CLINICAL EPIDEMIOLOGY AND DEVELOPMENT OF NOVEL MULTILOCUS SEQUENCE TYPING SCHEME FOR *Candida parapsilosis* ASSOCIATED WITH CATHETER-RELATED BLOODSTREAM INFECTIONS

DINA HUSSEIN MARZOUQ YAMIN

UNIVERSITI SAINS MALAYSIA

2022

CLINICAL EPIDEMIOLOGY AND DEVELOPMENT OF NOVEL MULTILOCUS SEQUENCE TYPING SCHEME FOR *Candida parapsilosis* ASSOCIATED WITH CATHETER-RELATED BLOODSTREAM INFECTIONS

by

DINA HUSSEIN MARZOUQ YAMIN

Thesis submitted in fulfilment of the requirements
for the degree of
Doctor of Philosophy

March 2022

ACKNOWLEDGEMENT

In the name of ALLAH, the most Merciful, the most Compassionate. All praise and thanks be to Him, by whose blessings all goods can be accomplished. May peace be upon Prophet Muhammad صلى الله عليه وسلم, his families and companions. I would like to express my gratitude to Allah SWT for giving me opportunity and help me endlessly and for giving me the patience and perseverance in finishing this project.

First, I would like to offer my sincerest gratitude to my dearest supervisor **Associate Professor Dr Azian Harun,** who kept her eyes on the progress of my project and always was available, for her continuous guidance, support, and patience. Her excellent supervision, expertise, interest, motivation, the genuine insight into the problems and her help to gain the deepest thoughts in research have made it possible for me to complete the research. A very special gratitude and thanks to my co-supervisors, **Associate Professor Dr Azlan Husin** and **Dr**. **Nur Waliyuddin Hanis Zainal Abidin**, for their encouragement, fruitful ideas, advice, and assistance, for commitment, patience in overcoming numerous obstacles. Moreover, in completing of the research, I had to take the help of some respected persons, who deserve gratitude; Assoc. Prof. Sarimah Abdullah, Dr. khalid, Dr. Dauda Ghoni, Dr. Nik Zuraina and Dr. Nisa. I am truly indebted and sincerely grateful to all lab members and friends. I am extending my warmest thankful to my family who never fail to support, care, and pray for my success. To my beloved parents, my gratitude for you can hardly be expressed by words. To my husband, Eng. Shadi Amer. I am always grateful for your infinite support. To my dear daughters, Aya and Maream. Last, I would like also to thank the research funding support received in the form of USM Research University Individual (RUI) grant and Tabung Insentif Pembangunan Pengajian Siswazah (TIPPS-PhD).

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SYMBOLS/ ABBREVIATIONS

Symbols/Abbreviations	Definition
-	negative or subtraction
%	percentage
/	division or 'or'
~	approximately
+	positive or addition
=	equal to
±	plus-minus
>	more than
<	less than
≤	less than or equal to
≥	more than or equal to
°C	degree Celsius
µl	microliter
µM	micromolar
A	adenine
A&E	Accident and Emergency
A_{260}	absorbance at 260 nm
A_{280}	absorbance at 280 nm
AFLP	Amplified fragment length polymorphism
AFST	Antifungal susceptibility testing
ALS	Agglutinin-like sequences
ANC	Absolute neutrophil count

APACHE	Acute physiology and chronic health evaluation
ARF	Acute renal failure
ATCC	American Type Culture Collection
$BaCl_2$	Barium chloride
BAM	Binary Alignment Map
BLAST	Basic Local Alignment Search Tool
BMD	broth microdilution
bp	base pair
BED	Browser Extensible Data
BSC	biological safety cabinet
BSI	Bloodstream infections
BQS	base quality score
BWA	Burrows–Wheeler alignment
C	cytosine
Ca^{2+}	calcium ions
$CaCl_2$	calcium chloride
CAPD	continuous ambulatory peritoneal dialysis
CBPs	clinical breakpoints
CCU	Cardiac care unit
CDC	Centers for Disease Control and prevention
CFU	colony forming unit
CI	confidence interval
CLSI	Clinical and Laboratory Standards Institute
cm	centimeter
CNV	copy-number variants

CRBSI	Catheter-related bloodstream infection
CRP	C- reactive protein
CTX	inter-chromosomal translocation
CVC	Central venous catheter
CVL	Central venous line
DEL	Deletion
df	degree of freedom
dH_2O	distilled water
DNA	Deoxyribonucleic acid
dN/dS	Ratio of nonsynonymous to synonymous amino acid changes
dNTPs	deoxyribonucleotide triphosphate
dsDNA	Double stranded DNA
DST	Diploid sequence type
DTTP	Differential time to positivity
eBURST	Based Upon Related Sequence Types
ECVs	epidemiological cutoff values
EDTA	ethylenediaminetetraacetic acid
EPIC-PCR	exon-primed intron-crossing PCR
et. al.	*et alia* (and others)
ER	Emergency room
ESPEN	European Society for Clinical Nutrition and Metabolism
ESRF	End stage renal failure
E test	Epsilometer test
ETT	Endotracheal tube

EUCAST	The European Committee on Antimicrobial Susceptibility Testing
F	forward or sense primers
FN	False negative
FP	False positive
g	gram
g	gravitational force
G	guanine
G+C	guanine-cytosine
GATK	Genome alignment tool kit
Gb	Gigabases
GB	Gigabite
GFF	General feature format
GUI	Graphical user interface
GTF	Gene transfer format
H_2SO_4	sulfuric acid
HCl	hydrochloric acid
HCWs	Health care workers
HDU	high dependency unit
Het	Heterozygous
HGP	Human Genome Project
Hom	Homozygous
HPC	High-Performance Computing
hr	Hour
HUSM	Hospital USM
IDSA	Infectious Disease Society of America

