# The Prevalence of Propofol Contamination in the Tygerberg Theatre Complex

Dr Carmen Drude

MBChB (Stell), DA(SA)

**In partial fulfilment of the degree**

**MMed Anaesthesiology**

**Promotor**

Dr Marianne Johnson

MBChB (Stell), DA(SA), MMed Anaes (Stell), FCA (SA)

**Statistician**

Michael McCaul and Nyasha Lovemore

Biostatistics Unit, Faculty of Health Sciences, University of Stellenbosch

## Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Signature: _____ Date: _____

## Acknowledgements

Dr Marianne Johnson has really sacrificed so much of her time and wisdom to bring this about. I have learned an enormous amount from her and definitely would not have been able to do this without her.

Prof Jeff Coetzee for his advice and input.

# Table of Contents

**1. ABSTRACT ..... 4**

**2. OPSOMMING ..... 5**

**3. LIST OF FIGURES AND TABLES ..... 6**

**4. LIST OF ABBREVIATIONS ..... 7**

**5. LITERATURE REVIEW ..... 8**

**6. STUDY OBJECTIVE ..... 14**

**7. METHODS ..... 15**
**7.1 Research design ..... 15**
**7.2 Specimen collection ..... 15**
**7.3 Data captured ..... 16**
**7.4 Quality control ..... 17**
**7.5 Strengths and limitations ..... 17**

**8. DATA MANAGEMENT AND STATISTICAL ANALYSIS ..... 18**
**8.1 Determination of sample size ..... 18**
**8.2 Statistical methods ..... 18**

**9. ETHICS AND FUNDING ..... 19**

**10. RESULTS ..... 20**
**10.1 Primary outcomes ..... 20**
**10.2 Secondary outcomes ..... 23**

**11. DISCUSSION ..... 27**

**12. CONCLUSION ..... 29**

**13. REFERENCES ..... 30**

**14. APPENDIX 1 ..... 36**

# 1. ABSTRACT

Propofol is a widely used intravenous anaesthetic agent. Soon after its introduction into the market, propofol-related postoperative infections were reported. It was determined that the emulsion supports growth and manufacturers provided strict aseptic guidelines with regards to propofol usage, but it has been shown that clinicians in South Africa do not adhere to these guidelines.

The primary objective of our observational study was to determine the prevalence of contamination of syringes containing propofol (Fresenius' Propoven® 1%, Fresenius Kabi, Sweden) in the Tygerberg Hospital Theatre Complex. Samples of syringes containing propofol were collected from various randomised operating theatres over a period of 15 days and specimens cultured and tested for growth of organisms.

The secondary objective was to differentiate between contamination occurring in emergency and elective surgery operating theatres and investigate the difference in propofol contamination when used by junior versus more senior anaesthetists.

We demonstrated an unacceptably high propofol contamination prevalence of 41.8% (95% CI: 32.5%, 51.6%). Coagulase negative staphylococcus (CONS) was the most prevalent organism. Overall, 58.18% of samples demonstrated no growth after 2 days. Regarding our secondary objective, there was no statistically significant difference regarding the prevalence of propofol contamination of samples taken from the emergency and elective operating theatres ($p = 0.95$; risk ratio 1.02; 95% CI: 0.55; 1.89). Propofol syringes handled by senior versus junior anaesthetists revealed a similar result ($p = 0.65$; risk ratio 0.90; 95% CI: 0.58; 1.41).

CONS are often contaminants and the bulk of organisms we cultured are non-pathogenic in healthy hosts but can be lethal in immunocompromised patients. Presence of commensals is also a warning that any other pathogens (including viruses) that may be present on the anaesthetist's hands or in the environment may also contaminate the propofol.

The presence of human commensals as well as environmental organisms in the propofol syringes are avoidable and a zero-contamination rate should be aspired to in all interactions with patients.

# 2. OPSOMMING

Propofol word tans algemeen as intraveneuse narkosemiddel gebruik. Propofol verwante postoperatiewe infeksies is egter gou nadat die middel bekendgestel is gerapporteer. Organismes kan groei in die emulsie en die vervaardigers het streng aseptiese riglyne vir die gebruik van propofol daargestel. Dit blyk egter dat praktisyns in Suid-Afrika nie die riglyne volg nie.

Die primêre doel van ons beskrywende studie was om die prevalensie van kontaminasie van spuite met propofol (Fresenius Propoven® 1%, Fresenius Kabi, Swede) in die Tygerberg Hospitaal Teater kompleks te bepaal. Monsters van spuite met propofol is versamel van verskeie gerandomiseerde operasieteaters oor ‘n periode van 15 dae. Die monsters is gekweek en evalueer vir die groei van organismes.

Die sekondêre doel was om te onderskei tussen kontaminasie in teaters waar nood en elektiewe chirurgie uitgevoer word en om te bepaal of daar ‘n verskil in propofol kontaminasie is tussen junior en meer senior narkose personeel.

Ons het ‘n onaanvaarbare hoë prevalensie van propofol kontaminasie van 41.8% (95% CI: 32.5%, 51.6%) gevind. Koagulase negatiewe stafilokokki was die mees prevalente organisme. Geen groei is gevind in 58.18% van die monsters na 2 dae. Wat betref ons sekondêre doel, was daar geen statisties beduidende verskil in die prevalensie van propofol kontaminasie in die monsters geneem in die nood en elektiewe teaters nie ($p = 0.95$; risk ratio 1.02; 95% CI: 0.55; 1.89). Daar was ook geen verskil in monsters van spuite gebruik deur junior en meer senior narkose personeel nie ($p = 0.65$; risk ratio 0.90; 95% CI: 0.58; 1.41).

Koagulase negatiewe stafilokokki is dikwels kontaminante en die meeste van die organismes wat ons gekweek het is nie patogenies in normale gashere nie. Dit kan egter dodelik wees in pasiënte wat imuunkompromiseerd is. Kontaminasie met kommensale organismes dien as a waarskuwing dat enige patogene (insluitend virusse) op die narkotiseur se hande of in die omgewing in die propofol mag land.

Die teenwoordigheid van menslike kommensale organismes sowel as omgewings organismes in die propofol spuite is voorkombaar. Daar moet gestreef word na geen kontaminasie in alle interaksies met pasiënte.

# 3. LIST OF FIGURES AND TABLES

**List of Figures**

Figure 10.1: Proportions of the various organisms cultured from 110 propofol-containing syringes

Figure 10.2: Numbers of bacterial species cultured from 110 propofol-containing syringes.

Figure 10.3: Comparison of proportions of contaminated propofol syringes obtained from operating theatres in which senior versus junior anaesthetists were the attending physicians

Figure 10.4: Comparison of proportions of contaminated propofol-containing syringes that were obtained from elective versus emergency operating theatres

**List of Tables**

Table 10.1: Organisms that were cultured

Table 10.2: Culture results

Table 10.3: Secondary outcomes

# 4. LIST OF ABBREVIATIONS

| | |
|---|---|
| BACSP | *Bacillus cereus* |
| BACCE | Bacillus species |
| BHI | brain heart infusion |
| CBA | chocolate blood agar |
| CFU | colony forming units |
| CLOHI | *Clostridium histolyticum* |
| CNS | central nervous system |
| CONS | coagulase negative Staphylococci |
| CI | confidence interval |
| EDTA | disodium ethylenediaminetetraacetate |
| FDA | Food and Drug Administration |
| GABA | gamma-aminobutyric acid |
| iNOS | inducible nitric oxide synthase |
| IV | intravenous |
| MCCSP | Micrococcus species |
| Pr | probability |
| RR | risk ratio |
| STRPA | *Streptococcus parasanguinis* |
| STAEP | *Staphylococcus epidermidis* |
| STRMO | *Streptococcus mitis/oralis* |
| TIVA | total intravenous anaesthesia |

# 5. LITERATURE REVIEW

Propofol, an intravenous anaesthetic agent, is commonly used for both in- and outpatient surgical procedures.[1]

Propofol's hypnotic effects result from its potentiation of the inhibitory function of the neurotransmitter gamma-aminobutyric acid (GABA). Quick onset of action and rapid elimination make it an ideal drug for monitored anaesthetic care in ambulatory surgery, sedation in intensive care units and for total intravenous anaesthesia (TIVA).[2-6]

Unfortunately, soon after introduction into clinical practice in 1989, various infections related to the intravenous use of propofol were reported and the lipophilic solution has since consistently been shown to support bacterial growth. [7]

**Biochemical data**

Propofol (2,6-diisopropylphenol) is dissolved in a hydrophobic emulsion manufactured as a 1% and a 2% solution in a glass ampule. The emulsion consists of 10% soybean oil, 1.2% egg phospholipid and 0.25% glycerine. The preparation is an isotonic solution and has a pH of 7 to 8.5, creating a favourable environment for bacterial growth.[8]

**Propofol uses**

At a dose of 2 to 2,5mg/kg, propofol reliably suppresses airway reflexes, producing optimal conditions for intubation or placement of a laryngeal mask.[9,10] Propofol has antipruritic as well as anti-emetic properties and can aid in terminating seizures. Expert opinion is still divided regarding the analgesic or anti-hypersensitivity effects of propofol, given conflicting reports in experimental and clinical pain.[11] Propofol is a safe anesthetic agent in malignant hyperthermia suspectable patients.

