Relationship between micro-organisms found in biliary cultures and subsequent surgical site infections after Pancreaticoduodenectomy at three University of the Witwatersrand academic hospitals.

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DECLARATION

I, Mariëtte Cordelia Mouton (student no. 1585051), am submitting this research report for the degree of Master of Medicine in Plastic and Reconstructive Surgery at the University of the Witwatersrand, Johannesburg. I declare that this research report has not been submitted for examination to this or any other university before.


……..

Mariëtte Cordelia Mouton

On this …9th……. Day of …August…….2024

DEDICATION

- This work is dedicated to my husband, Michael Premanathan, for his support and unfailing love.
- I would like to thank my mom, Cornelia Mouton, who supported me through all my years of study with prayer and motivation. This work is dedicated to my late father, Franciscus Mouton, who always believed in me and supported me.
- This research is dedicated to my daughter Atarah. She had to accept my long working hours.
- A special dedication to my father and mother-in-law, Gabriel, and Theresa Joseph, for their unwavering prayer and support.

PRESENTATIONS ARISING FROM THIS STUDY

Mouton MC, Bizos D, Fru P, Devar J, Schleicher G. Relationship between micro-organisms found in biliary cultures and subsequent surgical site infections after Pancreaticoduodenectomy at three University of the Witwatersrand academic hospitals. Bert Myburgh Research Forum at the University of the Witwatersrand Department of Surgery November 2023

ABSTRACT

Introduction

Surgical site infections (SSI) are common after pancreaticoduodenectomy. Intra-operative bactobilia is a reported risk factor for septic post-operative complications. In this study, we aimed to study the association between the micro-organisms in intra-operative biliary cultures and subsequent SSI at three University of the Witwatersrand academic hospitals.

Methodology

In this retrospective observational study, we included all patients who underwent a pancreaticoduodenectomy between January 2014 and December 2019. Demographic and peri-operative data were reviewed, and the causative organisms of superficial and deep-seated SSI were correlated with intra-operative biliary organisms.

Results

A total of 202 patients were included. Intraoperative bile sampling was performed in 94 (46.5 %) cases and bactobilia was detected in 62 (65.9 %) cases. Pre-operative biliary drainage was reported in 142 (70.3 %) patients. Intraoperative biliary cultures were resistant to Piperacillin/Tazobactam in 50 % (30) of the samples, and institutional resistance patterns were significantly different ($p = 0.006$). Similar micro-organisms in bile and surgical site samples were confirmed in 9/25 (36 %) patients with similar antimicrobial resistance patterns. There was no statistically significant association between bactobilia and surgical site infections ($p>0.05$).

Conclusion

Our study demonstrated heterogeneity in bile sampling, PBD, and biliary antimicrobial resistance patterns between the different institutions. Intraoperative biliary cultures assist in predicting the microbial species in postoperative SSI, it is therefore important to perform routine intraoperative bile sampling. We recommend that institutes review their internal practices regarding intraoperative bile sampling in patients undergoing PD, as this will allow guidance of antimicrobial selection in patients developing postoperative SSI. Further studies regarding the choice and duration of prophylactic antibiotics for PD are warranted.

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- Lastly, I would like to thank God for carrying me through my registrar training and is still leading me into my new future as a specialist.

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LIST OF ABBREVIATIONS

CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
CHBAH	Chris Hani Baragwanath Academic hospital
WDGMC	Wits Donald Gordon Medical Centre
PD	Pancreaticoduodenectomy
PBD	Pre-operative biliary drainage
SSI	Surgical site infection
MDR	Multi-drug resistant
WITS	University of Witwatersrand
NHLS	National Health Laboratory Service
HREC	Human Research Ethics Committee
Spp.	Species
SD	Standard deviation
N	Total number of observations

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1. INTRODUCTION

1.1 Background

Surgical site infections (SSI) have been identified as one of the leading causes of hospital-acquired infections and are associated with high morbidity and mortality. They may delay the commencement of adjuvant or systemic therapies for cancer patients (1). The management of patients with superficial or deep SSI differs and some may require more aggressive management in the form of surgical debridement, negative pressure wound therapy, subsequent secondary closure, or skin grafting. The incidence of SSI in Sub-Saharan Africa is between 10 and 20 %, as described by Fan et al in a systematic review (2). SSI represents a considerable financial and clinical burden on the patient and the healthcare system (3). The economic burden involves the additional costs associated with prolonged hospitalization, readmissions, additional investigations, and treatment. Treatment may include surgical intervention. Data from high-income countries indicate a doubling in the cost of treatment if complicated by an SSI (3). Health-related quality of life is negatively impacted due to SSI. This may be due to pain, anxiety, and mental stress due to the financial implications. Three distinct types of SSIs have been described by the United States Centres for Disease Control and Prevention (CDC). This classification is based on the level of tissue involvement, namely superficial incisional, deep incisional and organ-space infections (4).

The incidence of SSI after PD varies greatly in the literature. Rates between 7 % and in some instances up to 40 % have been reported (5, 6). Rates of SSI, thirty days after gastrointestinal surgery have been reported as 12.3 %, with a greater burden of SSI in countries with a low human development index according to the United Nations compared to a middle or high human development index (7).

Bactobilia is reported as a risk factor for septic post-operative complications including SSI and bacteraemia. Bile duct colonisation occurs commonly after manipulation of sterile bile ducts and after preoperative biliary drainage (PBD) in patients with obstructive jaundice, it has been identified as a strong predictor of subsequent bactobilia (8, 9, 10). The current literature suggests that routine PBD prior to pancreaticoduodenectomy (PD) should be reserved for specific indications due to its association with bactobilia and an increase in post-operative complications, be they infectious or overall morbidity. Bactobilia is a reported risk factor for septic post-operative complications including bacteraemia, wound infection, and intra-abdominal sepsis (9, 11, 12).

Several studies and a recent meta-analysis have shown that micro-organisms cultured from intra-operative bile specimens correlate with post-operative surgical site infective micro-organisms (1, 6, 13, 14, 15, 16, 17). The collection of intra-operative bile samples at PD may assist in identifying micro-organisms and antimicrobial susceptibility patterns which may help refine antibiotic usage. Institutional variability has been described for intra-operative bile and wound cultures, as well as antibiotic resistance patterns (1).

Pathogens commonly isolated from SSI after PD are gram-negative organisms, followed by gram-positive cocci. The most common species isolated from these patients are *Escherichia coli*, *Enterococcus species, Klebsiella species, Pseudomonas* and *Candida species* (13). Institutional comparison of micro-organisms cultured from wound and intraoperative bile samples has shown noticeable similarity (1). Rates of 21 % have been reported for bile and wound microorganism concordance, 15 % similar resistance patterns of micro-organisms in bile and wound cultures, and only 10 % of organisms susceptible to the antibiotic used as prophylaxis (13).

