same syringe were found to be sterile. This explanation

would support guidelines published by various bodies (6,7,29), recommending opening a new ampoule of drug for each patient rather than keeping drugs drawn up in syringes in theatre for fear of microbial contamination. Indeed, when taking into consideration the potentially devastating sequelae of introducing pathogens into the intrathecal space, the fact that one sample out of 119 yielded microbial growth is a concerning point.

5.3.1.2 The possibility that this was a spurious result

A second explanation for the growth of *S. aureus* in a single sample is that the contamination of the culture medium occurred at some stage before or after inoculation, rather than as a result of contamination of the contents of the syringe. Since the sample had been taken immediately after the preparation of the drug mixture, and subsequent samples drawn from the same culture medium yielded no microbial growth, this would seem plausible. Previous authors have found spurious positive cultures to be caused by specimen contamination, environmental pathogens in laboratory reagents, laboratory technical error and use of laboratory techniques which have not been validated (82). In this study, this particular tube of growth medium had undergone the same incubation as the others in order to ensure that there was no contamination prior to inoculation and no deviation from the prescribed aseptic technique in sample withdrawal or transfer was noted. However, it is possible that the there was contamination from external sources during the collection or transfer of the sample (for example, if somebody sneezed close to the investigator). Whilst the laboratory staff did not report any breach in laboratory processes, it is possible that the broth was accidently opened in the laboratory which could have allowed spurious contamination at that stage.

5.3.2 Possible explanations of why more samples were not found to be contaminated

The one sample which was found to be contaminated was from a multi-dose syringe taken at the beginning of the study period. No other contamination in either multi-dose or control syringes was noted. Possible explanations for this are considered below.

5.3.2.1 Lack of contamination as an explanation

Since syringes were prepared with clean hands in the theatre environment and all aliquots were withdrawn under strict aseptic conditions, it is possible that there simply was no contamination of the remaining syringes. As discussed in 2.6, the literature suggests that contamination of multi-dose drug containers is exceedingly rare in general.

5.3.2.2 Possibly microbes were unable to survive in the drug mixture

It is conceivable that some microbes may have been inoculated into the syringes under investigation, but were unable to survive or multiply in the drug mixture. As illustrated in Chapter 2, there is conflicting evidence in previous literature as to whether this drug mixture can be expected to support the growth of microbes. The final concentration of bupivacaine in the drug mixture investigated was $4mg.ml^{-1}$, which is greater than the minimum inhibitory concentration (MIC) found by many previous authors for several species (14,34,39,48-54). It is possible that the

antimicrobial activity of bupivacaine dominated over the potential for the dextrose in the mixture to provide nutritional support to any microbes which may have contaminated the syringe. The concern with this explanation, however, is that there are some pathogens which seem to be resistant to the antimicrobial effects of bupivacaine, such *Pseudomonas aeruginosa* (33,39,52,53), which may be allowed to multiply in the drug mixture should contamination take place.

5.3.2.3 The possibility that contaminants were not cultured

Since the final concentration of bupivacaine in the thioglycollate broth was 0.06%, it is unlikely that the bupivacaine would continue to inhibit microbial growth after inoculation into the broth since this is far below the MIC described by previous authors for various species (14,33,34,39,48-54). Thioglycollate broth was selected because it is an all-purpose growth medium which is useful in isolating a wide range of organisms and is good a yielding recovery from low numbers of microbes in the initial inoculum. However, there are some species which are fastidious and may not grow under the laboratory conditions presented. The broth was incubated in an aerobic incubator only, and this could conceivably have decreased the growth of any anaerobes present. However, it was assumed that the broth at the bottom of the tube would contain sufficiently low oxygen tensions to allow for the growth of anaerobic micro-organisms.

5.4 Discussion of secondary outcomes

This pilot study was not sufficiently powered to provide robust evidence to comment the secondary outcomes. Far greater sample numbers would be needed to show statistically significant correlations. However, the following were noted.

5.4.1 Discussion regarding theatre temperature

It was interesting to note that the sample which yielded microbial growth was taken with a theatre temperature of 22,6 °C, with the lowest recorded temperature during the study period being 22,4°C (Table 4.4). This could be coincidence (and would certainly be if this is a spurious result), or could be related to the decreased antimicrobial properties of bupivacaine at lower temperatures (2.3.5).

5.4.2 Discussion regarding numbers of cases

There was very little difference in the numbers of theatre cases having been commenced at corresponding sampling intervals, with the maximum range in any sampling interval being 3 cases (4.4.2). It is therefore not possible to comment upon any relationship between theatre traffic (for which number of cases was being used as a marker) and the risk of microbial contamination of the contents of the syringes. Between four and seven cases were done in the theatre over a twelve hour period. Therefore, with two obstetric theatres working, the estimate used in the design of this study of one caesarean section per hour was fairly accurate.