**Pharmacodynamics**

Following intravenous administration, propofol often induces apnoea, depending on the dose, rate of administration and presence of other respiratory depressive drugs. Ventilatory response to $CO_2$ and hypoxia is reduced. Propofol produces endothelium-independent vasodilatation, possibly through calcium channel antagonism. The cerebral metabolic rate is also lowered with resultant reduction in cerebral blood flow and intracranial pressure.[12]

**Pharmacokinetics**

Loss of consciousness occurs in 'one arm-brain circulation' after an intravenous bolus, with effects lasting approximately 5 to 10 minutes. After prolonged infusion, the context-sensitive half-time is quoted as 40 minutes by the manufacturer.[13]

Central nervous system (CNS) actions are primarily terminated by redistribution, due to the lipophilicity of the drug. Plasma elimination is mainly by hepatic metabolism (60%), with renal metabolism accounting for another 30% of extraction. Only 0.3% is secreted unchanged in the urine. Glucuronidation is the major metabolic pathway of propofol, with no active metabolites being produced.[14]

**Propofol as possible immunosuppressing agent**

Propofol is thought to impair the immune response of the host in various ways. Cytokines such as tumour necrosis factor alpha, which has pro-inflammatory properties, are reduced.[15]

Inducible nitric oxide synthase (iNOS) gene expression is an important factor involved in antimicrobial barriers. Exposure to propofol reduces expression of iNOS and inhibits macrophage functioning.[15-17]

**Incidence and distribution of reports of propofol-related infections**

Twenty outbreaks of propofol related infections were reported from 1989 to 2014. In total, 144 patients were infected of whom 10 patients died.[18] Vonberg and Gastmeier reported a mortality rate of 13.8% associated with propofol infections.[18]

Geographically, the distribution of outbreaks was widespread in developed countries. No outbreaks have been reported in developing countries, most likely due to lack of follow-up. The incidence of contaminated propofol cases is greater in operating theatres than in the ICU with endoscopic procedures being the most frequently associated procedure.[18]

**Morbidity and mortality**

Introducing contaminated agents into the patient's bloodstream can lead to systemic as well as surgical site infections. Length of hospital stay is increased by more than 7 days in patients with nosocomial bloodstream infections.[19]

Bennett et al reported followed 49 patients who developed infections due to contaminated propofol across 7 hospitals over a period of 32 months. Of these, 20 patients required readmission to hospital, 8 of

them had a prolonged hospital stay, 11 patients underwent additional surgical procedures and 2 of the patients died.[37]

**Mechanisms of contamination of propofol formulations**

Microbiological contamination of propofol can occur during manufacturing (intrinsic) or from the environment after opening the ampule or vial (extrinsic). It has been well-documented that extrinsic methods of contamination are the most common, with only one report of a batch of intrinsically contaminated propofol in the USA in 2009.[18] Extrinsic contamination may occur during storage and breakage of ampules or during drawing up into, and handling of syringes. Recent evidence suggest that unused syringes lying in operating theatres results in a contamination rate of 10%, which can increase to 26.5% with recapping of syringes.[19] Zacher et al. showed that swabbing the neck of propofol ampules with an alcohol swab prior to opening it, reduces bacterial contamination.[20] Hemingway et al. investigated 100 ampules of fentanyl and diamorphine that had been wiped with isopropyl alcohol and were compared with ampules that had not been wiped. Microorganisms were found on the inside neck of 18% of the ampules that had not been wiped and none on the wiped ampules.[21]

The majority of reports of extrinsic contamination occurred in previously used vials, syringes or microdroppers as well as intravenous (IV) injection port dead spaces that served as reservoirs for bacterial growth.[18] Environmentally exposed ampoules that are not used immediately have been identified as a contributing factor with an increase in contamination risk of 25% within 12 hours.[18] It has been shown that re-using vials and pump infusion lines as well as preparing multiple propofol syringes in advance increase the risk for bacterial contamination.[22, 23]

Price and Loftus described the transmission of various organisms from the surrounding environment, especially within the anaesthetic workstation.[24, 25] The patient's skin, frequently touched environmental sites around the patient, hands, gowns and gloves were all potential sources of pathogen spread. Despite having been proven to decrease spread of infection, adherence by operating theatre staff to hand-hygiene guidelines is significantly low and has been repeatedly shown to be the cause of bacterial contamination of propofol.[26-29] Anaesthetists' hands account for about 50% of contamination events.[19]

Following the first propofol associated post-surgical fevers and infections, in 1990, the United States Food and Drug Administration (FDA) issued recommendations for strict aseptic drug handling techniques. These guidelines are included in manufacturer's propofol package insert.

- Propofol handling guidelines as recommended by the FDA: Vials and ampules of propofol and prefilled syringes are intended for single-patient use.
- Strict aseptic technique must be practised when handling injectable medications.
- Vials and ampules should be inspected before use for particulate matter, discoloration, or evidence of separation of the emulsion. Do not use if contaminated.
- Fill syringes or spike the vial immediately before administration to each patient. Begin infusion immediately after drawing up or opening the vial/ampule.
- Disinfect vial rubber stoppers with 70% isopropyl alcohol.
- Discard unused portions within 6 hours of filling syringes or 12 hours after spiking a large volume vial/ampule for infusion.

The South African Society of Anaesthesiologists (SASA) recently published similar guidelines for the prevention of anaesthetic related infections.[30] Breedt et al. conducted an anonymous survey to determine whether SASA members are aware of existing guidelines, and if so, whether they adhere to aseptic handling techniques. They concluded that unsafe practices regarding handling and administration of propofol persisted and that the guidelines were of low impact.[30] Of the 542 members that participated in the survey, 61% were aware of the guidelines, with 47.3% having read and familiarized themselves with the guidelines. The general practice of participants revealed that 16% admitted to using the same propofol syringe on various patients while 21% of the participants reused 50ml syringes for infusions and 30% of the latter group used the same extension tubing for different patients. The authors noted no reports of infection associated with propofol use occurring where safe injection practices were followed.[30]

Seeberger et al. also studied the effect of educating staff and stringent implementation of correct aseptic handling of propofol. Practices were monitored by an infection control practitioner. They concluded that the aseptic precautions were efficient and with proper education, propofol-related infections could be prevented.[31]

**Microorganisms**

Numerous studies have shown propofol to provide an excellent growth medium for not only bacteria, but also for fungal growth.[32-35] Viral stability offered by the lipid emulsion is optimal for prolonged

survival of hepatitis C virus.[36] Zorrilla-Vaca et al. reported that 23% of published infection outbreaks were due to hepatitis C with hepatitis B virus demonstrated in 4.2% [18] 27.1% of infections were caused by Gram positive organisms and 20.1% by Gram negative organisms. *Staphylococcus aureus* remained the most common pathogen.[18]

According to Bennett et al. most postoperative infections traced to contaminated propofol were caused by *Staphylococcus aureus, Candida albicans, Moraxella osloensis, Enterobacter agglomerans* and *Serratia marcescens*.[37]

Other pathogens that have been grown from extrinsically contaminated propofol are the Gram negative organisms *Klebsiella pneumoniae* and *Serratia marcescens*.[37] Cilli and Henry reported isolating *S marcescens* from propofol syringes, blood, and respiratory cultures, after patients developed wound sepsis or bacteremia post-surgery.[38, 39] Endotoxin production has also been demonstrated.[19] A recent study by Zorilla-Vaca grew Corynebacterium species, *S. epidermidis*, Bacillus species, *Enterococcus faecalis*, Micrococcus and *Pseudomonas aeruginosa* from contaminated propofol vials.[40] Coagulase negative Staphylococci (CONS) other than *S. epidermidis* have been demonstrated as well.