The current guidelines for prophylactic antibiotics to prevent SSI do not include recommendations for the type and length of antibiotic administration for patients who have had PBD before PD (10, 18). It is important to ascertain the incidence of and the resistance patterns of organisms associated with PBD in specific institutions as these resistance patterns may vary (1). It is important in our setting to ascertain perioperative practices of patients undergoing PD. This knowledge may guide patient management, given that there are currently no South African data available on bile cultures and sensitivity patterns in patients undergoing PD. It is also not certain if bile sampling is routine practice at PD at Wits academic hospitals.

1.2 Aim and Objectives

This study aimed to better understand the relationship between micro-organisms found in biliary cultures and subsequent SSI after PD at three University of the Witwatersrand academic hospitals.

Primary objectives were:

1. To ascertain whether intra-operative bile specimens are being taken during PD for micro-biological assessment.
2. To compare intra-operative bile cultures in patients undergoing PD after PBD with those who did not receive preoperative drainage.
3. To compare intra-operative bile cultures and resistance patterns between three University of the Witwatersrand academic hospitals in Gauteng.
4. To assess the concordance of intraoperative bile cultures and subsequent surgical site infective organisms.

Our secondary objectives were:

1. To determine the association of intra-operative bactobilia with all septic post-operative complications.

2. RESEARCH METHODOLOGY

2.1 Study design and setting

This was a retrospective analysis of patient records; for all patients who underwent a PD between January 2014 and December 2019 at three University of the Witwatersrand academic hospitals. These were the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), Chris Hani Baragwanath Academic Hospital (CHBAH) and the Wits Donald Gordon Medical Centre (WDGMC). Included were all patients who underwent a pancreaticoduodenectomy in the specified period, irrespective of age and excluded were patients who did not have a completed PD. Ethical approval was obtained from the University of the Witwatersrand Human Research Ethics Committee (HREC). Furthermore, medical approval was obtained from the CEOs of CMJAH, CHBAH and WDGMC. Patients were not identified in any way since no identifier was recorded on the datasheets. Each patient was assigned a study number for data collection purposes.

2.2 Data collection

Patient demographics included age and gender. Perioperative data including pre-operative biliary drainage, intra-operative biliary cultures and sensitivities were recorded. Post-operative outcome data included microbiological confirmation of SSI with wound and fluid cultures within 30 days of surgery. Postoperative septic complications were confirmed as urinary tract infections and bacteraemia with appropriate cultures. These included all cultures submitted for 30 days postoperatively. Indication for surgery was recorded as benign or malignant disease from histopathological reports. Data collection was done by making use of a pre-existing hospital database, the National Health Laboratory Services (NHLS) database, as well as patient and theatre records. Patients who did not have completion of PD were excluded.

2.3 Statistical Analysis

The data were analysed using SPSS (version 27) and MS Excel software (Microsoft Office version 2010). Numeric (continuous) variables, such as age are reported as mean (± standard deviation). One way ANOVA was used to assess the relationship of the mean age of the three groups. Categorical variables were tested with the Pearson Chi-square test to assess the relationship between the two groups of patients. The Fisher's exact test was used if the criteria for

the Pearson Chi-square test were not met. A p-value of less than 0.05 was considered statistically significant.

3. RESULTS

3.1 Demographics

The total number of PD cases performed over the study period was 211. Nine were excluded due to incomplete patient records. The total number of patients included was 202. The institution at which the PD was done was as follows; WDGMC 106 (52.5 %), CHBAH 67 (33.2 %) and CMJAH 29 (14.4 %). The mean age was 57.7 (with a range from 12 to 86 years, SD ±12.42) and a gender distribution of 108 (53.5 %) males and 94 (46.5%) females. The indication for surgery was malignant disease in 165 (81.7 %) cases and benign disease in 37 (18.3%). No differences were seen between the institutions regarding gender distribution and the indication for surgery. See Table 3.1

3.2 Preoperative biliary drainage

In terms of PBD, 142 (70.3%) patients were drained. There was a statistically significant difference between the three institutions in the percentage of patients receiving PBD (p = 0.001) (Table 3.1). Statistically fewer patients at WDGMC were drained.