ICU	Intensive care unit
IDT	Integrated DNA Technology
IFIs	invasive fungal infections
IGV	Integrative Genomics Viewer
IMWs	Internal medical wards
INDEL	Insertion/deletion
INV	Inversion
IQR	Interquartile Range
ITS	Internal Transcribed Spacer
ITX	intra-chromosomal translocation
IUPAC	International Union of Pure and Applied Chemistry
Kb	Kilobases
kDa	kilodaltons
L	liter
LAMP	loop-mediated isothermal amplification
LIS	laboratory information system
M	molar
MALDI-TOF MS	Matrix-assisted laser desorption ionization–time of flight mass spectrometry
Mb	million base pair/ megabases
MDS	multidimensional scaling
ME	2-mercaptoethanol
MEGA	Molecular Evolutionary Genetics Analysis
mg	milligram
Mg^{2+}	magnesium ions

$MgCl_2$	magnesium chloride
MIC	Minimum inhibitory concentration
min	minute
ml	milliliter
MLST	Multilocus sequence typing
mm	millimeter
mM	millimolar
MLR	Multiple logistic regression
MST	minimum spanning tree
MTL	mating type loci
MV	Mechanical ventilation
n	frequency or total
N	grand total
Na+	Sodium ion
NaCl	sodium chloride
NaOH	sodium hydroxide
NCBI	National Centre for Biotechnology Information
NCCLS	National Committee for Clinical Laboratory Standards
ng	Nanogram
NGS	Next-generation sequencing
NHGRI	National Human Genome Research Institute
NICU	Neonatal ICU
NIH	National Institutes of Health
NJ	Neighbor joining

nm	Nanometer
nM	Nanomolar
No.	Number
nr	non-redundant
OD	Optical density
O&G	Obstetrics and Gynaecology
OR	Odd ratio
ORFs	open reading frames
PBS	Phosphate buffered saline
PCR	polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
pg	Picogram
PICC	Peripherally inserted central catheter
PICU	Pediatric intensive care unit
PLB	Phospholipases B
PMM	polymorphic microsatellite marker
PO_3^-	Phosphate
PS	Power and Sample size
Q	Quality score
QC	Quality control
R	Resistance
R	reverse or antisense primers
RAPD	Random amplified polymorphic DNA
RBC	Red blood cell
rDNA	Ribosomal DNA

RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
ROC	Receiver Operation Characteristics
rpm	Round per minute
rRNA	Ribosomal RNA
s	Second
S	Sensitive
SAM	Sequence alignment map
SD	standard deviation
SDA	Sabouraud dextrose agar
SDD	Susceptible dose dependent
SDS	Sodium dodecyl sulfate
SE	standard error
SGD	*Saccharomyces* Genome Database
SLR	Simple logistic regression
SNPs	single nucleotide polymorphisms
SNVs	Single nucleotide variants
SOFA	Sequential Organ Failure Assessment
SPSS	Statistical Package of Social Science
SRA	Sequence read archive
ST	sequence type
START	Sequence type analysis and recombinational tests
SV	Structural Variation
T	Thymine
T_A	annealing temperature

Taq	*Thermus aquaticus*
Tb	Terabases
TBE	Tris-Borate-EDTA
TE	Tris-EDTA
T_m	Melting temperature
TPN	Total parenteral nutrition
ts	Transition
tv	Transversion
TWBC	Total white blood cell count
U	Unit
UPGMA	unweighted pairgroup method with arithmetic average
UV	Ultraviolet
USA	United States of America
USM	Universiti Sains Malaysia
V	Volts
VCF	Variant call format
w/v	Weight per volume
WBC	White blood cell count
WGA	Whole-genome assembly
WGS	whole-genome shotgun/ sequence
WHO	World Health Organization
x	times or multiply
YPD	yeast extract peptone dextrose

EPIDEMIOLOGI KLINIKAL DAN PEMBANGUNAN SKIM PENJENISAN JUJUKAN MULTILOKUS BAHARU UNTUK CANDIDA PARAPSILOSIS YANG MENYEBABKAN JANGKITAN ALIRAN DARAH BERKAITAN DENGAN KATETER

ABSTRAK

Candida parapsilosis dikesan sebagai satu patogen jangkitan dalam penjagaan kesihatan termasuklah jangkitan salur darah yang berpunca daripada kateter (CRBSI). Mengambil kira kekurangan maklumat mengenai CRBSI, objektif penyelidikan ini adalah menentukan kadar kebolehjangkitan dan faktor-faktor risiko CRBSI yang berpunca daripada *C. parapsilosis.* Sebanyak 208 kandidemia *C. parapsilosis* telah dikumpul dan direkodkan dalam kajian ini. Daripada jumlah ini, 31 sampel tidak melibatkan kateter telah dikecualikan daripada analisis, manakala 177 kes memenuhi kriteria penyelidikan, terdiri daripada 30 kes melibatkan CRBSI, dan 147 kes yang tidak melibatkan CRBSI merangkumi ciri-ciri demografi, penyakit tersirat, prosedur perubatan invasif, dan nilai-nilai ujian makmal. Kadar prevalens, ciri-ciri klinikal dan faktor-faktor risiko CRBSI yang berpunca daripada *C. parapsilosis* berjaya ditentukan. Identifikasi *C. parapsilosis* telah disahkan dengan penjujukan gen RNA ribosom pada bahagian ITS. Satu skim penjenisan jujukan multilokus (MLST) baharu untuk pengenotipan populasi isolat klinikal *C. parapsilosis* telah berjaya dibangunkan. Semasa fasa pembangunan MLST, beberapa lokus telah disaring berdasarkan data kajian terdahulu dan data penjujukan seluruh genom. Tujuh lokus bervariasi tinggi telah dipilih berdasarkan lima isolat *C. parapsilosis* yang berbeza. Ujian keneutralan telah dijalankan ke atas lokus terpilih dan kuasa diskrimasi skim tersebut telah ditentukan. Kepelbagaian genetik populasi *C. parapsilosis* dan perkaitan dengan hasil klinikal dan