5.5 Legal implications of the protocol

The drug mixture under consideration contains fentanyl, which is a schedule six drug. As stated in the General Regulations in terms of the Medicines and Related Substances Act, 1965 (Act No 101 of 1965), published by the Department of Health in 2003, all schedule six substances need to logged in a register where they are dispensed (79). According to Regulation 11 of this Notice, the following information needs to be kept:

(a) the name of the medicine or scheduled substance;

(b) the date on which the prescription was dispensed;

(c) the dosage form and quantity of the medicine or scheduled substance;

(d) the name and address of the patient, or, in the case of a prescription issued by a veterinarian, the name and address of the person to whom the medicine or scheduled substance was sold;

(e) where applicable, the name of the medical practitioner, dentist, veterinarian or any other authorised person who issued the prescription; and

(f) prescription reference number (79).

However, the Regulations do *not* state that each ampoule must be registered to a single patient. Therefore, it would fulfil legal requirements if each patient who received a portion of the mixture were registered as having received the equivalent amount of fentanyl. The concentration of fentanyl in the mixture is $10\mu g.ml^{-1}$. If ten patients each receive 2ml of the mixture, the register should document that they each

received 20μg of fentanyl. In this way, all fentanyl dispensed will be accounted for and the names of all patients who received fentanyl will appear in the register.

5.6 Suggestions for future studies

The purpose of this pilot study was to establish whether there is a clear and undeniable risk to patients in using this protocol, in which case further investigation would be unwarranted and the protocol should be immediately abandoned. However, this clear risk was not established with the simple methodology used in this pilot study. To avoid ambiguity in results, the methodology of future work will necessarily be more complicated and sample numbers will be greater. In view of the outcomes of this pilot, the following recommendations are made for future work.

5.6.1 Recommendations to make results more universally applicable

5.6.1.1 Involvement of multiple investigators

Since it would appear that aseptic technique is important (17), but not standardised (27), it would seem prudent to include samples from drug mixtures prepared by many different anaesthetists. Alternatively, one investigator could repeat the procedure using different interpretations of the aseptic technique.

5.6.1.2 Investigations carried out in different theatres

Likewise, samples taken from syringes prepared in many different theatres which service different patient populations would provide evidence which would be applicable to more clinical situations.

5.6.1.3 Use of drugs produced by various manufacturers

The drugs used in the study should be obtained from as many different suppliers as possible, since differences in manufacturing processes may alter results.

5.6.1.4 Use of more diverse microbiological investigations

To ensure that all possible organisms causing contamination are cultured, a greater variety of growth media should be used, and these should be incubated in both aerobic and anaerobic incubators.

5.6.2 Recommendations to decrease the chance of ambiguous results

5.6.2.1 Increased sample size

Part of decreasing the ambiguity of the results obtained in this pilot study will include using greater sample numbers. As explained in 5.2.3.2, the results obtained in this study suggest that 581 syringe pairs would be needed in order to assess a 5% difference in contamination rates between control- and multi-dose syringes.

5.6.2.2 Transfer of samples in a sterile environment

It is of great benefit to prepare the drug mixtures and withdraw samples from the syringes in the theatre environment as this exposes the contents to conditions akin to those in the clinical context. However, transferring the samples into culture media in the theatre environment offers additional opportunity for spurious contamination of the culture medium. Therefore, the samples withdrawn from the syringes should be sealed in an air-tight container and then transported to the laboratory. Here, the samples can be inoculated into growth medium under a laminar flow hood, which would decrease the risk of extrinsic contamination.

5.6.2.3 Processing of samples at multiple different laboratories

Ideally, multiple samples should be withdrawn at each time period and sent to different laboratories. This would give a comparison in the event of a positive culture result. If all laboratories give the same result, it can be assumed that this is a true positive result. Should only one of the laboratories give a positive result, it would be more likely that this is a spurious result. Practicality and funding would dictate how many laboratories could be utilised.

5.7 Summary of discussion

In this chapter, the conclusions drawn from the results of this pilot study have been explored. This pilot study does not show conclusively that the protocol being examined poses risk to patients. In order to gain a more conclusive result, methodology of a future study would have to be more complicated than this pilot to

avoid ambiguous results, and the sample size will need to be greater. Further, in the clinical setting, care would need to be taken in keeping a register of the fentanyl used in order to comply with legislation.

This pilot study has achieved the intention of testing methodology and providing some guidelines for sample sizes needed for future studies. However, in order to gain a clear understanding of the topic, a great deal of further research is required.