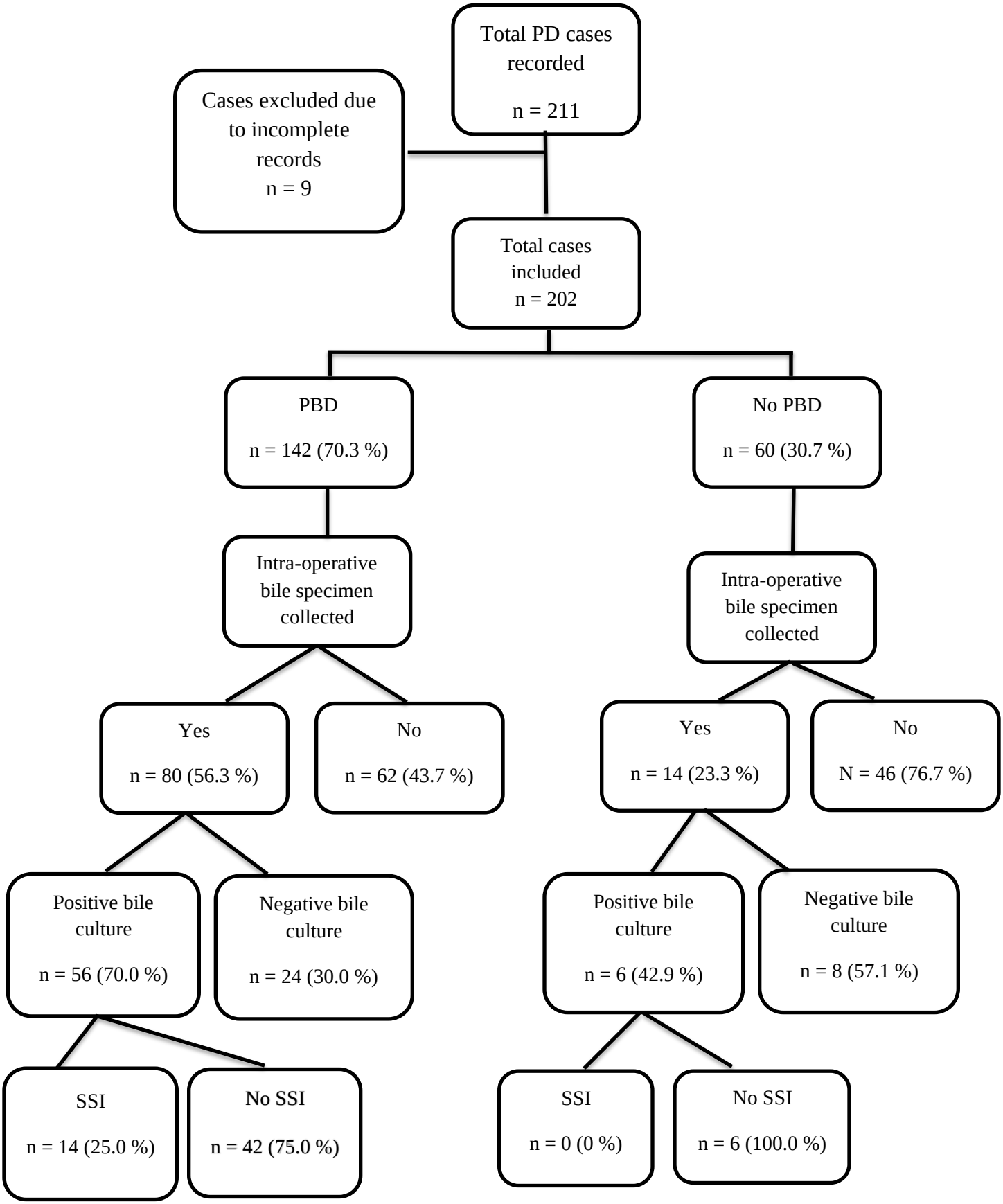
Total PD cases recorded
n = 211
Cases excluded due to incomplete records
n = 9
Total cases included
n = 202
PBD
n = 142 (70.3 %)
No PBD
n = 60 (30.7 %)
Intra-operative bile specimen collected
Intra-operative bile specimen collected
Yes
n = 80 (56.3 %)
No
n = 62 (43.7 %)
Yes
n = 14 (23.3 %)
No
N = 46 (76.7 %)
Positive bile culture
n = 56 (70.0 %)
Negative bile culture
n = 24 (30.0 %)
Positive bile culture
n = 6 (42.9 %)
Negative bile culture
n = 8 (57.1 %)
SSI
n = 14 (25.0 %)
No SSI
n = 42 (75.0 %)
SSI
n = 0 (0 %)
No SSI
n = 6 (100.0 %)

Figure 3. 1 Diagram of patients with PBD and no PBD.

Table 3. 1 Perioperative data

		Total Population	CHBAH	CMJAH	WDGMC	p-value
N (%)		202	67 (33.2)	29 (14.4)	106 (52.5)	0.230
Age in years (Mean ± SD)		57.7±12.4	55.4±12.4	56.5±13.0	59.5±12.1	
Gender	Female	94 (46.5)	36 (53.7)	12 (41.4)	46 (43.4)	0.346
	Male	108 (53.5)	31 (46.3)	17 (58.6)	60 (56.6)	
Histology	Benign	37 (18.3)	10 (14.9)	5 (17.2)	22 (20.8)	0.619
	Malignant	165 (81.7)	57 (85.1)	24 (82.8)	84 (79.2)	
PBD	Yes	142 (70.3)	53 (79.1)	26 (89.7)	63 (59.4)	0.001
	No	60 (29.7)	14 (20.9)	3 (10.3)	43(40.6)	
Intraoperative bile specimen collected	Yes	94 (46.5)	43 (64.2)	17 (58.6)	34 (32.1)	0.000
	No	108 (53.5)	24 (35.8)	12 (41.4)	72 (67.9)	
Intraoperative bile culture result	Positive	62 (66.0)	31 (72.1)	10 (58.8)	21 (61.8)	0.503
	Negative	32 (34.0)	12 (27.9)	7 (41.2)	13 (38.2)	
Bile classification	Polymicrobial	40 (64.5)	21 (67.7)	6 (60.0)	13 (61.9)	0.864
	Monomicrobial	22 (35.5)	10 (32.3)	4 (40.0)	8 (38.1)	
Biliary organisms N = 121	Gram-negative organisms	92 (76.0)	48 (73.8)	16 (84.2)	28 (75.6)	0.804
	Gram-positive organisms	24 (19.8)	16 (24.6)	2 (10.5)	6 (16.2)	0.468
	Fungal organisms	5 (4.1)	1 (1.5)	1 (5.3)	3 (8.1)	

Biliary resistance to Piperacillin/Tazobactam	Yes	30 (50.0)	21 (70.0)	4 (40.0)	5 (25.0)	0.006
	No	30 (50.0)	9 (30.0)	6 (60.0)	15 (75.0)	
SSI		50 (24.8)	24(48.0)	8 (16.0)	18 (36.0)	0.019
	Incisional SSI	11 (22.0)	4 (16.7)	0 (0)	7 (38.9)	0.004
	Deep-space infection	39 (78.0)	20 (83.3)	8 (100)	11 (61.1)	0.682

3.3 Intraoperative Bile culture analysis

A total of 94 (46.5%) intraoperative bile specimens were submitted for analysis. Intraoperative bile cultures were more likely to be taken at CHBAH, 43 (64.2 %), compared to 17 (58.6%) at CMJAH and 34 (32.1%) at WDGMC (p = 0.000) (Table 3.1). The overall rate of positive bile cultures (bactobilia) was 62 (66%), there was no difference in bactobilia across the three institutions (p = 0.503). Polymicrobial growth made up most of the bile specimens 40 (64.5%), compared to monomicrobial growth 22 (35.5%). Polymicrobial specimens were the majority group at all three institutions. There was a significant relationship between PBD and bactobilia with 56 (39.4%) patients developing bactobilia post PBD (p = 0.048). (Table 3.4)

Most biliary organisms were gram-negative organisms 92 (76.0%), the rest were gram-positive organisms 24 (19.8%) and fungal 5 (4.1%). The main organisms cultured from intraoperative biliary specimens were *Klebsiella ssp., Enterococcus ssp.*, *Escherichia Coli,* and *Enterobacter ssp.* Similar organisms were cultured across the three institutions (Figure 3.2). *Candida albicans* species were seen in 4 cases, two at WDGMC, and one each for CHBAH and CMJAH. One *Candida non-albicans* species was isolated at WDGMC.

We assessed the resistance of biliary organisms to Piperacillin/Tazobactam. Overall, the resistance to Piperacillin/Tazobactam was 30 (50%) of the bile specimens. A significant variation was identified between the different hospitals and biliary culture resistance, with 21 (70 %) at CHBAH, 4 (40%) at CMJAH and 5 (25%) at WDGMC specimens resistant to Piperacillin/Tazobactam (p = 0.006) (Table 3.1).