kerintangan antikulat telah ditentukan. Dapatan penyelidikan ini menunjukkan *C. parapsilosis* adalah spesis *Candida* utama yang menyebabkan kandidemia sebanyak 29.2% daripada keseluruhan kes. Kadar prevalens CRBSI *C. parapsilosis* adalah 14.4% daripada keseluruhan kandidemia, dan 17.7% adalah daripada kalangan pesakit yang menggunakan kateter. Daripada 208 kes yang melibatkan kandidemia *C. parapsilosis*, 30 kes adalah CRBSI, 112 adalah berkaitan dengan BSI dan 66 merupakan pengkoloni kateter. Kemasukan ke unit rawatan rapi (ICU) dan nutrisi parenteral total (TPN) telah dikenalpasti sebagai faktor-faktor yang tidak bersandar ke atas CRBSI *C. parapsilosis*. Kemasukan selain unit rawatan rapi dan TPN menunjukkan perkaitan yang signifikan dengan perkembangan CRBSI *C. parapsilosis*. Sebanyak 19.1% kematian pesakit disebabkan kandidemia *C. parapsilosis* telah dicatatkan. Skim MLST yang telah dibangunkan dapat mengkelasifikasikan 19 jenis *C. parapsilosis* sensu stricto kepada 15 DST yang berbeza dengan kuasa diskriminasi 0.942. Analisis filogenetik berdasarkan rantaian 4735 nukleotida 19 jenis *C. parapsilosis* sensu stricto telah mengkategorikan isolat tersebut kepada empat kumpulan. Namun begitu, perkaitan DST *C. parapsilosis* dengan kesudahan penyakit dan kerintangan antikulat tidak dapat ditentukan berikutan saiz sampel yang kecil. Kesimpulannya, kadar prevalens kandidemia *C. parapsilosis* adalah yang tertinggi dalam kalangan spesis *Candida*, sementara prevalens CRBSI *C. parapsilosis* adalah rendah berbanding keseluruhan kandidemia *C. parapsilosis*. Kemasukan ke ICU dan penerimaan TPN adalah faktor-faktor risiko tidak bersandar ke atas CRBSI *C. parapsilosis*, di mana kemasukan selain ICU dan TPN menunjukkan perkaitan yang signifikan dengan perkembangan CRBSI. Skim MLST yang julung kali dibangunkan ini adalah mampan dengan kadar diskriminasi yang tinggi untuk pengenotipan *C. parapsilosis* seluruh dunia.

CLINICAL EPIDEMIOLOGY AND DEVELOPMENT OF NOVEL MULTILOCUS SEQUENCE TYPING SCHEME FOR *Candida parapsilosis* ASSOCIATED WITH CATHETER-RELATED BLOODSTREAM INFECTIONS

ABSTRACT

Candida parapsilosis has been increasingly reported as an important pathogen causing healthcare associated infections, including catheter-related bloodstream infections (CRBSIs). Given the paucity of information on this pathogen, this study aimed to determine the prevalence of *C. parapsilosis* CRBSI and the associated risk factors. As there is lack of genotyping method to study the molecular characteristics of *C. parapsilosis*, this study included the development of multilocus sequence typing (MLST) scheme for *C. parapsilosis* sensu stricto population. In this study, data for 208 *C. parapsilosis* candidemia were collected and recorded in a standard proforma. After exclusion of 31 cases that were not catheterized, 177 episodes of *C. parapsilosis* candidemia were included in analysis, from which, 30 cases of CRBSI were compared to 147 non-CRBSI cases in terms of demographic, underlying diseases, invasive medical procedures, and laboratory test values. The prevalence, clinical characteristics and risk factors of *C. parapsilosis* CRBSIs were determined. Species identification of *C. parapsilosis* sensu stricto was confirmed by sequencing the ITS region of the ribosomal RNA genes. A novel MLST scheme was developed for genotyping clinical *C. parapsilosis* population. Five representative non-related *C. parapsilosis* isolates were used to test the potentially polymorphic loci for the presence of polymorphism. In MLST development phase, a number of loci were screened based on previously published MLST schemes and data from *C. parapsilosis* whole genome sequences. Seven most polymorphic loci were selected. Neutrality test was performed on the selected loci and

discriminatory power of the scheme was determined. Genetic diversity among *C. parapsilosis* population and correlation with clinical outcomes and antifungal resistance were determined. Results of this study revealed that *C. parapsilosis* was the most predominant *Candida* species causing candidemia contributing to 29.2% of all candidemia cases. The prevalence of *C. parapsilosis* CRBSI was 14.4% of all *C. parapsilosis* candidemia, and 17.7% of catheterized candidemia patients. Out of 208 cases of *C. parapsilosis* candidemia, 30 cases were CRBSI, 112 were BSI and 66 were catheter colonizer. Intensive care unit (ICU) admission and receipt of total parenteral nutrition (TPN) were reported as independent risk factors of *C. parapsilosis* CRBSI. Admission in non-ICU and receipt of TPN were significantly associated with the development of *C. parapsilosis* CRBSI compared to non-CRBSI. Death due to *C. parapsilosis* candidemia was reported in 19.1% of patients. The developed MLST scheme demonstrated the ability to discriminate 19 *C. parapsilosis* sensu stricto strains into 15 different DSTs with a discriminatory power of 0.942. Phylogenetic analysis based on 4735 concatenated nucleotides of 19 *C. parapsilosis* sensu stricto strains were grouped into four clusters. The associations of *C. parapsilosis* DSTs with outcomes and antifungal resistance cannot be determined due to small number of strains in each genotype. In conclusion, the prevalence of *C. parapsilosis* candidemia was the highest among other *Candida* species, while the prevalence of *C. parapsilosis* CRBSI was low compared to all *C. parapsilosis* candidemia. Admission to ICU and receiving TPN were independent risk factors for *C. parapsilosis* CRBSI, where non-ICU admission and TPN receipt were significantly associated with development of CRBSI compared to non-CRBSI. A novel MLST scheme for *C. parapsilosis* has been successfully developed. This robust, highly discriminatory novel MLST scheme could be used as a molecular genotyping tool for *C. parapsilosis* sensu stricto strains worldwide.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Fungal infection is a crucial problem causing healthcare associated diseases among leukemic patients, bone marrow transplant patients and other immunocompromized patients worldwide (Levin *et al.*, 1998; Pagano and Mayor, 2018; Zancope-Oliveira *et al.*, 2000). *Candida* is one of the main causative agents especially for bloodstream infection (Al-Musawi *et al.*, 2021; Raja, 2021; Rudramurthy and Singh, 2020; Tóth *et al.*, 2019). Even though *Candida albicans* remains the most common pathogenic *Candida* species, several *Candida* species have recently emerged such as *Candida tropicalis*, *Candida parapsilosis*, *Candida glabrata* and *Candida krusei* (Al-Musawi *et al.*, 2021; Barbedo *et al.*, 2017; Ben-Ami, 2018; Chen *et al.*, 2010; Diekema *et al.*, 1997; Martini *et al.*, 2020; Ricotta *et al.*, 2021; van Asbeck *et al.*, 2009b; Zheng *et al.*, 2021). So far, more than 17 different *Candida* species have been identified as bloodstream pathogen, but *C. albicans* remains the mostly studied (Tseng *et al.*, 2018), where *C. parapsilosis* rank next (Ben-Ami, 2018; Hernández-Castro *et al.*, 2010; Noni *et al.*, 2019). *C. parapsilosis* was the predominant cause of bloodstream infection among neonate (Magobo *et al.*, 2020). While in other studies, candidemia in neonate was predominantly caused by *C. albicans*, followed by *C. parapsilosis* (Hajjeh *et al.*, 2004; Jantarabenjakul *et al.*, 2021). *C. parapsilosis* is second or third most common yeast species causing nosocomial bloodstream infections (Ataides *et al.*, 2015; Kocsube *et al.*, 2007; Lockhart *et al.*, 2008; Silva *et al.*, 2009; Tay *et al.*, 2009; Vigezzi *et al.*, 2019; Zheng *et al.*, 2021), or fourth (Pfaller *et al.*, 2001) with relatively low virulence (Brillowska-Dabrowska *et al.*, 2009;