CHAPTER 6: CONCLUSIONS

6.1 Implications of this study of resource utilisation

The enormous case load which is placed upon public hospitals in developing countries mandates that any measures which save costs without decreasing the quality of health care should be considered. At CHBH, there is an average of over 600 caesarean sections performed per month (77). With such a great number of caesarean sections, the protocol for mixing and using drugs reviewed in this study could potentially save the South African Department of Health over R54 000,00 per annum (Appendix F). Purely from a financial view, if there is a real risk to patient safety in using this protocol, then the cost-saving implications in terms of the drugs used would be negated by the far greater financial burden of treating a patient with an infectious complication of spinal anaesthesia. Naturally, there are also ethical and philanthropic considerations to be taken aside from the purely financial factors. However, if it can be shown that the risk is no greater using this protocol than in opening new ampoules for each patient, consideration should be given to instituting this protocol as a means of sound resource utilisation.

6.2 Future work to be done

This initial study failed to show conclusively that the use of this protocol increases risk to patient well-being. However, there is a great deal more work which needs to be done in order to assess what risk there is. In order to do this, a far broader study will need to be undertaken. This will include larger sample numbers, as discussed in Chapter 4, and altering methodology employed, as discussed in Chapter 5.

Should such studies indicate that the risk to patients is no greater when using the multi-dose protocol than when using single-dose ampoules, the protocol may be accepted for clinical use in resource-limited environments. In this case, ongoing audit should be carried out to survey for infectious complications as discussed in 2.2. At this stage, it would be useful to compare incidence of complications between centres which use single-dose ampoules for individual patients only (6,7), those which use single-dose ampoules for multiple patients (8) and those which use the multi-dose protocol under investigation in this study.

6.3 Closing remarks

Currently accepted guidelines on the use of multi-dose syringes in the anaesthetic environment are based largely upon the assumption of good practice rather than evidence (6,7,29). However, the reality in resource-limited settings is that drugs used for spinal anaesthesia are often in short supply and, as a result, practitioners regularly use single-dose ampoules for more than one patient (8). Therefore, it is of

utmost importance to establish appropriate, evidence-based guidelines to optimise resource utilisation without compromising patient safety.

This pilot study is the first step taken towards establishing this evidence in connection with the protocol for preparing a drug mixture for spinal anaesthetic in a multi-dose syringe. It is hoped that future work based upon this pilot study will assist in establishing such appropriate guidelines, which take cognisance of the reality of constraints in resource-poor environments whilst striving to ensure patient safety and well-being.

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APPENDICES

<u>APPENDIX A:</u> Protocol for drug preparation for spinal anaesthesia at Chris Hani Baragwanath Hospital

OPTIONS FOR INTRATHECAL INJECTIONS FOR CAESAREAN SECTION

OPTION 1

2.5ml heavy bupivacaine (0.5%)

Use premixed, or mix 2.25ml bupivacaine with 0.25ml 50% dextrose.

OPTION 2

2.0ml volume, with heavy bupivacaine and fentanyl 12-20µg

Using a 20ml syringe, draw up 16ml of heavy bupivacaine. Add 1-2amps (100-200µg) fentanyl.

If you added 1 amp (total 18ml), then a 2.0ml spinal will contain 8.88mg of bupivacaine, and 11.11µg of fentanyl.

If you added 2 amps (total 20ml), then a 2.0ml spinal will contain 8mg of bupivacaine, and 20µg of fentanyl.

SOME INFORMATION

Adding fentanyl to intrathecal bupivacaine improves both the ***quality*** and ***duration*** of the block. It allows for lower doses of bupivacaine to be used, thus decreasing the ***haemodynamic*** effects of the sympathetic block achieved by bupivacaine alone.

Concerns regarding the use of fentanyl relate to its side effects. There may be ***pruritus*** (usually limited to the nose, for some reason, and for a short period only), ***nausea/vomiting*** (which is minimal with doses below 50µg, and a lot less of a problem than the nausea associated with hypotension), and there is always a concern about ***respiratory depression***. Intrathecal doses of up to ***20µg*** have repeatedly been found to be ***safe***, and patients are not required to be monitored postoperatively for longer than the usual recovery period.

Dr P Penfold
Senior Consultant #6722
Obstetric Anaesthesia

APPENDIX B: Ethics Waiver

University
of the Witwatersrand,
Johannesburg

Human Research Ethics Committee (Medical)
(formerly Committee for Research on Human Subjects (Medical)

Secretariat: Research Office, Room SH10005, 10th floor, Senate House • Telephone: +27 11 717-1234 • Fax: +27 11 339-5708
Private Bag 3, Wits 2050, South Africa

Ref: W-CJ-090930-1
30/09/2009

TO WHOM IT MAY CONCERN:

Waiver: This certifies that the following research does not require clearance from the Human Research Ethics Committee (Medical).

Investigator: **Dr Gwen Morgan. MMed candidate, Student No 9802209D**

Project title: **Microbial growth in a mixture of hyperbaric bupivicaine and fentanyl prepared in a multi-dose syringe in the operating theatre environment.**

Reason: This is a wholly laboratory study. There are no humans involved.