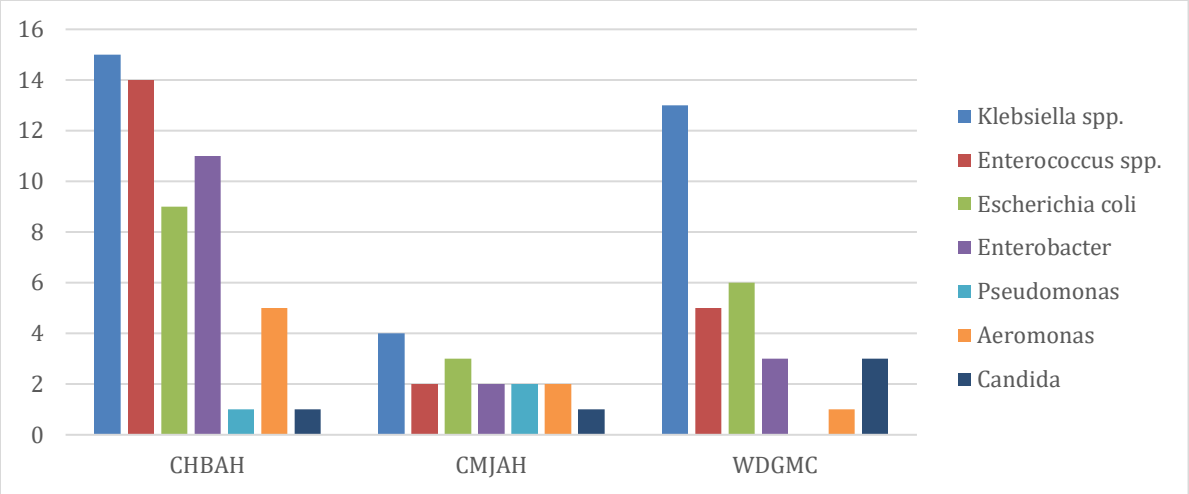

Figure 3. 2 Bar graph of biliary micro-organisms compared per institution.

3.4 Surgical Site Infections and Microbiological Analysis

Surgical site infection was detected in 50 (24.8%) patients. Of these 39 (78%) were deep-seated and 11 (22%) were superficial incisional infections (Table 3.1). The rate of SSI between institutions varied, 24 (48%) were detected at CHBAH, 18 (36%) at WDGMC and 8 (16%) at CMJAH (p = 0.019). In the SSI group, 25 (50%) had no bile specimens collected. The rate of bile positivity for the group with SSI was 14 (56 %). There was no relationship between a positive bile culture and SSI (p = 0.220). No relationship was found between PBD and SSI (p = 0.083) (Table 3.2). The main organisms detected in SSIs were *Klebsiella spp.* (29 cultures), *Enterococcus spp*. (20) and *Escherichia Coli* (14) (Figure 3.3). Similar organisms were detected across the three institutions.

Table 3. 2 Comparison between the group with surgical site infection and no surgical site infection.

Variable		Total population N = 202 (%)	SSI N = 50 (%)	No SSI N = 152 (%)	p-value
Gender	Female	94 (46.5)	26 (52.0)	68 (44.7)	0.372
	Male	108 (53.5)	24 (48.0)	84 (55.3)	
PBD	Yes	142 (70.3)	40 (80.0)	102 (67.1)	0.083
	No	60 (29.7)	10 (20.0)	50 (32.9)	
Intraoperative bile specimen	Yes	94 (46.5)	25 (50.0)	69 (45.4)	0.571
	No	108 (53.5)	25 (50.0)	83 (54.6)	

Intraoperative bile culture result	Positive	62 (66.0)	14 (56.0)	48 (69.6)	0.220
	Negative	32 (34.0)	11 (44.0)	21 (30.4)	

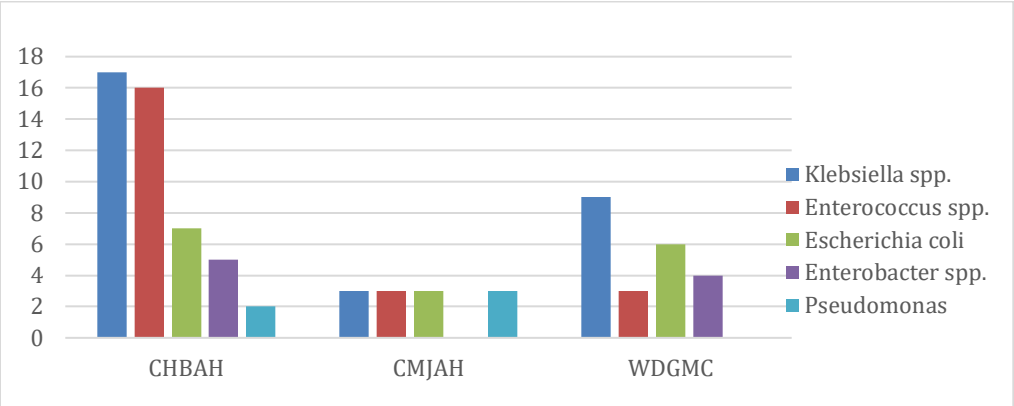

Figure 3. 3 Bar graph of micro-organisms in surgical site infections across three institutions

Concordance of biliary organisms and the organisms responsible for SSI can be seen across the three institutions (Figure 3.4). A total of 9 patients out of 25 patients (36%) had concordance between the bile culture and the organism responsible for SSI. These 12 organisms were mostly gram-negative organisms. They were *Enterobacter spp.*, *Klebsiella spp.*, *Enterococcus spp.*, *Morganella morganii*, *Citrobacter species* and *Pseudomonas* (Table 3.3). All concordant organisms had a similar resistance pattern between bile and SSI. The patients were distributed as follows, CHBAH (5 cases), WDGMC (3 cases) and CMJAH (1 case).