**Improving propofol formulations**

Many proposed antimicrobial agents have been suggested to retard the growth of microorganisms should accidental contamination occur. Most investigated agents have been rejected due to poor efficacy, adverse side effects and high costs. The FDA requires that additives must retard microorganism growth to <10 fold at 24 hours after contamination. Adding ionically charged solutions to propofol can also destabilise the lipid emulsion, which prohibits the use of some additives.

June 1996 brought about the addition of a preservative; disodium edetate (EDTA), a water-soluble chemical which retards microbial proliferation. It removes divalent and trivalent metal cations by chelation, leading to bursting of the microbial cell membrane. Although it does slow bacterial growth, it does not inhibit growth completely, and adhering to aseptic techniques are still emphasised. Propofol preparations containing EDTA are very expensive, further limiting use.[18, 41-44]

Adding lidocaine to propofol also has antibacterial effects.[45, 46] However, even when present in effective concentrations, it does not exert a sufficient retarding effect on microbial proliferation and poses the risk of emulsion deterioration.[47]

Fospropofol, a water-soluble prodrug of propofol, used mainly for sedation, has been shown to be a less favourable medium for bacterial growth. Transient paraesthesia and pruritus on injection are common side effects.[48, 49] Benzyl alcohol has antimicrobial activity at low concentrations, but its use is limited by toxicity. Hall et al. demonstrated the efficacy of a 0.4 micron filter (EmulSiv™ filter) which would serve as an additional precaution to prevent bacteria from entering the solution.[50] The EmulSiv filter is a potentially viable solution to the problem of extrinsic contamination, if production costs can be lowered.

Due to multiple concerns regarding bacterial contamination as well as pain on administration and hyperlipidaemia, propofol has undergone various transformations over the years in attempts to overcome these unwanted adverse effects. Focus has been geared towards the addition of external compounds. However, major safety concerns continue to require further research.[51] Newer developments have not yet been proven to possess safer side effect profiles or more ideal pharmacological properties.

# 6. STUDY OBJECTIVE

Breedt et al.[30] has shown that general adherence to the manufacturer's guidelines regarding propofol usage is low among anaesthetists in South Africa. The objective of this study was to investigate the prevalence of extrinsic contamination and resulting bacterial growth that occurs with use and possibly reuse of Fresenius Propoven 1% (10mg/ml, 20ml) (Fresenius Kabi, Sweden) in the Tygerberg Hospital operating theatres.

Secondary objectives were formulated around modifiable aspects that could potentially influence results and shed light on target groups and areas for intervention. Hence the experience and seniority of the attending doctor was included. The assumption was also made that emergency theatres will yield a higher prevalence of contamination as opposed to the more organised and structured approach in theatres with elective surgery.

# 7. METHODS

## 7.1 Research design

We conducted an observational study to determine the prevalence of extrinsic propofol contamination in the Tygerberg Hospital operating theatres.

After ethical approval was granted, we collected a total of 110 samples from propofol-containing syringes in randomly selected operating theatres, using a strictly aseptic technique. Specimens were obtained over a period of three weeks from the 25$^{th}$ of November 2019 and sent for laboratory analysis. We randomized the operating theatres by drawing the theatres to be sampled (A to Z) from a hat each day. We collected samples at different times of day between 07:00 and 15:30, in order to reduce performance bias.

During the three-week study period, we took 17 samples from the four emergency theatres and 93 samples from theatres dedicated to elective procedures. As the theaters from which specimens were taken were randomly chosen, the surgical disciplines as well as the seniority of the anaesthetists can be considered to be random. Senior anaesthetists in our study were defined as specialist anaesthetists and registrars who have completed intensive care and cardiothoracic rotations. Juniors included registrars in their first two years of the program as well as medical officers. Elective surgery included procedures performed by plastic, neuro, general, urology, paediatric, orthopaedic, gynaecological and cardiac surgical teams. In order to minimize the Hawthorne effect, theatre staff was not made aware of the study prior to sample collection.

All specimens were collected by the author. By not directly involving patients in the study, we avoided multiple confounding factors and the possibility of infringing patient autonomy.

## 7.2 Specimen collection

We withdrew 1ml propofol specimens from any propofol syringe that has already been used for drug administration, using sterile gloves, a sterile 5ml syringe and an 18G needle. We injected the samples into culture bottles and sent them to the Tygerberg National Health Laboratory Service (NHLS) Microbiology laboratory for testing for growth of organisms. The bottled medium comprised a nutrient broth, consisting of oxoid brain heart infusion (BHI) (manufactured by Diagnostic Media, Johannesburg,

South Africa). The broth is a buffered beef extract containing Lab-Lemco powder, a specially selected meat extract and raw materials refined to a powder consistency.

The laboratory protocol for incubation of the specimens each consisting of a 1ml sample of propofol in 5ml BHI Broth (a one in six dilution) was as follows:

- On receipt of the specimen, 100µl of the sample was plated onto a chocolate blood agar (CBA) plate. CBA was chosen as this grows the largest variety of aerobic organisms.
- The CBA plate was incubated for 24 hours at 35°C in ambient air.
- A colony count was done after incubation (Day one).

Colony count formula: number of colonies on plate x 10 = number of colonies per ml x 6 (dilution 1:6 above) = total colony count of original 1ml of propofol.

- The original sample also incubated for 24hrs at 35°C in ambient air

Day 1:

- Sample was then plated onto a tryptose blood agar, CBA and McConkey agar. All plates were incubated for 24 hours at 35°C in ambient air.
- Colony count done as above.

Day 2:

Growth was evaluated and identification of organisms done as follows:

- Conventional biochemical tests
- Automated identification on a Vitek®2 machine (bioMèrieux, Marcy l'Etoile, France) machine - a microbial identification system.

## 7.3 Data captured

We entered the following information into an Excel® spreadsheet:

- Most senior attending anaesthetist: Junior vs Senior Registrar
- Emergency or elective theatre
- The day's case number for that theatre

- Estimated time of opening of propofol ampule and time the sample was taken
- Whether lignocaine had been added or not
- Culture result and colony count

No samples were taken from specimens where the above information could not reliably be attained. As such, none of our collected specimens were excluded from the study.

## 7.4 Quality control

We took care to ensure that specimens were collected correctly, using strict aseptic technique. Equipment, including swabs, syringes and gloves were standardized and all expiry dates checked prior to usage. Delivery to the laboratory was timeous and standard laboratory protocols were followed as per the National Health Laboratory Service. Entry of data in spreadsheet was also checked for accuracy.

## 7.5 Strengths and limitations

Multiple confounding factors and the possibility of infringing on patient autonomy has been avoided due to the laboratory nature of the study.

It is the first study investigating propofol contamination done at our institution and the results will have a major impact on patient management. Clinicians will be more aware of their contribution towards postoperative infection and by them changing their practice, the incidence of morbidity and subsequent length of hospital stay may be significantly reduced.

Limitations to this study include the fact that it is a single center study and cannot be extrapolated to other institutions. Another is that we have no control group, yet there is not quite a need for that.

# 8. DATA MANAGEMENT AND STATISTICAL ANALYSIS

## 8.1 Determination of sample size

Sample size estimation was done using openepi.com software.[52] Based on an anticipated contamination prevalence of 6%, we established that a sample size of 100 specimens would enable us to detect the rate of contamination.

## 8.2 Statistical methods

We analysed the data using Stata version 15 (Statacorp, College Station, TX, USA). We employed Pearson's Chi-square test of independence to assess the presence of any significant relationship between exposure variables and the outcome variable, (propofol contamination.) We calculated risk ratios using 2x2 tables.

| **Primary / Secondary Objectives** | **Outcome** | **Study** | **Data type** | **Predictor / Comparator** | **Test** | **Measure of occurrence / Effect** |
|---|---|---|---|---|---|---|
| Primary | Prevalence of propofol contamination in Tygerberg operating theatres | Observational study | Binary | Not applicable | Not applicable | Prevalence (%) and 95% CI |
| Secondary | Different prevalences regarding emergency versus elective theatres | Observational study | Binary | Theatre: emergency vs elective | Chi Square | Difference between prevalences (%) and the 95% CI of the difference<br>RR with 95% CI |
| Secondary | Different prevalences regarding junior vs senior anaesthetists | Observational study | Binary | Seniority of anaesthetist: registrar/medical officer vs consultant | Chi Square | Difference between prevalences (%) and the 95% CI of the difference<br>RR with 95% CI |

# 9. ETHICS AND FUNDING

The Health Research Ethics Committee of the University of Stellenbosch granted approval to perform the study (Protocol number:S18/10/269). This study was conducted in compliance with the submitted protocol, the International Council for Harmonisation, Good Clinical Practice guidelines and the applicable regulatory requirement(s). Formal informed consent was waived on the grounds that the research design involved no more than minimal risk posed to the patient by the collection of samples and no personal information was captured.