Mroczyńska and Brillowska-Dąbrowska, 2021), particularly in neonate intensive care units (Barbedo *et al.*, 2017; de Paula Menezes *et al.*, 2020; Mirhendi *et al.*, 2010; Nosek *et al.*, 2009; van Asbeck *et al.*, 2009b; Vaz *et al.*, 2011). Because of the different pattern of antifungal resistance among these species, it is necessary to identify the causative agent of candidemia to species level. Moreover, rapid diagnosis and proper selection of antifungal drug worth to be considered for better outcomes, reduce costs and hospital admission days (Lamoth *et al.*, 2021; Salazar *et al.*, 2020). Using the conventional diagnostic laboratory tests is not enough to confirm the identity of fungal pathogens. Recently, advance in molecular field as an alternative for conventional diagnostic protocols including macroscopy, microscopy, culture and many commercially available chemical assimilation tests, has been observed. Even though the fungal culture remains the gold standard diagnostic test, assimilation tests are needed for confirmation. Collectively, these tests consume several days for results to be released, and such delay may affect patient outcome severely (Fallahi *et al.*, 2020; Malik *et al.*, 2021). For that reason, treatment with antifungal drugs is often empirical since it relies on presumption and on epidemiological data obtained from previous studies if available.

The prevalence of *C. parapsilosis* candidemia has risen during the last three decades especially catheter-related bloodstream infection (CRBSI). There are few studies which determine the prevalence of *C. parapsilosis*, especially in Malaysia. *C. parapsilosis* has the ability to adhere to prosthetic materials such as blood catheters, and subsequently colonizes the catheter surface. These colonized catheters may introduce the pathogen to bloodstream leading to much complicated bloodstream fungal infection that can be resolved by immediately changing catheter and proper antifungal treatment (Phua *et al.*, 2019; Trofa *et al.*, 2008). *C. parapsilosis* can be also

transmitted to the patient bloodstream from the hands of health care workers (HCWs) through the process of catheter exchange and medical check-up (da Silva *et al.*, 2021a; Mareković *et al.*, 2021). The adherence of *C. parapsilosis* to surfaces is enhanced by the ability of this organism to form biofilm which adhere to prosthetic surfaces. The presence of biofilm protects the yeast cells from patient immune system as well as provide resistance to antifungal treatments (Gómez-Molero *et al.*, 2021.; Zuo *et al.*, 2021b). In addition, *C. parapsilosis* is able to grow in solutions with high glucose concentration like total parenteral nutrition solutions (TPN) that is usually given to patients via central catheters (da Silva *et al.*, 2021b; Raja, 2021). Even though these virulence factors have been described, there is paucity of studies focusing on the prevalence and risk factors of *C. parapsilosis* CRBSI, particularly in Malaysia.

Diagnosis and treatment of *C. parapsilosis* CRBSI affecting inpatients suffering immunosuppression or other comorbidities is a medical challenge that requires quick decision making and knowledge of its aetiology (Phua *et al.*, 2019).

Candida parapsilosis complex has been divided into three distinct groups (group I, II and III), which were then identified as separate species namely, *C. parapsilosis* sensu stricto (recently known as *C. parapsilosis*), *C. orthopsilosis* and *C. metapsilosis* respectively. *C. parapsilosis* is the most prevalent and have higher morbidity and mortality than the latter two species that are rarely encountered (Tavanti *et al.*, 2005a). Because of that, much more concern should be directed to *C. parapsilosis*. Differentiation among these species can be accomplished molecularly by sequencing RNA ribosomal genes such as ITS1 and ITS2 and D1/D2 region (White *et al.*, 1990).