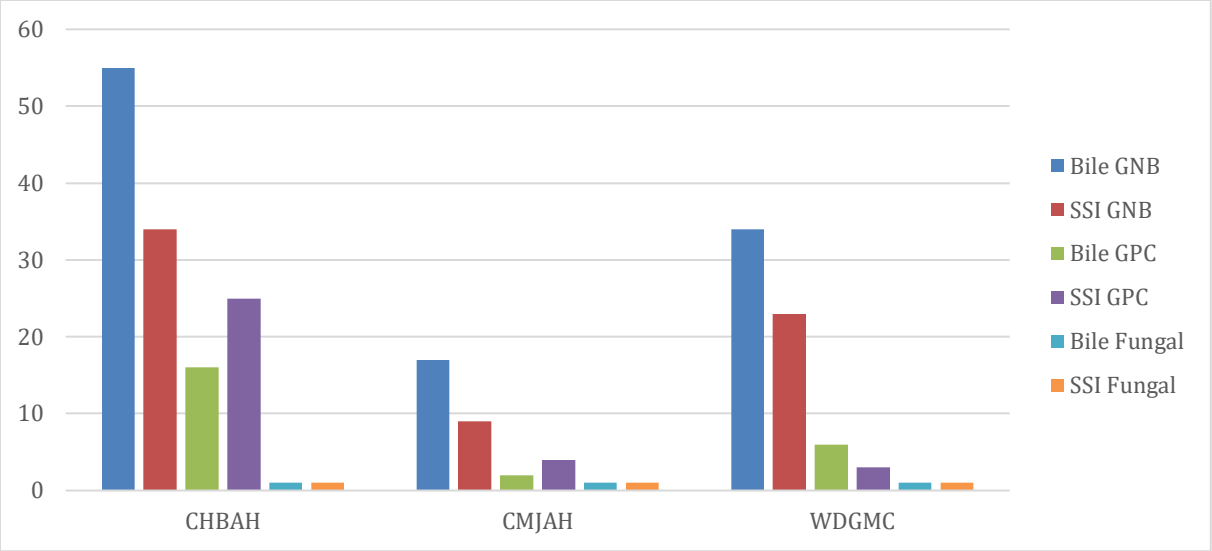

Figure 3. 4 Bar graph of microorganism comparison between bile cultures and surgical site cultures per institution

Table 3. 3 Microbiology comparing bile cultures and surgical site cultures.

Organism		Bile culture organisms	Fluid cultures	Wound cultures	Correlating organisms	Similar resistance pattern
Gram-negative organisms	*Acinetobacter baumannii*		3	1	-	-
	Aeromonas spp.	8	-	-	-	-
	Citrobacter spp.	6	1	-	1	1
	Enterobacter spp.	16	7	2	4	4
	Escherichia Coli	19	9	5	-	-
	Klebsiella spp.	32	17	12	3	3
	Morganella morganii	1	1	1	1	1
	Pantoeae agglomerans	2	-	-	-	-
	Proteus mirabilis	4	-	-	-	-
	Pseudomonas aeruginosa	2	4	1	1	1
	Prevotella buccae	-	-	1	-	-
	Raoultella ornithinolytica	1	-	1	-	-
	Serratia plymuthica	1	-	-	-	-
	Stenotrophomonas maltophilia	-	1	-	-	-
Gram-positive organisms	*Enterococcus spp.*	21	13	7	2	2
	Clostridium perfringens	1	-	-	-	-
	Staphylococcus spp.	1	4	4	-	-
	Streptococcus spp.	1	2	2	-	-
Candida albicans		4	-	-	-	-
Candida non albicans		1	2	2	-	-
Total		121	65	37	12	12

3.5 Septic postoperative complications

Positive post-operative urine cultures were reported in 18 (8.9%) patients. There was no significant relationship between urinary tract infection and bactobilia (p = 0.280). The main organisms detected in urinary tract infections were *Klebsiella spp.* (6) and *Escherichia coli* (3). Positive blood cultures were reported in 14 (6.9%) patients. The main organisms detected in blood cultures were *Klebsiella spp.* (5) and *Escherichia Coli* (4). There was no significant relationship between bacteraemia and bactobilia (p = 1.000). (Table 3.4)

Table 3. 4 Influence of bactobilia on post-operative septic complications.

Variable	Total	Positive Bile result	Negative bile result	No bile specimen collected	p-value
PBD	142 (70.3)	56 (39.4)	24 (16.9)	62 (43.7)	0.048
SSI	50 (24.8)	14 (28.0)	11 (22.0)	25 (50.0)	0.220
Incisional SSI	11 (22.0)	6 (54.5)	4 (36.4)	1 (9.1)	0.251
Deep-space infection	39 (78.0)	11 (28.2)	9 (23.1)	19 (48.7)	1.000
Urinary tract infection	18 (8.9)	4 (22.2)	3 (16.7)	11 (61.1)	0.280
Bacteraemia	14 (6.9)	4 (28.6)	3 (21.4)	7 (50.0)	1.000

4. DISCUSSION

Intra-operative bile sampling has become routine in high-volume centres performing PD, as demonstrated in a recent meta-analysis (14). In our study, we found that bile sampling was not routinely performed during the study period, as 53.5 % of cases did not have an intraoperative bile specimen. There was a significant variation in routine bile sampling between the three institutions.

The current literature indicates that PBD is a risk factor for bactobilia, and our data was consistent with this finding (8, 9, 10). PBD rates between the three institutions were significantly different, with WDGMC draining a lower percentage of cases. Biliary micro-organisms known to colonise bile are usually polymicrobial in up to 70% of cases, with *Enterococcus spp.* (58%), *Klebsiella spp.* (29%), *Escherichia coli* (27%), *Candida spp.* (23%) *and Enterobacter spp. (20%)* (15). Our findings were consistent with the reported literature with 64.5 % polymicrobial bile specimens with similar organisms cultured (*Klebsiella spp.*, *Enterococcus spp.* and *Enterobacter spp.* being the top 3 organisms).

The use of perioperative antibiotics is standard in PD. Commonly used antimicrobial prophylaxis includes second generation cephalosporins and metronidazole (15). An increasing rate of resistance to this group of antibiotics has led to the use of broad-spectrum antibiotics. A significant decrease in SSI after PD was demonstrated with longer courses of broad-spectrum antibiotics (16). In a recent systematic review, the authors adapted their perioperative antibiotic prophylaxis to Piperacillin/Tazobactam instead of Cefuroxime and Metronidazole due to intraoperative biliary resistance patterns identified (15). In our setting, Piperacillin/Tazobactam is used in perioperative prophylaxis if the patient had previous biliary stenting. Biliary resistance patterns were evaluated and in our setting 50.0 % of biliary specimens exhibited resistance to Piperacillin/Tazobactam. The difference in resistance patterns for the three institutions was significant. CHBAH expressed the most resistant biliary profile. A newly recognised threat that has become evident is multi-drug resistant (MDR) enteric gram-negative bacteria (19). These organisms may influence SSI management. Some studies propose tailoring antimicrobial prophylaxis after PBD (8). In the setting of a high prevalence of MDR, it has been recommended not to change prophylaxis to Meropenem and Colistin. Close follow-up is recommended, with escalation of antimicrobials based on clinical and laboratory findings (13).