UNIVERSITY OF THE WITWATERSRAND
PROF PE CLEATON - JONES
HREC (MEDICAL)
2009 -09- 30
JOHANNESBURG

Professor Peter Cleaton-Jones
Chair: Human Research Ethics Committee (Medical)

copy: Anisa Keshav, Research Office, Senate House, Wits

APPENDIX C: Faculty of Health Sciences Post-Graduate Committee Approval

Faculty of Health Sciences
Medical School, 7 York Road, Parktown, 2193
Fax: (011) 717-2119
Tel: (011) 717-2745

Reference: Ms Tania Van Leeve
E-mail: tania.vanleeve@wits.ac.za
23 November 2009
Person No: 9802209D
PAG

Dr G Morgan
Postnet Suite 6
Private Bag X1
Northcliff
2115
South Africa

Dear Dr Morgan

Master of Medicine (in the specialty Anaesthesia): Approval of Title

We have pleasure in advising that your proposal entitled "*Microbial growth in a mixture of hyperbaric bupivacaine and fentanyl prepared in a multi-dose syringe in the operating theatre environment*" has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

APPENDIX D: Permission from Medical Superintendent to conduct research at Chris Hani Baragwanath Hospital

PERMISSION TO CONDUCT RESEARCH AT CHRIS HANI BARAGWANATH HOSPITAL

PRINCIPAL RESEARCHER:

FULL NAME: DR GWEN MORGAN

DESIGNATION: ANAESTHETICS REGISTRAR

CONTACT NUMBER: 082 895 0176

EMAIL: gmorgansa@gmail.com

DEPARTMENT/S: Anaesthetics

HEAD/S OF DEPARTMENT/S: Prof LUNDGREN

TITLE OF RESEARCH: Microbial Growth in a Mixture of Hyperbaric Bupivacaine and Fentanyl Prepared in a Multi-Dose Syringe in the Operating Theatre Environment

OBJECTIVES OF RESEARCH: To determine whether there is microbial growth in the drug mixture of hyperbaric bupivacaine and fentanyl prepared in multi-dose syringes

STUDY SITE/S: Obstetrics Theatre, Chris Hani Baragwanath Hospital

BRIEF OUTLINE OF METHODOLOGY: Syringes will be prepared in the theatre with aliquots withdrawn and transferred to culture media for further microbiological investigation. No patients will be involved.

EXPECTED START DATE: 30/11/2009 (if possible, small pilot study on 28/11/09) EXPECTED DURATION: 1 week.

ETHICS CLEARANCE ? (Y) / N / PENDING

CONFLICTS OF INTEREST ? Y / (N) DETAILS

COSTS TO HOSPITAL AND/OR PATIENTS ? Y / (N) - WHLS account code D9002 billed directly to Creditor's Department

SOURCE OF FUNDING: Faculty of Health Sciences University of the Witwatersrand.

PERMISSION GRANTED (Y) / N

SIGNATURE | M.R. Bhugu: DIRECTOR NAME IN PRINT + DESIGNATION | OFFICIAL STAMP+DATE

MEDICAL SUPERINTENDENT
P.O. BERTSHAM 2013, RSA
2009 -11- 20
CHRIS HANI BARAGWANATH HOSPITAL

APPENDIX E: Data sheet

INVESTIGATION INTO MICROBIAL GROWTH IN MULTI-DOSE SYRINGE
DATA SHEET

SYRINGE PAIR STUDY NUMBER:
Date:

Sample number	**Lab Request Bar Code**	**Lab number**	**Time sample taken**	**Temp (°C) when sample taken**	**Time sample at lab**	**Microbial growth**	**Species Identification**
CS – 00							
MS – 00							
MS – 04							
MS – 08							
MS – 12							
CS – 12							

APPENDIX F: Calculation on costs saved using the multi-dose syringe

At current costs, each ampoule of Micro bupivacaine 0.5% with dextrose 4ml costs R10,50 on government tender, whilst each ampoule of Fresenius Fentanyl 100 µg/2ml costs R1,40 on government tender. According to the protocol in Appendix 1, each 20ml syringe contains four ampoules of the bupivacaine and two ampoules of the fentanyl, which comes to a total cost of R44,80. Assuming a total volume of 2ml of drug mixture used for each intrathecal injection, a syringe containing 20ml of drug mixture as described in Appendix 1 may be used for 10 patients. Therefore, using the multi-does syringe protocol, the cost per patient is R4,48.

If new ampoules of bupivacaine and a new ampoule of fentanyl are opened for each patient, the cost per patient is R11.90

The average number of caesarean sections performed at Chris Hani Baragwanath Hospital over the 18 month period starting January 2008 was 608 caesarean sections per month. According to this calculation, the use of the multi-dose syringe protocol would save R54 136,32 per annum.