Fresenius Kabi, Cape Town, consented to provide funding for this research project. Fresenius Kabi is a global health care company that provides a variety of products, including Fresenius Propoven 1% and Propofol 2% Fresenius.

# 10. RESULTS

## 10.1 Primary outcomes

Forty-six of the 110 propofol samples produced bacterial cultures (41.8%; 95% CI:32.5%; 51.6%). Of these, 31% of the 110 samples produced cultures of coagulase negative staphylococcus organisms (e.g., *Staphylococcus epidermidis, Staphylococcus haemolyticus*).

The remaining cultures consisted of mixed growths of environmental flora each comprising 2% of the sample. Bacillus species and *Clostridium histolyticum* comprised 2%. Table 10.1 lists the organisms that were cultured.

**Table 10.1: Organisms that were cultured**

| Culture results | Proportion |
|---|---|
| Coagulase Negative Staphylococcus | 30.91% |
| No growth after 2 days | 58.18% |
| Bacillus species , Clostridium histolyticum | 1.82% |
| Bacillus species | 0.91% |
| Bacillus cereus | 1.82% |
| Streptococcus parasanguinis | 0.91% |
| Staphylococcus epidermidis, Bacillus species, Streptococcus mitis/oralis | 0.91% |
| A mixed growth isolated: Environmental organisms isolated | 1.82% |
| A mixed growth isolated, Environmental flora isolated | 1.82% |
| Micrococcus species | 0.91% |