The rate of SSI in our setting is 24.8 %, which is within the range as described in the literature (1, 5, 6). The SSI rates across the three institutions did vary, with a lower rate of SSI observed at CMJAH. Very few studies showed no statistically significant relation between bactobilia and SSI, as was the case in our setting (1, 17). More than half of the patients (56.0 %) with SSI had bactobilia, but no statistically significant relationship was noted. Half of the patients with SSI had no intraoperative bile specimen collected, this may have influenced the statistical analysis.

Biliary micro-organisms from intraoperative bile cultures have been known to have similar organisms in wound cultures (1, 15). In our setting, there were similar organisms found in biliary and surgical site cultures. This concordance was seen at all three institutions.

PBD is known as a strong predictor of SSI, in our setting there was no statistically significant relationship between SSI and PBD, but notably, 80.0 % of patients with SSI had PBD performed. This is consistent with rates in the literature, where PBD is seen as a risk factor for SSI (6).

In our study, there was no relationship between bactobilia and septic postoperative complications, namely urinary tract infections and bacteraemia. This is similar to some studies that did not show a significant relationship between bactobilia and infectious postoperative complications (17, 20, 21). The limitations of our study were retrospective data, a small sample size, as well as incomplete data regarding obstructive jaundice and the duration between PBD and surgery.

5. CONCLUSION

Our study demonstrated heterogeneity in bile sampling practice, preoperative biliary drainage, and biliary antimicrobial resistance patterns between the different institutions. Pre-operative biliary drainage was associated with bactobilia which was mainly polymicrobial. There was no statistically significant association between bactobilia and overall septic postoperative complications. There was concordance between intraoperative biliary micro-organisms and those cultured from SSI in a third of patients. Biliary resistance to Piperacillin/Tazobactam, the antimicrobial of choice for prophylaxis at these three institutions, was high and institutional variation was significant.

We recommend that institutes review their internal practices regarding intraoperative bile sampling in patients undergoing PD, as this will allow guidance of antimicrobial selection in patients developing postoperative SSI. Further studies regarding the choice and duration of prophylactic antibiotics for PD are warranted.

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APPENDICES

APPENDIX 1: Approved protocol

Relationship between micro-organisms found in biliary cultures and subsequent surgical site infections after Pancreaticoduodenectomy at 3 University of the Witwatersrand academic hospitals.

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Wits Donald Gordon Medical Centre

Summary:

The current role of pre-operative biliary drainage (PBD) for patients who are going to undergo pancreaticoduodenectomy (PD) has recently been questioned. Recent literature suggests PBD should not routinely be performed but reserved for selected cases. PBD is associated with an increase in post-operative complications. Bactobilia associated with PBD, is linked to an increase in infectious post-operative complications and even morbidity, although not mortality. The collection of intra-operative bile samples at PD assists in culture directed antimicrobial therapy, which has recently been shown to decrease post-operative complications.

In this study, we aim to ascertain if bile sampling is performed, to compare bile cultures between drained and undrained patients and to compare resistance patterns between three University of the Witwatersrand academic hospitals. All patients undergoing PD in the period of January 2014 until December 2019 will be included. All data will be collected from patient folders, theatre registers, NHLS databases and discharge summary databases. The variables including age, sex, major co-morbidities (diabetes mellitus, chronic obstructive airway disease, chronic kidney disease), total bilirubin, malignant/benign disease, jaundice, cholangitis, white cell count, c-reactive protein, prophylactic antibiotic dose and duration, septic post-operative complications (wound culture, blood culture, urine and fluid culture), thirty day

mortality will be collected. Data will be entered on an Excel sheet or Redcap database and analysed accordingly.

In this study, we aim to indicate that not all patients undergoing PD has had bile samples taken and that there might be a difference in the resistance patterns between the different institutions. Therefore, there is a need for institution specific bile cultures and sensitivity patterns to assist in potentially decreasing the post-operative infectious complications, with the correct antimicrobial management.

Background:

Pancreaticoduodenectomy (PD), also known as the Whipple's procedure, became well known after Whipple described his two-stage procedure in 1935 (1). This procedure was infrequently performed due to the high associated morbidity and mortality. Initial mortality was as high as 25%. Since then, it has gained popularity in the management of patients with benign or malignant disease of the periampullary region. Currently mortality has decreased to about 3% in high volume expert centres (2, 3).

Operating in the presence of obstructive jaundice was thought to be a contributory factor to morbidity and mortality. Jaundice causes severe systemic pathophysiological changes of which hyperbilirubinaemia and endotoxaemia has been shown to influence prognosis in surgical patients. Various peri-operative interventions were recommended to relieve the obstruction. This included fluid replacement and decreasing endotoxaemia by ensuring decreased bacterial load in the gut by using oral lactulose and neomycin (4). As jaundice was felt to be responsible for the substantial morbidity and mortality, pre-resection drainage of the biliary system was investigated as a possible solution. Whipple's original operation consisted of two stages, the initial phase being a cholecystoenterostomy to drain the bile followed by a resection and reconstruction once the jaundice had settled.

Preoperative biliary drainage, initially with percutaneous ultrasound guided drainage and thereafter with stents and nasobiliary tubes placed at ERCP, was then introduced. The initial

prospective trial from Cape Town comparing preoperative percutaneous drainage showed no benefit (5). Other trials of prophylactic stenting (by ERCP) have been performed and in an initial meta-analysis on the efficacy of PBD indicated that there is no benefit and should not be routinely performed (6). It was associated with an increase in post-operative infections, wound infections and delayed gastric emptying. In the most recent meta-analysis, it was concluded again that PBD does not have a beneficial effect on post-operative outcome. It was even associated with an increase in overall complication rates and not just infectious complications (7). Preoperative biliary drainage may be prophylactic and most of the prospective studies excluded patients presenting with a bilirubin level of > 250 micromoles per litre, of whom were drained preoperatively. In addition, patients presenting with features of cholangitis that required drainage were also excluded.

Bactobilia is a serious risk factor for septic post-operative complications like bacteraemia, wound infection and intra-abdominal sepsis (8, 9, 10). The current guidelines for prophylactic antibiotics to prevent surgical site infections (SSIs) do not include recommendations for the type and length of antibiotic administration for patients who have had prophylactic biliary drainage prior to PD (11, 12). A recent study evaluated the effect of seventy-two hours of broad-spectrum antibiotic coverage on outcomes of PD, compared to routine twenty-four hours of peri-operative antibiotics. A significant decrease in surgical site infections (SSI) after PD was demonstrated with longer broad-spectrum antibiotics (13). In another study patients classified as high risk (PBD), received routine prophylactic antibiotics, which was then switched to culture directed therapy and continued for five days. There was a statistically significant decrease in overall infectious complications (urinary tract infections, pulmonary infections and septicaemia) (9).