For epidemiological purposes, many studies have been performed on *Candida* species in general, or specifically for *C. albicans*, *C. tropicalis* or *C. glabrata* and

C. krusei, while they are rarely done on *C. parapsilosis*. The purpose of such studies is to genotype these species, study the association of their genotypes with different clinical outcomes, and to study the association of genotypes and antifungal resistance (Arastehfar *et al.*, 2020; Gharaghani *et al.*, 2021; Gong *et al.*, 2018; Wu *et al.*, 2021). To achieve similar goals on *C. parapsilosis*, a reliable, rapid, easy, stable, reproducible and with high discriminatory power molecular genotyping protocol is needed. Many molecular protocols have been applied on *C. parapsilosis* such as random amplified polymorphic DNA (RAPD) (Hernández-Castro *et al.*, 2010; Madhavan *et al.*, 2019; Tay *et al.*, 2009), polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) (Asadzadeh *et al.*, 2009; Ataides *et al.*, 2015; Barbedo *et al.*, 2017; Cantón *et al.*, 2011; Lockhart *et al.*, 2008; Marcos-Arias *et al.*, 2020; Mirhendi *et al.*, 2010; Tavanti *et al.*, 2005a; van Asbeck *et al.*, 2009a), amplified fragment length polymorphism (AFLP) (Tavanti *et al.*, 2007; Tavanti *et al.*, 2010), PCR fingerprinting (Garzillo *et al.*, 2017), and karyotyping (Gómez-Molero *et al.*, 2020). While some of them were able to detect interspecies differences, but none was able to detect intraspecies differences until the introduction of microsatellite genotyping protocol (Sabino *et al.*, 2010) that was able to genotype *C. parapsilosis* with high discriminatory power (Barbedo *et al.*, 2017; Zhang *et al.*, 2020). Nevertheless, this technique has disadvantages include a prolonged optimization process and increased expenses, which are particularly problematic in the creation of multiplex systems. Additionally, automation constraints and data management requirements might obstruct the transfer of technology across various labs and institutions (Pimentel *et al.*, 2018).

Multilocus sequence typing (MLST) has been initially established for genotyping of bacteria (Maiden, 1998), and subsequently MLST schemes have been

developed for several fungi including *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. krusei* and *C. dubliniensis* (Odds and Jacobsen, 2008; Urwin and Maiden, 2003). An attempt for developing MLST scheme for *C. parapsilosis* has been done by Tavanti *et al*. in 2005, studied 11 loci which are mostly housekeeping genes, but that scheme was only able to differentiate between the three species of the *C. parapsilosis* complex but failed to genotype *C. parapsilosis* into different strains (Tavanti *et al.*, 2005a). No more attempts to construct MLST scheme for *C. parapsilosis* have been done up to date. MLST has many advantages, including easy to be conducted, rapid, reliable, reproducible, and has high discriminatory power. Besides, MLST data can be deposited in internet-based MLST databases constructed for any organism that has established MLST scheme, and data from different epidemiological areas can be compared. With the advances in molecular techniques worldwide, sequencing cost has significantly reduced over the last decades and MLST becomes more affordable. MLST work does not need large working area, and no extra training other than conventional PCR skill is needed. In contrast, the most important drawbacks are that the results need relatively long period of time to be obtained, starting from sample processing, followed by DNA extraction, PCR amplification of seven loci, sequencing, sequence editing, sequence alignment and analysis, to the final interpretation of the results, which are clear and accurate, and data can be shared on the internet. Besides that, the genome sequence of organism needs to be known in advance to infer the housekeeping genes of the species. Even though that MLST does not cost a lot, the sequencing and technical requirements is of relatively high cost to resource-limited countries (Hu *et al.*, 2021; Uelze *et al.*, 2020).

In order to provide appropriate therapy and adequate prevention of the disease, early identification of fungal pathogens from bloodstream infected patients is critical,

particularly in neonates and immunocompromized patients (Clancy and Nguyen, 2018; Sanguinetti *et al.*, 2019). Without a fast and reliable diagnostic tool, the uncertainty of causative pathogens predisposes to improper use of antifungal medicines, this leads indirectly in possibly excessive expenses, undesirable pharmacological effects and the emergence of multidrug-resistant fungi (Clancy and Nguyen, 2018). Consequently, assays for the detection of candidemia in general and *C. parapsilosis* CRBSI in particular that are quick, sensitive, and specific are required for accurate and timely diagnosis, which could result in more effective therapy.

1.2 Justification of the study

Invasive candidiasis is the most commonly reported invasive fungal infection worldwide and *C. parapsilosis* is one of the most important non-albicans pathogens.

Given the increasing frequency rate of nosocomial *C. parapsilosis* bloodstream infection among intensive care patients, with geographical variation of the distribution of *C. parapsilosis* complex species, it is of paramount importance to conduct epidemiological studies in different regions worldwide, including Malaysia.

Candida parapsilosis has been implicated in CRBSI, and since central venous catheters are widely used in critically ill patients, data on the risk factors and associated comorbidities must be obtained to aid in the formulation of infection prevention and patients management.

Although the rate of mortality caused by *C. parapsilosis* is often less than recorded for other *Candida* species, *C. parapsilosis* complex has various distinctive characteristics, including its potential for biofilm formation on the surface of intravascular devices and its strong affinity to parenteral nutrition. and hence signifies its further studies. Infections due to *C. parapsilosis* have been associated with different

presentations and outcomes. Therefore, it is necessary to understand the phenotypic and genotypic characteristics of this pathogen. Given the robustness of MLST method for studying the molecular characteristics of pathogens, this method is expected to be useful for *C. parapsilosis* population as well. Unfortunately, there is no existing MLST scheme for this pathogen yet. Therefore, it is strongly justifiable to develop a new method for genotyping *C. parapsilosis*. It is hoped that the novel MLST scheme will provide a highly discriminatory method for such purpose and can be applied for *C. parapsilosis* population worldwide.