**Figure 10.1: Proportions of the various organisms cultured from 110 propofol-containing syringes**

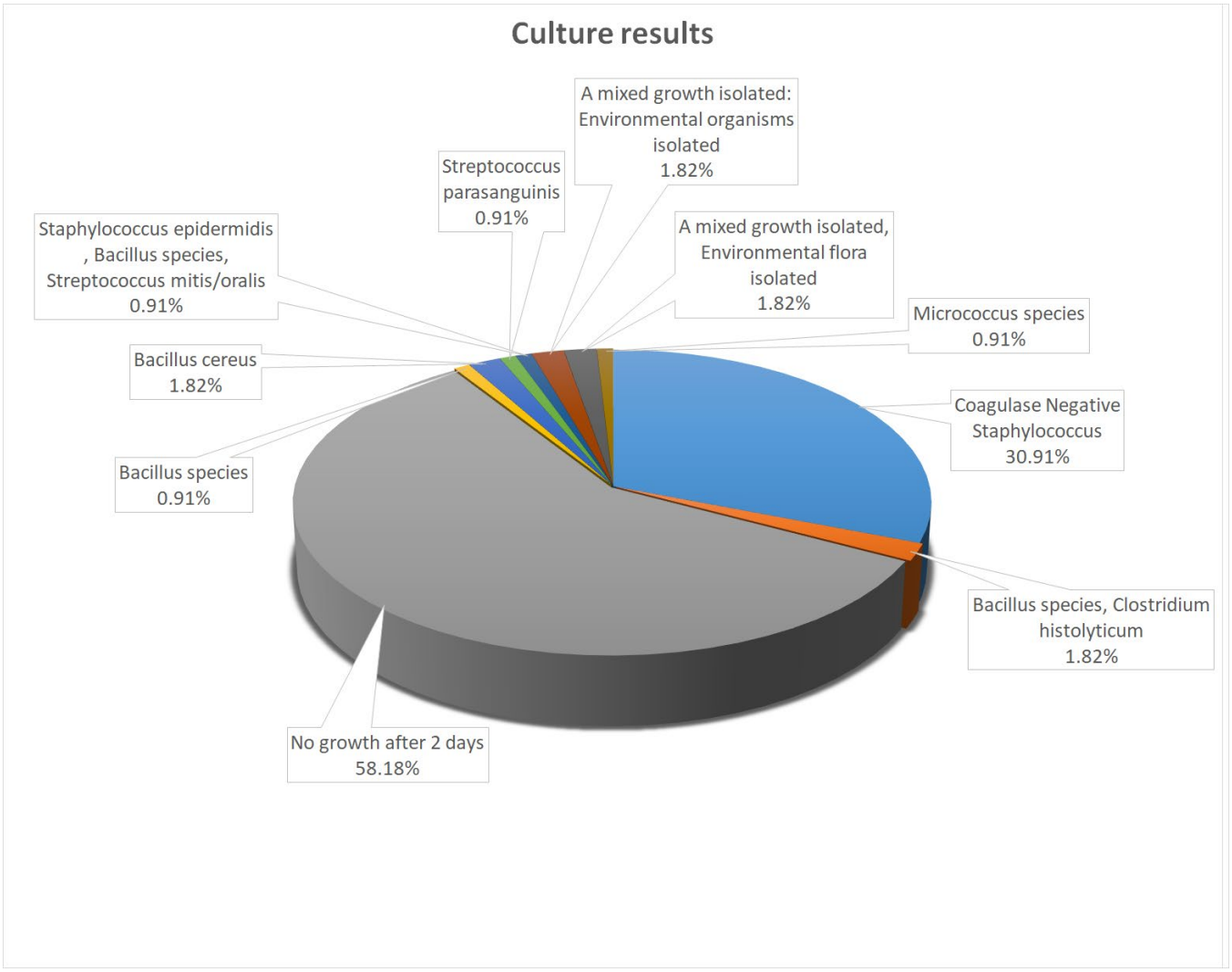


**Figure 10.2: Numbers of bacterial species cultured from 110 propofol-containing syringes.**

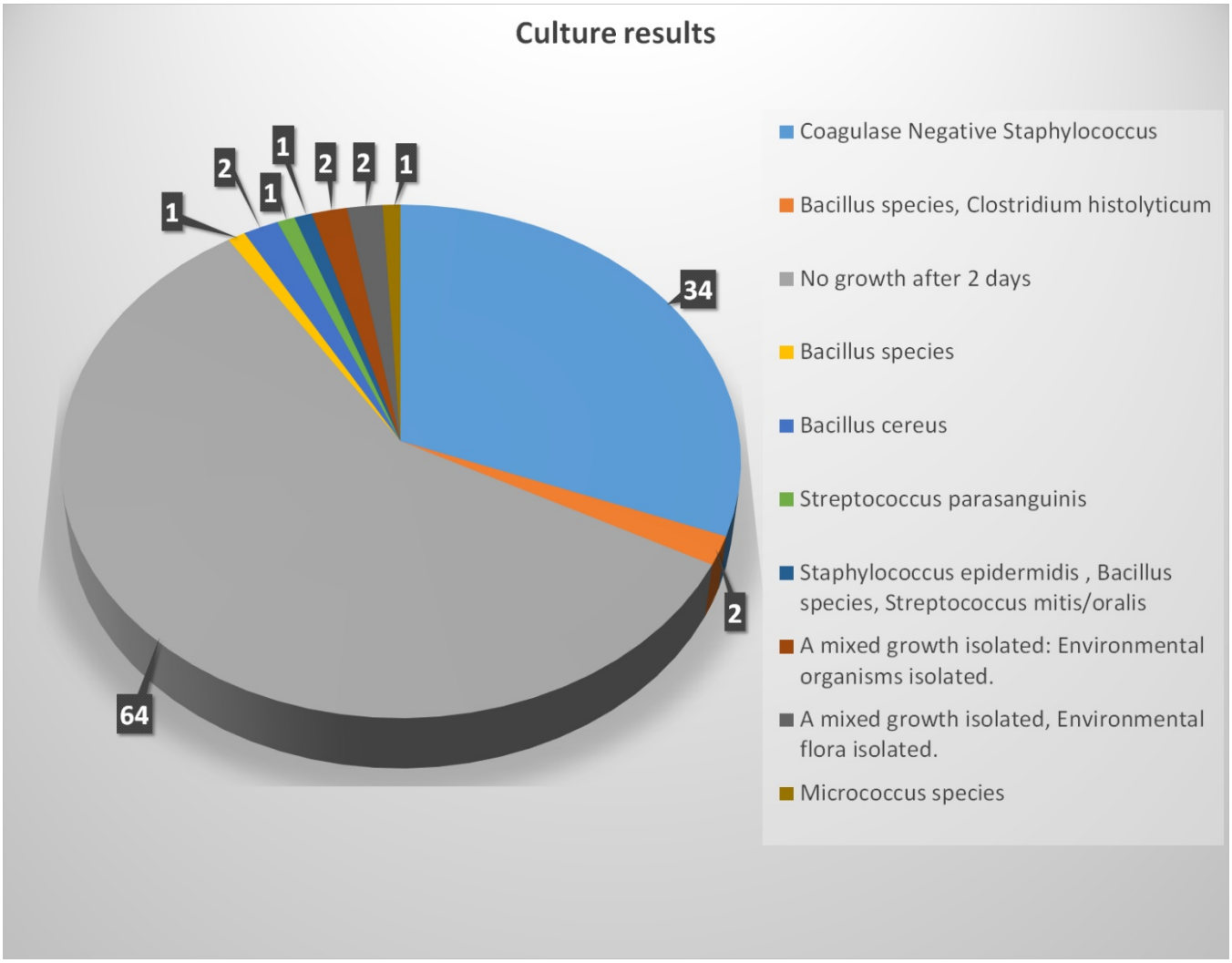
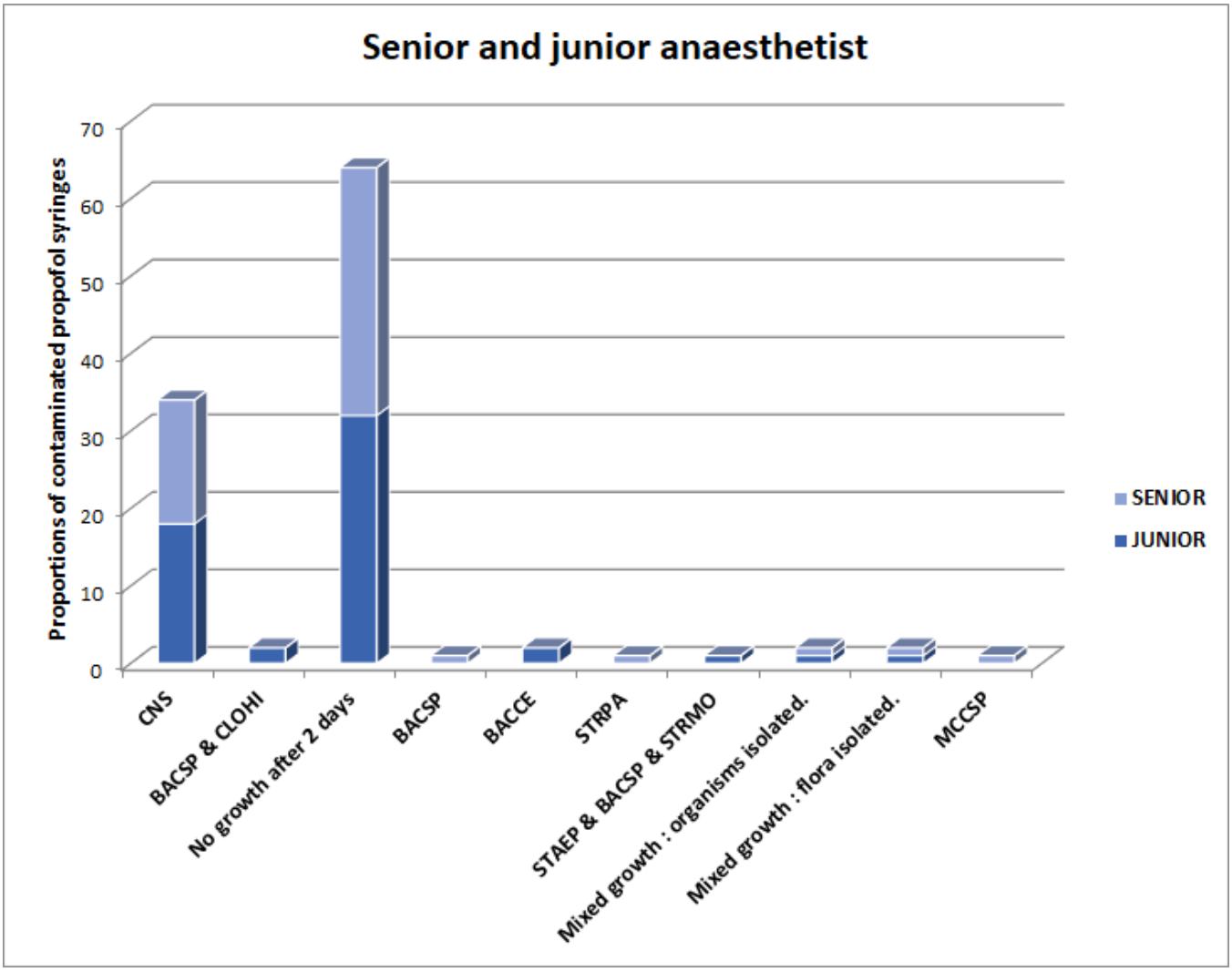



**Table 10.2: Culture results**

| Culture Results | <60 cfu/ml | 60 cfu/ml | 120 cfu/ml | 180 cfu/ml | 240 cfu/ml | 300 cfu/ml | 480 cfu/ml | 780 cfu/ml | 840 cfu/ml | 1500 cfu/ml | 1560 cfu/ml | 1680 cfu/ml | 2820 cfu/ml | 3060 cfu/ml | No count |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A mixed growth isolated, Environmental flora isolated | 2 | | | | | | | | | | | | | | |
| A mixed growth isolated: Environmental organisms isolated | | | | | 1 | | | 1 | | | | | | | |
| Bacillus cereus | 1 | 1 | | | | | | | | | | | | | |
| Bacillus species | | 1 | | | | | | | | | | | | | |
| Bacillus species , Clostridium histolyticum | | | | | | | | | | | 1 | | 1 | | |
| Coagulase Negative Staphylococcus | 2 | 18 | 6 | 1 | | 1 | 1 | | 1 | 1 | | 1 | | 1 | 1 |
| Micrococcus species | | 1 | | | | | | | | | | | | | |
| No growth after 2 days | 64 | | | | | | | | | | | | | | |
| Staphylococcus epidermidis, Bacillus species, Streptococcus mitis/oralis | | | | | | | | | | | | | | | 1 |
| Streptococcus parasanguinis | 1 | | | | | | | | | | | | | | |
| Grand Total | | | | | | | | | | | | | | | |