Intra operative bile sampling is still not routine at all centres where PD is regularly performed (14). It has been shown that preoperative biliary drainage (PBD) is associated with bactobilia and there is an association with subsequent increased post-operative complications. It is therefore important to ascertain the incidence of and the resistance patterns of organisms associated with post-stenting bactobilia in specific institutions as these resistance patterns may vary (14).

Bile ducts are sterile and have been shown to become colonised in up to 25 % after obstruction, 75 % after ERCP and up to 100 % after stenting (15). In multiple studies PBD has been shown to be the strongest predictor of bactobilia (7, 9, 12, 15). Interestingly the association of PBD with infectious post-operative complications and morbidity is controversial. The influence

of PBD on morbidity and mortality has been studied with diverse findings. Some studies show that there is an increased risk of infectious complications (8, 9) while others did not (16). Similar morbidity and infection between stented and non-stented patients have also been demonstrated (14).

The current literature suggests that routine PBD should be reserved for specific indications. It is associated with bactobilia and an increase in post-operative complications, be it infectious or overall. There are no standardised guidelines for antibiotic prophylaxis, although recent studies suggest longer, broad-spectrum antibiotics to be associated with fewer complications.

It is suggested that there is a need for institution specific bile cultures and sensitivity patterns in patients undergoing PD who have had PBD. Currently there are no South African data available on bile cultures and sensitivity patterns of patients undergoing PD. It is not certain if bile sampling is part of routine practice at PD at Wits Academic hospitals.

Objectives:

Primary objectives

1. To ascertain whether intra-operative bile specimens are being taken at time of PD for micro-biological assessment.
2. To compare bile cultures in patients undergoing PD after PBD with those who did not receive preoperative drainage.
3. To compare bile cultures and resistance patterns between three University of the Witwatersrand academic hospitals in Gauteng.
4. To Correlate the causative organism of superficial and deep-seated surgical site infection

Secondary objective:

- To determine the association of bactobilia with septic post-operative complications.

Methods:

Design: Observational retrospective study

Site of study: Charlotte Maxeke Johannesburg Academic Hospital, Chris Hani Baragwanath Hospital and Wits Donald Gordon Medical Centre.

Study population: Patients undergoing pancreaticoduodenectomy over the period January 2014 until December 2019 at three University of the Witwatersrand academic hospitals.

Sampling: Sample size estimated at 50 cases

Inclusion criteria: All patients who underwent a pancreaticoduodenectomy in the specified period

Exclusion criteria: Pancreaticoduodenectomy not completed

Data collection: Data will be collected from theatre registers, case notes, discharge summary databases and the NHLS database. All variables included as per datasheet – Appendix A.

Statistical Analysis: Data would be entered into an EXCEL sheet or Redcap database and statistical analysis will be done using the Statistica Software package. Results will be presented as mean values $\pm$ standard deviation or median (range) with numbers (n) in each group in Tables or as graphs.

Comparisons between groups would be using the student's t test or Mann-Whitney tests with ordinal data compared using a Chi-squared or Fisher exact test. Statistical significance would be set at p=0.05.

Ethics:

Ethical issues during this study: All patient names and folder numbers will be kept confidential and only random allocated numbers will be used. Ethics approval will be obtained from the Human Research Ethics Committee of the University of the Witwatersrand prior to starting the study.

Problems:

Expected problems in this study is retrieving patient files and collecting adequate data. Lost files and incomplete data in files are a possibility. Finding enough cases of PD with bile cultures taken at surgery.

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APPENDIX 2: Data sheet

Date of surgery	Age	Gender	Data number	Preop Biliary drainage (Y/N)	Date of drainage	Interval to surgery

Intra-op Bile culture (Y/N)	Date and time culture received	Delay of specimen	Bile culture result/Sensitivity	Fluid culture	Urine culture	Blood culture	Wound Culture	Histology

APPENDIX 3: Change of title approval

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Private Bag 3 Wits, 2050
Fax: 027117172119
Tel: 02711 7172076

Reference: Mrs Sandra Benn
E-mail: sandra.benn@wits.ac.za

06 January 2023
Person No: 1585051
TAA

Dr MC Mouton
14 Hermes Road
Impala Park
1459
South Africa

Dear Dr Mariette Mouton

Master of Medicine in Plastic and Reconstructive Surgery: Change of title of research

I am pleased to inform you that the following change in the title of your Research Report for the degree of **Master of Medicine in Plastic and Reconstructive Surgery** has been approved:

From:	**Influence of preoperative biliary drainage on resistance patterns of biliary micro-organisms at three University of the Witwatersrand Academic Hospitals**
To:	**Correlation between micro-organisms found in biliary cultures and subsequent surgical site infections after Pancreaticoduodenectomy at 3 University of the Witwatersrand academic hospitals**

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

APPENDIX 4: Ethics clearance certificate 2018

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Dr M Mouton

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M170836

NAME: (Principal Investigator)	Dr M Mouton
DEPARTMENT:	School of Clinical Medicine Department of Surgery Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	Influence of preoperative biliary drainage on resistance patterns of biliary micro-organisms at three University of the Witwatersrand academic hospitals
DATE CONSIDERED:	25/08/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Professor D Bizos and Dr P Fru-Fonteh
APPROVED BY:	Professor CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	16/01/2018

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on 3rd floor, Phillip V Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **August** and will therefore be due in the month of **August** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature — Date: 22/01/2018

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX 5: Ethics Clearance Certificate 2023

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R49 Dr M Mouton

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M2211124

NAME: (Principal Investigator)	Dr M Mouton
DEPARTMENT:	School of Clinical Medicine Department of Surgery Division of Plastic and Reconstructive Surgery Medical School University
PROJECT TITLE:	*Correlation between micro-organisms found in biliary cultures and subsequent site infections after Pancreaticoduodenectomy at three University of the Witwaterstand academic hospitals*
DATE CONSIDERED:	Ad hoc
DECISION:	Approved unconditionally
CONDITIONS:	Renewal of M17/08/36 - expires on 2023/01/15
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Professors D Bizos & P Fru; Drs J Devar & G Schleicher
APPROVED BY:	Dr N Naran, Co-Chairperson, HREC (Medical)
DATE OF APPROVAL:	2023/01/05

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **November** and therefore reports and re-certification will be due in the month of **November** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator

06/01/2023
Date

APPENDIX 6: Approval to do research from CMJAH CEO.