1.3 The research questions of the study

1. What is the prevalence of *C. parapsilosis* candidemia in Hospital USM from 2001-2018?

2. What is the prevalence of *C. parapsilosis* CRBSI in Hospital USM?

3. What are the risk factors of *C. parapsilosis* CRBSI?

4. What is the genetic variability of *C. parapsilosis* population in Malaysia?

5. Is there an association between certain *C. parapsilosis* genotype(s) and the clinical outcome of CRBSI?

6. Is there an association between *C. parapsilosis* genotype(s) and antifungal resistance among CRBSI?

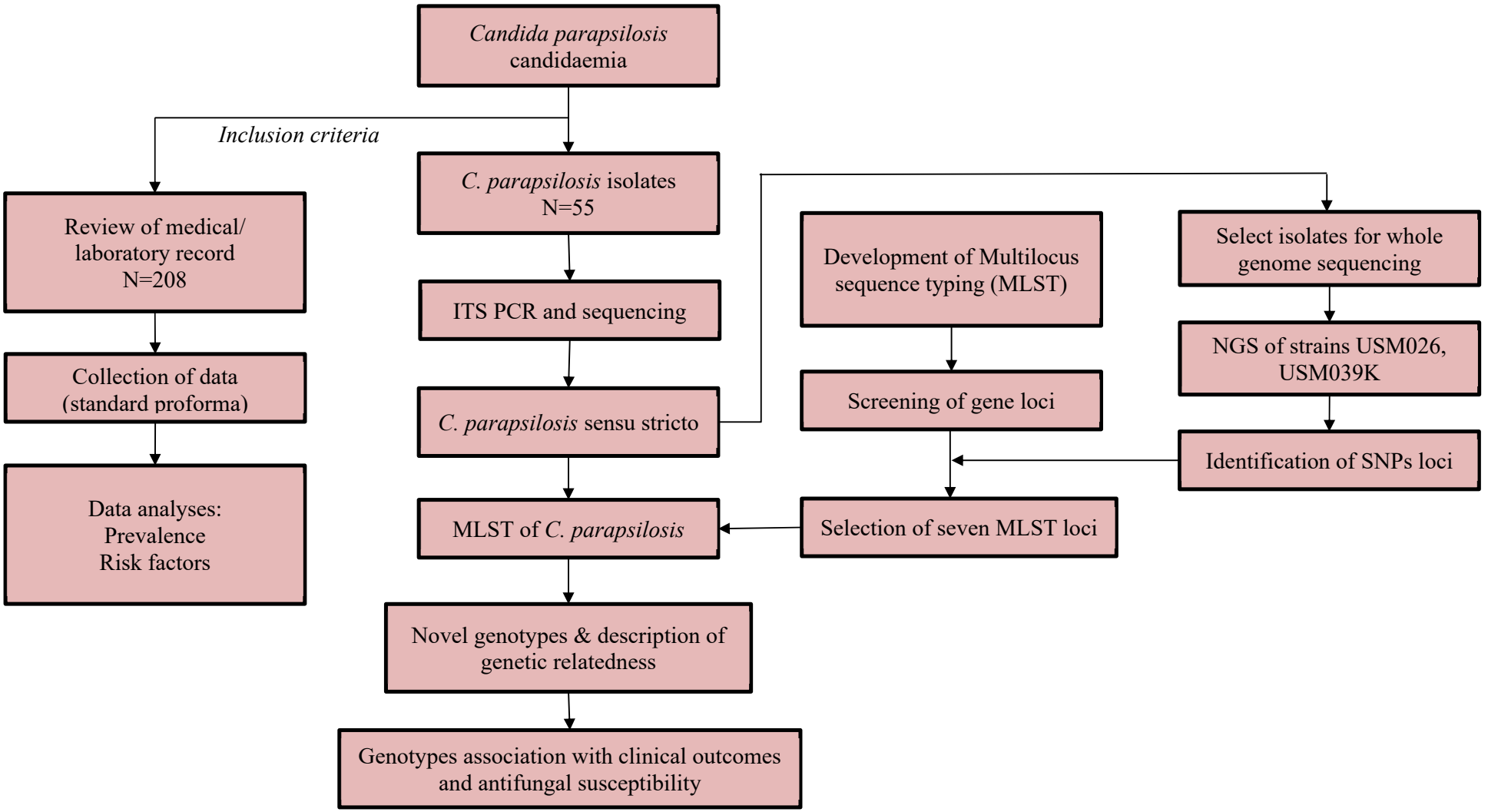
1.4 The objectives of the study

1.4.1 General objective:

This study aimed to determine the prevalence, clinical characteristics and risk factors of *C. parapsilosis* CRBSI, and to develop a multilocus sequence typing (MLST) scheme for *C. parapsilosis* sensu stricto population.

1.4.2 Specific objectives:

1. To determine the prevalence of *C. parapsilosis* bloodstream infections.
2. To determine the prevalence of CRBSI caused by *C. parapsilosis*.
3. To determine the risk factors for *C. parapsilosis* CRBSI.
4. To develop a novel sequence-based genotyping scheme for *C. parapsilosis*.
5. To determine the genetic variability of *C. parapsilosis*.
6. To determine the association between genotype and clinical outcome among *C. parapsilosis* CRBSI.
7. To determine the association between genotype and antifungal resistance of *C. parapsilosis* CRBSI.


Candida parapsilosis candidaemia
Inclusion criteria
Review of medical/ laboratory record N=208
Collection of data (standard proforma)
Data analyses: Prevalence Risk factors
C. parapsilosis isolates N=55
ITS PCR and sequencing
C. parapsilosis sensu stricto
MLST of *C. parapsilosis*
Novel genotypes & description of genetic relatedness
Genotypes association with clinical outcomes and antifungal susceptibility
Development of Multilocus sequence typing (MLST)
Screening of gene loci
Selection of seven MLST loci
Select isolates for whole genome sequencing
NGS of strains USM026, USM039K
Identification of SNPs loci

CHAPTER 2

LITERATURE REVIEW

2.1 History of taxonomy and nomenclature

Candida parapsilosis was first isolated in 1928 by Ashford from the feces of a patient with diarrhea in Puerto Rico (Ashford, 1928) and were considered nonpathogenic. The type strain of the species ATCC22019 (with the acronyms CBS604, CLIB214, IFM46829, IFO1396, JCM1785, MCO478, NRRL-Y12969) was initially classified as *Monilia parapsilosis*, species unable to ferment maltose, to differentiate it from *Monilia psilosis* (currently known *C. albicans*), and reclassified as *C. parapsilosis* in 1932 (Langeron and Talice, 1932). In 1940, *C. parapsilosis* was reported as the causative agent of a fatal case of endocarditis in an intravenous drug user (Joachim and Polayes, 1940). At this early stage, researchers have related *C. parapsilosis* with an exogenous introduction that cleverly foreshadows the link between *C. parapsilosis* and invasive medical devices and hyperalimentation solutions. *C. parapsilosis* is ubiquitous in the environment and is one of the fungal agents most frequently isolated from the subungual space. Isolates of this yeast are commonly detected in blood, skin, and nails, particularly in the hands of HCWs, as well as on the surfaces of medical plastics and prosthetic equipment. *C. parapsilosis* is a new fungal pathogen that is increasingly linked to a variety of illnesses, including fungemia, vaginitis, endocarditis, endophthalmitis, septic arthritis, and peritonitis (Nosek *et al.*, 2009; Pappas *et al.*, 2018; Trofa *et al.*, 2008).