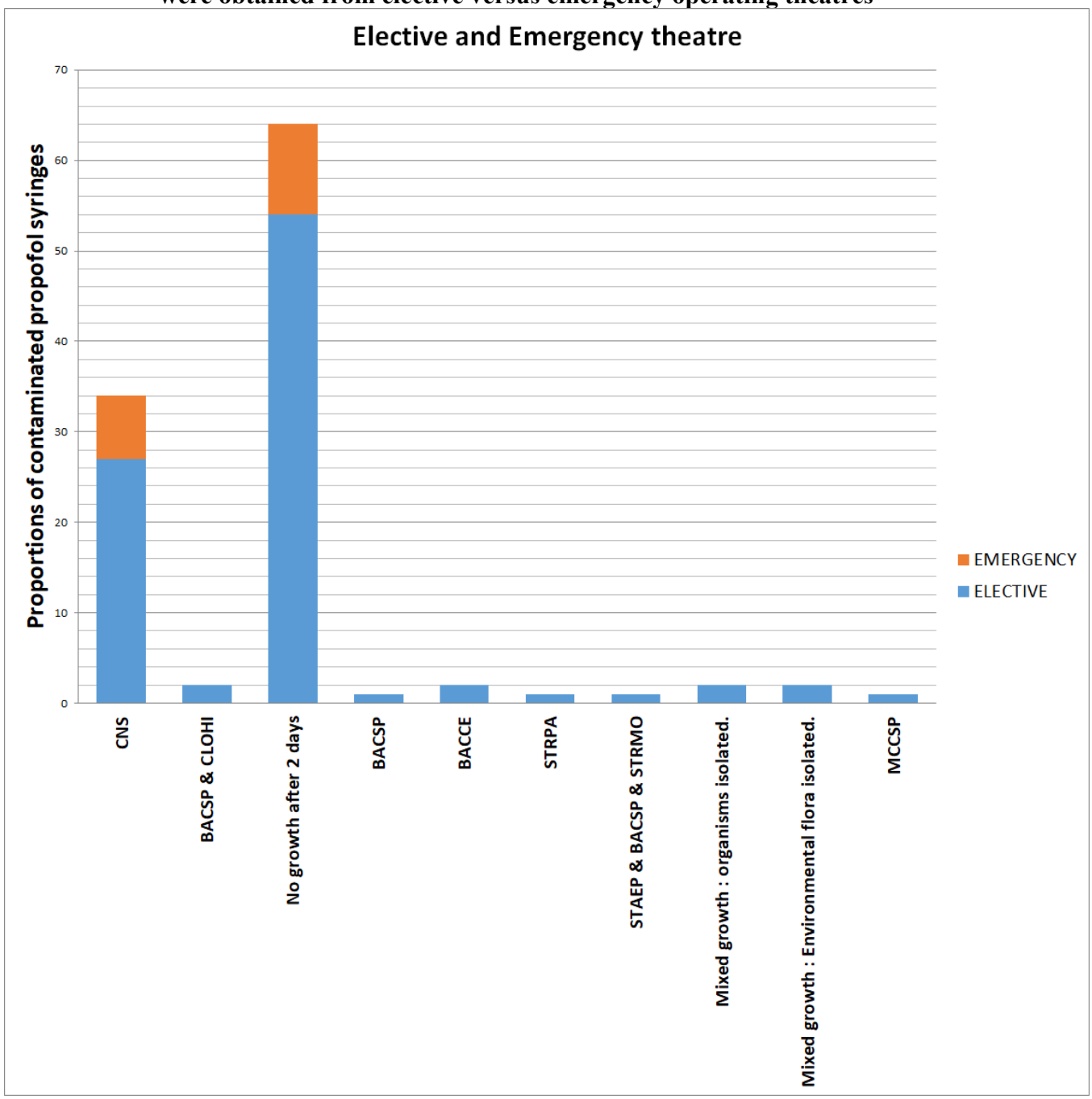
## 10.2 Secondary outcomes

**Figure 10.3: Comparison of proportions of contaminated propofol syringes obtained from operating theatres in which senior versus junior anaesthetists were the attending physicians**




(CNS: Coagulase negative Staphylococcus; BACSP: Bacillus species; CLOHI: *Clostridium histolyticum*; BACCE: *Bacillus cereus*; STRPA: *Streptococcus parasanguinis*; STAEP: *Staphylococcus epidermidis*; STRMO: *Streptococcus mitis/oralis*; MCCSP: Micrococcus species).

**Figure 10.4: Comparison of proportions of contaminated propofol-containing syringes that were obtained from elective versus emergency operating theatres**




(CNS: Coagulase negative Staphylococcus; BACSP: Bacillus species; CLOHI: *Clostridium histolyticum*; BACCE: *Bacillus cereus*; STRPA: *Streptococcus parasanguinis*; STAEP: *Staphylococcus epidermidis*; STRMO: *Streptococcus mitis/oralis*; MCCSP: Micrococcus species)

**Table 10.3: Secondary outcomes**

| Group | Syringes contaminated | Proportions contaminated (95% CI) | Difference between proportions (95% CI) | P (Chi sq) | Risk Ratio (95% CI) |
|---|---|---|---|---|---|
| **Elective theatre** | 54/93 | 58.1% (47.9%; 67.6%) | 0.8% (-24.7%; 26.2%) | 0.9535 | 1.02 (0.55; 1.89) |
| **Emergency theatre** | 7/17 | 41.2% (21.6%; 64.0%) | | | |
| | | | | | |
| **Senior** | 21/53 | 39.6% (27.6%; 53.1%) | 4.2% (-21.9%; 13.8%) | 0.6526 | 0.90 (0.58; 1.41) |
| **Junior** | 25/57 | 43.9% (31.8%; 56.7%) | | | |

In this study, 31% of organisms isolated were coagulase negative staphylococci (CONS), which include *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* species. *Staphylococcus epidermidis*, a normal skin and mucous membrane colonizer, is benign in its interaction with its host and has not been reported to lead to severe infection or disease in immunocompetent individuals.[53] Opportunistic infections do however occur and often involve indwelling medical devices and intravascular catheters, leading to bacterial sepsis.[54] *Staphylococcus haemolyticus* is known to cause meningitis, soft tissue infections, prosthetic joint infections or blood stream infections.[55] CONS's inherent ability to cause a clinically manifested infection is determined by specie- and strain-specific virulence factors. One such factor is the ability to form biofilm, which promotes adherence and colonization of the organism on indwelling devices such as central venous catheters. CONS also contain genes for multiple drug resistance and is therefore complex to treat.[56] Host specific defense mechanisms also affect susceptibility to infection.[57] Sidhu et al. have demonstrated that the greater proportion (69.0%) of isolates of CONS were culture contaminants.[58] They conducted a study to determine the rate of contamination of blood cultures in a tertiary hospital and isolated CONS from 307 of blood cultures. Only 74 out of the 307 cases (24.1%) were pathogenic. In order to determine true pathogenicity, certain clinical and laboratory criteria had to be fulfilled. Clinical criteria included: persistent fever >/= 38˚C or temperature below

36˚C, hypotension (BP<90mmHg), neutropenia or disseminated intravascular coagulopathy. Laboratory criteria included: 2 sets of blood cultures yielding the same bacteria or isolating the same species in one set of initial blood samples and same duration for bacterial growth as a culture result from a different site. The clinical significance of these organisms is thus difficult to determine, considering the detection of CONS bactereamia has to be clinically correlated. Laboratory molecular identification as well as the number of colony-forming units (CFU) need to also be considered. The CFU/ml serves as a representation of the number of microorganisms in the sample that was plated.[59, 60] The CFU ranged between no count up to 3060 CFU/ml. CONS yielded the highest CFU/ml (>4000CFU/ml). Bacillus species and CLOHI followed second highest with 2820CFU/ml.

The correlation between the CFU and the number of bacteria present is not absolute as the addition of broth and solid media increases the complexity.

Bacillus species, such as *Bacillus cereus* (2%), were among the cultured organisms. Their primary habitat is soil although they can be detected in water, decaying organic matter and in normal flora. It has been shown that most bacillus species have not been linked with major diseases in humans or animals, implying unimportant pathogenic activity although food poisoning and trauma induced infections involving bacillus species have been reported. Unfortunately, immunocompromised patients or the critically ill are at risk of falling victim to these opportunistic infections caused by bacilli. [61, 62]

*Clostridium histolyticum* were 1,8% of the contaminants. This species, a Gram-positive anaerobe, is found in soil and sometimes in faeces. It is can cause gas gangrene, releasing exotoxins resulting in necrosis and proteolysis. It has been found to play a role in ulcerative colitis and can also result in infective endocarditis among intravenous drug users.[63]

*Streptococcus parasanguinis* formed 0.91%% of the contaminants. It is an atypical viridans Gram-positive bacterial species, colonizes various body parts, most commonly the oral cavity, and is commonly associated with valvular endocarditis.[64.65]

The micrococcus species are aerobic Gram-positive organisms and colonize the skin, mucosa and oropharyngeal space. Immunocompromised or neutropenic patients are vulnerable to clinically significant infections caused by micrococci. They have occasionally been identified as the infective cause of meningitis, pneumonia, septic arthritis and systemic bacteraemias.[66]

# 11. DISCUSSION

Our investigation of propofol syringes in Tygerberg Hospital theatre complex, revealed a contamination prevalence of 41.84% (95% CI: 32.5%, 51.6%), with coagulase negative staphylococci being the most prevalent organism. This is higher than the expected prevalence of 3 – 6.3%, as reported in the literature.[40] These studies were however conducted in developed countries, making comparison difficult. Organisms cultured in Tygerberg Hospital are similiar to previous studies done, with the exception of Staphylococcus aureus which was not found. This organism was a common finding in most of the previous studies done. [18, 37, 40]

The majority of studies have shown that CONS are often contaminants and may likely not cause a clinically significant infection in affected patients. Admittingly, the bulk of organisms we cultured are non-pathogenic in normal hosts but can be lethal in immunocompromised patients. A follow up study can be done to identify the specific individual organisms, and patients can be followed up to determine whether this high rate of contamination does cause clinically significant infections postoperatively. Importantly, contamination by commensals is certainly a warning that any pathogens (including viruses) that may be present on the anaesthetist's hands or in the environment may also end up in the propofol.[62, 66]

No conclusion can be drawn from our secondary outcomes' results, with wide confidence intervals. It is thus difficult to interpret whether the contamination rate is higher with more junior or more senior personnel. The majority of specimens were taken from elective theatres, and although we suspected emergency surgery and the associated urgency to influence aseptic handling of drugs, we cannot reliably indicate this.