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

University
of the Witwatersrand
Johannesburg

Department of Surgery:

Charlotte Maxeke Johannesburg Academic Hospital

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7th July 2017

Ms. G Bogoshi
Office of the CEO
Charlotte Maxeke Johannesburg Academic Hospital

Dear CEO

Re: Research Project:

Good day. This is to inform you that Dr Mariëtte Mouton of is planning to conduct a study titled:

- Influence of preoperative biliary drainage on resistance patterns of biliary micro-organisms at three University of the Witwatersrand Academic Hospital.

We are aware of the study and fully support it. There will be no cost implication to the Hospital. Dr Mariëtte Mouton has already applied for ethical approval from the Human Ethics Committee of University of the Witwatersrand.

Yours sincerely

Prof T E Luvhengo
Clinical Head of Surgery Department
Charlotte Maxeke Johannesburg Academic Hospital

APPENDIX 7: Approval to do research from CHBAH CEO.

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

MEDICAL ADVISORY COMMITTEE
CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

PERMISSION TO CONDUCT RESEARCH

Date: 4 Dec 2017

TITLE OF PROJECT: Influence of preoperative biliary drainage on resistance patterns of biliary micro-organisms

UNIVERSITY: Witwatersrand

Principal Investigator: M Mouton

Department: Surgery

Supervisor (If relevant): D Bizos, P Fru-Fonteh

Permission Head Department (where research conducted): Yes

Date of start of proposed study: Dec 2017
Date of completion of data collection: Dec 2019

The Medical Advisory Committee recommends that the said research be conducted at Chris Hani Baragwanath Hospital. The CEO /management of Chris Hani Baragwanath Hospital is accordingly informed and the study is subject to:-

- Permission having been granted by the Human Research Ethics Committee of the University of the Witwatersrand.
- the Hospital will not incur extra costs as a result of the research being conducted on its patients within the hospital
- the MAC will be informed of any serious adverse events as soon as they occur
- permission is granted for the duration of the Ethics Committee approval.

.....
Recommended
(On behalf of the MAC)
Date: 04 December 2017

.....
Approved/Not Approved
Hospital Management
Date: 7/12/17

APPENDIX 8: Approval to do research from WDGMC CEO.

Donald Gordon
Medical Centre

National centred. Independent. Academic.

MEDICLINIC

27 September 2017

To whom it may concern

Re: Permission to do research at the Wits Donald Gordon Medical Centre

This letter serves to confirm that Dr Mariëtte Mouton has been granted permission to conduct research for the study titled:

"Influence of Preoperative Biliary Drainage on Resistance Patterns of Biliary Micro-Organisms", at the Wits Donald Gordon Medical Centre.

Yours Faithfully,

Dr Sue Tager
CEO
Wits Donald Gordon Medical Centre
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Mobile: 082 457 7134
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Revision Date: 25 April 2012 M2549

APPENDIX 9: Approval to do research from NHLS.

NATIONAL HEALTH LABORATORY SERVICE

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Web: www.nhls.ac.za

10 November 2017

Applicant: Dr Mariëtte Mouton
Institution: University of the Witwatersrand
Department: Surgery
Email: mariette_mouton@yahoo.com
Tel: 011 481 2104
Cell: 071 610 6879

Re: Provisional Approval to access National Health Laboratory Service (NHLS) Data

Your application to undertake a research "**Influence of Preoperative Biliary Drainage on resistance Patterns of Biliary Micro-Organisms at Three University of the Witwatersrand Academic Hospitals**" using data from the NHLS database has been reviewed. This letter serves to advise that the application has been provisionally approved.

Please note that final approval will be granted on your compliance with the NHLS conditions of service and that the study can only be undertaken provided that the following conditions have been met.

- Ethics approval is obtained from a recognised SA Health Research Ethics Committee.
- Processes are discussed with the relevant NHLS departments (i.e. Information Management Unit and Operations Office) and are agreed upon.
- Confidentiality is maintained at participant and institutional level and there is no disclosure of personal information or confidential information as described by the NHLS policy.
- A final report of the research study and any published paper resulting from this study are submitted and addressed to the NHLS Academic Affairs and Research office and the NHLS has been acknowledged appropriately.
- NHLS Data cannot be used to track patients as no pre-approval/consent is obtained from Patients.

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research Office. Once Ethics approval has been granted please send it through to academic.research@nhls.ac.za. Any data related queries may be directed to NHLS Corporate Data Warehouse, contact number: 011 386 6074 email: zarina.sabat@nhls.ac.za

PP: Ramvadhela

Dr Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research

NATIONAL HEALTH LABORATORY SERVICE

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11 November 2021

Applicant: Mariette Mouton
Institution: University of the Witwatersrand
Department: Surgery
Email: mariette_mouton@yahoo.com
Tel: 011 488 4911 **Cell:** 084 999 2654

Re: Approval to access National Health Laboratory Service (NHLS) Data

Your application to undertake a research project **"Influence of preoperative biliary drainage on resistance patterns of biliary micro-organisms at three University of the Witwatersrand academic hospitals" Ref No: PR2119450** using data from the NHLS database has been reviewed. This letter serves to advise that the application has been approved and the required data will be made available to you ***without patient names*** to conduct the proposed study as outlined in the submitted application. Submissions should be made annually on the AARMS system – https://aarms.nhls.ac.za.

Please note that approval is granted on your compliance with the NHLS conditions of service and that the study can only be undertaken provided that the following conditions have been met.

- Processes are discussed with the relevant NHLS departments (i.e. Information Management Unit and Operations Office) and are agreed upon.
- Confidentiality is maintained at participant and institutional level and there is no disclosure of personal information or confidential information as described by the NHLS policy.
- NHLS Data cannot be used to track patients as no pre-approval/consent is obtained from Patients.
- All data requested should be in accordance with the research protocol submitted and approved by the relevant Ethics Committee.
- Request for the inclusion of the NHLS as a source of data in the original protocol to be approved by Ethics as NHLS does not have a Human Research Ethics Committee.
- A final report of the research study and any published paper resulting from this study are submitted and addressed to the NHLS Academic Affairs and Research office and the NHLS has been acknowledged appropriately.

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research Office. Any data related queries may be directed to NHLS Corporate Data Warehouse, contact number: 011 386 6074 email: zarina.sabat@nhls.ac.za

Dr Malokong

Dr Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research

APPENDIX 10: Plagiarism Report.

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ORIGINALITY REPORT

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SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

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