The *Candida* genus encompasses heterogeneous anamorphic yeasts which include around 196-200 species that are connected to ascomycetes or basidiomycetes physiologically. The name is derived from the tradition in ancient Rome for a candidatus, a candidate for public office, to dress in white. *Albico* means “to be white,”

so the name *C. albicans* is redundant (Vincent, 2012). The most important pathogenic species, *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *C. lusitaniae* and *C. glabrata*, are phylogenetically related to the *Ascomycetes* (Cortegiani et al., 2018). The genus *Candida* is classified as follows: Phylum: *Deuteromycota,* Class: *Blastomycetes*, Order: *Cryptococcales*, Family: *Cryptococcaceae*, Genus: *Candida* (Vincent, 2012). *C. parapsilosis* belongs to *Lodderomyces- Spathaspora* clade in the family *Debaryomycetaceae* (Alanio et al., 2017).

2.2 *Candida parapsilosis* associated risk factors

The commonest presentation of invasive candidiasis is candidemia (Wang *et al.*, 2019) representing less than 75% of all invasive infections. Of these candidemias 36% occur in intensive care unit (ICU), and a third of them were community acquired (Quindós, 2014).

Recently, implementation of invasive monitoring and offensive treatment techniques has been escalated in the ICUs, and this eventually elevated the ratio of patients prone to fungal infections (Yapar, 2014). Many risk factors associated with the development of invasive candidiasis mainly candidemia have been identified, including age (Sutcu *et al.*, 2016), receiving broad-spectrum antibiotics, the presence of a central venous catheter (CVC), receipt of mechanical ventilation (MV), the administration of total parenteral nutrition (TPN), hematological and solid organ malignancies, neutropenia, acute renal failure (ARF) (Al Thaqafi *et al.*, 2014; Fesharaki *et al.*, 2018), prior fungal colonization (Blumberg *et al.*, 2001; Fesharaki *et al.*, 2018), immunosuppressive therapies (e.g., chemotherapy, corticosteroids), ICU admission, complicated surgery, prolonged use of CVCs (Li *et al.*, 2016; Oliver *et al.*, 2019) and the use of other invasive devices (Bassetti *et al.*, 2015; Chander *et al.*, 2013).

Assessing these risk factors is integral for early administration of proper antifungal therapeutics resulting in decrease the aggressiveness of the infection (Al Thaqafi *et al.*, 2014; Oliver *et al.*, 2019).

A variety of new medical devices have been used as a result of introduction of modern technology, that is likely an attributable factor for medical complications that can be disastrous such as potentially life- threatening systemic infections and device malfunctions that may need removal of device and often tissue destruction complicate the process (Asadzadeh *et al.*, 2019; Delfino *et al.*, 2014).

Many implantable biomaterials could provide surfaces for *C. parapsilosis* colonization such as: CVCs, implantable venous access ports, hemodialysis and peritoneal dialysis catheters, urinary catheters, intrauterine devices, endotracheal tubes, intracardiac prosthetic devices, neurosurgical shunts, prosthetic joints, voice prostheses and dentures (Borges *et al.*, 2018; Fais *et al.*, 2017; Jain *et al.*, 2018; Keuning *et al.*, 2019; Sharma *et al.*, 2020; Talpaert *et al.*, 2015).

Candida parapsilosis bloodstream infection (BSI) possible risk factors were underlying disease including diabetes, chronic renal function insufficiency, tumor, cerebrovascular accident and multiple trauma, the use of broad-spectrum antibiotics and central venous catheterization. Other risk factors such as MV, gastrointestinal surgery and the use of immunosuppressant such as corticosteroids and chemotherapeutic drugs. Among the underlying diseases, diabetes, tumors and chronic renal function insufficiency were the most frequent diseases, which also pointing to relative lower immunity was an essential risk factor for *C. parapsilosis* BSI. Main finding of a study was that the central venous catheterized patients accounted for more than 90%, strongly indicating that central venous catheterization was one of the important risk factors for *C. parapsilosis* candidemia (Liu *et al.*, 2018).

In a cross-sectional study, the patients' demographic data, previous clinical history, clinical and laboratory risk factors, parenteral nutrition, intravenous antibiotic, other parenteral drugs, proven neutropenia, and type and number of catheters used during the hospital stay were collected, in which the frequency and duration of catheterization suggested a direct relationship with fungal catheter colonization and incidence of primary candidemia. The duration of hospitalization, diabetes, and renal problems were the most common risk factors for fungemia among patients with cardiovascular disorders (Fesharaki *et al.*, 2018).

In North China, surveillance study showed the possible factors of candidemia in patients with cancer from 2006 – 2010. This study indicated that distant organ metastasis and surgery during the last month were independent risk factors for non-albicans *Candida* and *C. albicans* candidemia, respectively (Li *et al.*, 2013).

In a retrospective study, clinical information of all candidemia patients were retrieved from tertiary care hospital and analyzed. Candidemia found to be significantly associated with the longer duration of ICU admission (Chander *et al.*, 2013).

In a study involving 252 patients with 258 candidemia episodes, a non-albicans *Candida* candidemia cases were compared to *C. albicans* candidemias, only malignancy was significantly independently associated with non-albicans *Candida* candidemia development (Al Thaqafi *et al.*, 2014).

At tertiary care pediatric hospital, the following data were recorded on pediatric intensive care unit (PICU) stay for all patients: age, gender, underlying conditions, cause of admission, referral site (emergency department, medical ward, or other hospital), length of admission period in the referral site and treatments used. The use of corticosteroids and antifungal therapy within the last two weeks was emphasized.

Information of vascular access (central or peripheral, insertion site, duration and number of replaced CVCs), along with the presence of intravenous catheters and endotracheal tubes (ETT) or tracheostomies were also collected. Age greater than one year, isolation of *Candida* species from CVC, and isolation of *Candida* from ETT were significantly associated with non-albicans *Candida* candidemia compared to *C. albicans* candidemia (Hegazi *et al.*, 2014).