The prevalence of contamination in this study is still unacceptably high, considering that almost half of the syringes were contaminated. The presence of human commensals as well as environmental organisms in the propofol syringes are avoidable and zero contamination rate should be aspired to in all interactions with patients. Breedt et al. has reliably shown that there is awareness regarding the aseptic handling guidelines, and thus adherence to these recommendations seems to be the major problem[30]. With the aid of infection control, regular staff training should be implemented to create awareness and change behaviour. There should be easy and quick access to hand sanitizing agents in and around theatres and visible reminders for healthcare workers to clean their hands. Propofol ampules could be

packaged with a small tag around the neck, emphasising aseptic handling techniques. While the use of pre-filled syringes could avoid some contamination, it still requires aseptic handling technique with administration.

# 12. CONCLUSION

The severity of contamination of propofol in the Tygerberg Hospital operating theaters was demonstrated. Guidelines have been set in place to prevent complications caused by possible contaminants. Anaesthetists need to be made aware of their contribution to postoperative bacteraemias and wound infections in order to change their practice. Emphasis needs to be placed on regular hand hygiene, strict aseptic propofol handling and safe injection practices. Patient safety should be our top priority.

# 13. REFERENCES


1. Cole DC, Baslanti TO, Gravenstein NL, Gravenstein N. Leaving more than your fingerprint on the intravenous line: A Prospective study on propofol anesthesia and implications of stopcock contamination. Anesth Analg. 2015 Apr;120(4):861-7.
2. Matsuki A. A Review of recent advances in total intravenous anesthesia. The Japanese journal of anesthesiology.1991 May;40(5):684-91.
3. Short CE, Bufalari A. Propofol anesthesia. Vet Clin North Am: Small Animal Practice 1999;29(3):747-78.
4. White PF. Clinical uses of intravenous anesthetic and analgesic infusions. Anesthesia Analgesia 1989;68(2):161-71.
5. Bensel BM, Guzik-Lendrum S, Masucci EM, Woll KA, Eckenhoff R, Gilbert SP. Common General anaesthetic propofol impairs kinesin processivity. Proceedings of the National Academy of United States of America. 2017 May 23;114(21):E4281 - E4287.
6. Mackenzie N, Grant IS. Propofol for intravenous sedation. Anaesthesia. 1987 Jan;42(1):3-6.
7. Centers for Disease Control (CDC). Postsurgical infections associated with an extrinsically contaminated intravenous anesthetic agent--California, Illinois, Maine, and Michigan, 1990. MMWR Morb Mortal Wkly Rep. 1990 Jun 29;39(25):426-7, 433.
8. Sklar GE. Propofol and postoperative infections. Ann Pharmacother. 1997 Dec;31(12):1521-3.
9. Koenig SJ, Lakticova V, Narasimhan M, Doelken P, Mayo PH. Safety of propofol as an induction agent for urgent endotracheal intubation in the medical intensive care unit. J Intensive Care Med. 2015 Dec;30(8):499-504.
10. Keaveny JP, Knell PJ. Intubation under induction doses of propofol. Anaesthesia. 1988 Mar;43 Suppl:80-1.
11. Bandschapp O, Filitz J, Ihmsen H, Berset A, Urwyler A, Koppert W, Ruppen W. Analgesic and antihyperalgesic properties of propofol in a human pain model. Anesthesiology 8 2010; Vol:113, 421-28.

12. Chang KS, Davis RF. Propofol produces endothelium-independent vasodilatation and may act as a Ca2+ channel blocker. Anesth Analg.1993 Jan;76(1):24-32.

13. https://www.pharmacology2000.com/General/Pharmacokinetics/kinobj5.htm

14. Hiraoka H, Yamamoto K, Miyoshi S, Morita T, Nakamura K, Kadoi Y, Kunimoto F, Horiuchi R. Kidneys contribute to the extrahepatic clearance of propofol in humans, but not lungs and brain. Br J Clin Pharmacol.2005 Aug;60(2):176–82.

15. Wu GJ, Chen TL, Chang CC, Chen RM. Propofol suppresses tumor necrosis factor-alpha biosynthesis in lipopolysaccharide-stimulated macrophages possibly through downregulation of nuclear factor-kappa B-mediated toll-like receptor 4 gene expression. Chem Biol Interact. 2009 Aug 14;180(3):465-71.

16. Chiu WT, Lin YL, Chou CW, Chen RM. Propofol inhibits lipoteichoic acid-induced iNOS gene expression in macrophages possibly through downregulation of toll-like receptor 2-mediated activation of Raf-MEK1/2-ERK1/2-IKK-NFkappaB. Chem Biol Interact. 2009 Oct 30;181(3):430-9.

17. Ruei-Ming Chen, Chih-Hsiung Wu, Huai-Chia Chang, Gong-Jhe Wu, Yi-Ling Lin. Propofol Suppresses Macrophage Functions and Modulates Mitochondrial Membrane Potential and Cellular Adenosine Triphosphate Synthesis. Anesthesiology 5 2003, Vol.98, 1178-85.

18. Andrés Zorrilla-Vaca, Jimmy J. Arevalo, Marek A. Mirski. Infectious Disease Risk Associated with Contaminated Propofol Anesthesia, 1989–2014. Emerg Infect Dis. 2016 Jun;22(6):981–92.

19. Lloyd E. Kwanten. Anaesthetists and syringe hygiene: getting to the pointy end-a summary of recommendations. British Journal of Anaethesia, Volume 123, issue 4, E475-E479, 2019 October 01.

20. Zacher AN, Zornow MY, Evans G. Drug contamination from opening drug ampoules. Anaesthesiology 1991 November;75(5):893 – 5.

21. Hemingway CJ, Malhotra S, Almeida M, Azadian B, Yentis SM. The effect of alcohol swabs and filter straws on reducing contamination of glass ampoules used for neuroaxial injections. Anaesthesia 2007; 62:286-88.

22 Mehta U, Gunston GD, O'Connor N. Serious consequences to misuse of Propofol Anaesthetic. SAfr Med J. 2000;90(3):240.

23. Rongrong Rueangchira-Urai, Panthila Rujirojindakul, Alan Frederick Geater, Edward McNeil. Bacterial contamination of Anaesthetic and Vasopressor drugs in the Operating Theatres. Turkish Journal of Anaesthesiology and reanimation 2017 Feb;45(1):47-52.

24. Silvia Munoz-Price L, Weinstein RA. Fecal patina in the anaesthesia work area. Anaesthesia and analgesia 2015April;120(4):703-05.

25. Loftus RW, Koff MD, Burchman CC, Schwartzman JD, Thorum V, Read ME, Wood TA, Beach ML. Transmission of pathogenic bacterial organisms in the anesthesia work area. Anesthesiology 2008;109:399-407.

26. Krediet AC, Kalkman CJ, Bonten MJ, Gigengack AC, Barach P. Hand-hygiene practices in the operating theatre: an observational study. Br J Anaesth. 2011 Oct;107(4):553-8.

27. Nichols RL, Smith JW. Bacterial contamination of an anesthetic agent. N Engl J Med. 1995 Jul 20;333(3):184-5.

28. Trépanier CA, Lessard MR. Propofol and the risk of transmission of infection. Can J Anaesth. 2003 Jun-Jul;50(6):533-7.

29. Muller AE, Huisman I, Roos PJ, Rietveld AP, Klein J, Harbers JB, Dorresteijn JJ, van Steenbergen JE, Vos M. Outbreak of severe sepsis due to contaminated propofol: lessons to learn. J Hosp Infect. 2010 Nov;76(3):225-30.

30. Breedt A, Coetzee JF, Kluyts H, Scheepers P. A survey of propofol injection practices reveals poor knowledge of and unsatisfactory adherence to the SASA Guidelines for Infection Control. Southern African Journal of Anaesthesia and Analgesia 2017;23(4):102–13.

31. Seeberger MD, Staender S, Oertli D, Kindler CH, Marti W. Efficacy of specific aseptic precautions for preventing propofol-related infections: analysis by a quality-assurance programme using the explicit outcome method. J Hosp Infect. 1998 May;39(1):67-70.

32. Tessler M, Dascal A, Gioseffini S, Miller M, Mendelson J. Growth curves of Staphylococcus aureus, Candida albicans, and Moraxella osloensis in propofol and other media. Can J Anaesth. 1992 May;39(5 Pt 1):509-11.

33. Sosis MB, Braverman B. Growth of Staphylococcus aureus in Four Intravenous Anesthetics. Anesth Analg. 1993 Oct;77(4):766-8.

34. Thomas DV. Propofol supports bacterial growth. Br J Anaesth. 1991 Feb;66(2):274.

35. McNeil MM, Lasker BA, Lott TJ, Jarvis WR. Postsurgical Candida albicans Infections Associated with an Extrinsically Contaminated Intravenous Anesthetic Agent. J. Clin. Microbiol. 1999 May;37(5):1398-403.

36. Eike Steinman, Sandra Ciesek, Martina Friesaland, Thomas J. Erichsen, Thomas Pietschmann. Prolonged Survival of Hepatitis C Virus in the Anesthetic Propofol. Clinical Infectious Diseases, Volume 53, Issue 9, 1 November 2011: 963-64.

37. Bennett SN, McNeil MM, Bland LA, Arduino MJ, Villarino ME, Perrotta DM, Burwen DR, Welbel SF, Pegues DA, Stroud L. Postoperative infections traced to contamination of an intravenous anesthetic, Propofol. N Engl J Med. 1995 Jul 20;333(3):147-54.

38. Feriha Cilli, Arzu Nazli-Zeka, Bilgin Arda, Oguz resat Sipahi, Sukran Aksit-Barik, Nurhayat Kepeli, Mehmet Ali Ozinel, Zeynep Gulay, Sercan Ulusoy. Serratia Marcescens Sepsis Outbreak Caused by Contaminated Propofol. Am J Infect Control.2019 May;47(5):582-84.

39. Bonnie Henry, Cindy Plante-Jenkins, Krystna Ostowska.An Outbreak of Serratia Marcescens Associated with the Anesthetic Agent Propofol. Am J Infect Control. 2001 Oct;29(5):312-5.

40. Andrés Zorrilla-Vaca, Kevin Escandón-Varga, Vanessa Brand-Giraldo, Tatiana León, Mónica Herrera, Andrey Payán. Bacterial contamination of propofol vials used in operating rooms of a third-level hospital. Am J Infect Control. 44 (2016) e1-e3.

41. Marik PE. Propofol: therapeutic indications and side effects. Current Pharmaceutical Design 2004 Dec;10(29);3639-49.

42. Jansson JR, Fukada T, Ozaki M, Kimura S. Propofol EDTA and reduced incidence of infection. Anaesth Intensive Care. 2006 Jun;34(3):362-8.

43. Fukada, T. and Ozaki,M. Microbial growth in propofol formulations with disodium edetate and the influence of venous access system dead space. Anaesthesia. 2007 Jun;62(6):575-80.

44. Meyer TA. The Propofol Safety Review. The official Journal of the Anesthesia Patient Safety Foundation Volume 22, No.2, Circulation 81,489.

45. Vidovich MI, Peterson LR, Wong HY. The effect of lidocaine on bacterial growth in propofol. Anesth Analg. 1999 Apr;88(4):936-8.

46. Fault Bazaz BS, Salt WG. Local anaesthetic as antimicrobial agents: structure-action considerations. Microbios. 1983;37(147):45-64.

47. Wachowski I, Jolly DT, Hrazdil J, Galbraith JC, Greacen M, Clanachan AS. The growth of Microorganisms in Propofol and Mixtures of Propofol and Lidocaine. Anesth Analg. 1999 Jan;88(1):209-12.

48. Telletxea S, Lauzirika Z, Etxebarria A, Ortega LF. Fospropofol: A new prodrug of Propofol. Rev Esp Anestesiol Reanim. 2012 Nov;59(9):497-502.

49. Pergolizzi JV, Gan TJ, Plavin S, Labhsetwar S, Taylor R. Perspectives on the role of fospropofol in the monitored anesthesia care setting. Anesthesiology Research and Practice 2011 Dec;2011:458920.

50. Hall WCE, Jollyg DE, Hrazdil J, Galbraith JC, Greacen M, Clanachan AS. The Emulsiv filter removes microbial contamination from propofol, but is not a substitute for aseptic technique. Canadian Journal of Anaesthesia June 2003; 50(6): 541-6.

51. Baker MT, Naguib M. Propofol: the challenges of formulation. Anesthesiology 2005 Oct;103(4):8860-76.

52. https://www.openepi.com

53. Michael Otto. Staphylococcus epidermidis – the “accidental” pathogen. Nat Rev Microbiol. 2009 Aug; 7(8): 555-67.

54. Rogers KL, Fey PD, Rupp, M.E. Coagulase-negative staphylococcal infections. Infect Dis Clin North Am. 23,73-98.doi: 10.1016/j.idc.2008.10.001.

55. Marco Falcon, Floriana Campanile, Maddalena Giannella, Sonia Borbone, Stefania Stefani, Mario Venditti. Staphylococcus haemolyticus endocarditis: clinical and microbiologic analysis of 4 cases. Diagn Microbiol Infect Dis.2007 Mar;57(3):325-331

56. KR Soumya, Suja Philip, Sheela Sugathan, Jyothis Mathew and EK Radhakrishnan. Virulence factors associated with Coagulase Negative Staphylococci isolated from human infections. 3 Biotech. 2017 Jun; 7(2): 140.

57. Becker K, Heilmann C, Peters G. Coagulase-negative staphylococci. Clin Microbiol Rev. 2014;27(4):870.

58. Shailpreet K. Sidhu, Sita Malhotra, Pushpa Devi, and Arpandeep K. Tuli. Significance of coagulase negative Staphylococcus from blood cultures: persisting problems and partial progress in resource constrained settings. Iran J Microbiol. 2016 Dec; 8(6): 366-71.

59. Asiye Karakullukçu, Mert Ahmet Kuşkucu, Sevgi Ergin, Gökhan Aygün, Kenan Midilli, Ömer Küçükbasmaci. Determination of clinical significance of coagulase negative staphylococci in blood cultures.Diagnostic Microbiology and Infectious disease,Volume 87,Issue 3, March 2017, pages 291-4.

60. T Cundell. The limitations of the colony-forming unit in microbiology. European pharmaceutical Review 2015.

61. Carmelita U. Tuazon, M.D., M.P.H. Bacillus species. Infectious Disease and antimicrobial agents.(http://www.antimicrobe.org/b82.asp)

62. Fatma Deniz Aygun, Faith Aygun, Halit Cam. Successful Treatment of Bacillus cereus Bacteremia in a patient with a Propionic Acidemia. Case Report: Open Access Volume 2016|Article ID 6380929| 2 pages.

63. Hatheway, CL (1990). Toxigenic Clostridia. Clin Microbiol Rev. 3: 86-7.

64. J.F Fernȧndez-Garayzȧbal, E. Fernȧndez, A. Las Heras, C. Pascualt, M.D. Collinst, and L. Dominguez. Streptococcus parasanguinis: New Pathogen Associated with Asymptomatic Mastitis in Sheep. Volume 4, Number 4-December 1998.

65. Qiurong Chen, Guojun Wu, Hui Chen, Hui Li, Shuo Li, Chenhong Zhang, Xiaoyan Pang, Linghua Wang, Liping Zhao and Jian Shen. Quantification of Human Oral and Fecal Streptococcus parasanguinis by Use of Quantitative Real-Time PCR Targeting the groEL Gene. Front. Microbiol.,20 December 2019.

66. Miquel B. Ekkelenkamp, Marc J.M. Bonten. Staphylococci and micrococci, in Infectious Diseases (Third Edition), 2010.

# 14. APPENDIX 1

## 14.1 Data captured