A cancer center in China conducted an eight-year case-control study and reported age more than or equal to 65 years old, surgery, CVCs more than seven days, TPN more than five days, urinary catheter more than two days, nasogastric tube more than three days, gastrointestinal cancer, distant organ metastasis of cancer and previous antibiotics medications were associated with candidemia. Admission in medical wards looked like a predictor for candidemia. Whereas a TPN more than five days, urinary catheter more than two days, gastrointestinal cancer and metastasis of cancer to distant organs were the independent risk factors for candidemia (Li *et al.*, 2017).

In Turkey, the study variables were obtained from patients' files in a tertiary care hospital. Data of age, sex, underlying diseases, prior infection, length of hospitalization, antimicrobial therapy 30 days preceding candidemia, history of surgery whether abdominal or non-abdominal within 30 days before candidemia, ICU admission, TPN receipt, dates for CVC indwelling and dates for withdrawal were collected. Only the first episodes during the same hospitalization period were included. Among patients with non-albicans *Candida* candidemia, significantly more patients had previous antifungal therapy in the preceding month before candidemia and concomitant bacteraemia compared with *C. albicans* candidemia. Totally, the time from CVC insertion to candidemia was 13 days and the difference was not significant in between *C. albicans* vs non-albicans *Candida* candidemia (Yeşilkaya *et al.*, 2017).

While in other study, in terms of frequency, tracheotomy, femoral artery catheterization, TPN, RBC transfusion, abdominal surgery, and previous use of antibiotics were significantly elevated in candidemia group, but only TPN and previous use of more than two of broad spectrum antibiotic in combinations were associated with a high risk of candidemia (Yapar *et al.*, 2019).

Almirante *et al.* (2006) found that the risk factors significantly associated with *C. parapsilosis* candidemia compared to *C. albicans* candidemia were neonate patients, prior antifungal therapy, transplant recipients, and receipt of parenteral nutrition.

A prospective study for eight years detected significant differences in the mean duration of MV and TPN before the diagnosis of systemic infection with *C. albicans* and *C. parapsilosis*, respectively. Also, the duration of total hospitalization of patients with *C. parapsilosis* was significantly longer than in those with *C. albicans*, comparison revealed that underlying illness, prematurity retinopathy and bronchopulmonary dysplasia were more common in patients with *C. parapsilosis* than in those with *C. albicans* (Celebi *et al.*, 2012).

In 2019, a study concerning candidemia risk factors has assessed the possible clinically significant differences between *C. parapsilosis* and other *Candida* species. Receipt of chemotherapy and parenteral nutrition were associated with *C. parapsilosis* candidemia, whereas, older age and surgery were correlated with non-*C. parapsilosis* candidemia (Sun *et al.*, 2019).

The risk factor of candidemia were compared between *C. albicans, C. parapsilosis* and others, where APACHE II scores, central venous catheterization and the use of broad-spectrum antibiotics were closely related to *C. parapsilosis* candidemia (Zuo *et al.*, 2021a).

A five-year retrospective study among ICU patients was held to investigate the most essential predisposing factors for *Candida* BSI including TPN, receiving broad-spectrum antibiotics, immuno-suppressants like chemotherapy and radiotherapy, mucosal barriers disruption due to surgery, ICU admission and intravenous medical devices such as CVCs. Patients received TPN; duration of ICU admission and duration of MV before blood culture were independently related to *C. albicans* infections (Rajendran *et al.*, 2016).

Barchiesi *et al.* (2016) carried out a study of 270 patients with *Candida* BSI. BSIs due to *C. parapsilosis* were significantly more commonly associated with the CVC usage in comparison with *C. albicans*.

In case-control study, risk factors of *C. parapsilosis* BSI in neonatal ICU have been studied. The clinical conditions associated with *C. parapsilosis* BSI in the NICU were gestational age, low birthweight and time to exposure to invasive devices, with predominance of assisted ventilation (Garzillo *et al.*, 2017).

A study in China focused on studying the risk factors of CRBSI in ICU. Patients who had multiple CVCs were almost six times more likely to develop a CRBSI than patients who only had one CVC. In patients with more than three applications of antibiotics before CRBSI, the diagnoses were seven times more likely to develop CRBSI (Peng and Lu, 2013). While in China-SCAN study, 294 patients divided to CRCBSI and NCRCBSI, were assessed and revealed that the SOFA score on the time of candidemia diagnosis was the only independently associated with CRCBSI. Age, solid tumors, chronic hepatic insufficiency and body weight were not associated with CRCBSI (Hu *et al.*, 2014).

In Malaysian tertiary hospital, case-control study has been conducted and yielded that only renal insufficiency, antimicrobial therapy whether antibacterial or

antifungal, steroid therapy and urinary catheter were significant predictors of candidemia (Tzar *et al.*, 2015). Other relative study indicated that *C. parapsilosis* fungemia has been associated with vascular catheterization and parenteral nutrition (Haydar, 2018).

In 2019, a systematic review concluded that risk factors specifically for *Candida* CRBSIs among parenteral nutrition patients are not broadly published but include underlying haematological disease and prior history of fungaemia (Phua *et al.*, 2019).

2.3 Mode of acquisition

Candida parapsilosis could be isolated from blood, skin and soft tissues from patients in medical, surgical, ICU and from dermatologic devices (Neji *et al.*, 2017).

Candida parapsilosis in severely debilitated subjects could be transmitted through contaminated infusions given as parenteral nutrition, catheter colonization and as a cause of prosthetic valve endocarditis (Herek *et al.*, 2019). It is also frequently isolated from hands of HCWs indicating cutaneous origin as a result of its ability to proliferate at high glucose concentrations and production of biofilms on prosthetic materials (Thomaz *et al.*, 2018).

2.4 Pathology and pathogenesis

As an exogenously acquired pathogen, *C. parapsilosis* does not require colonization of mucosal surfaces prior to cause infection thus causes fungemia from exogenous source directly like the hands of HCWs or from the surrounding environment (Trofa *et al.*, 2008) and because of its ability to proliferate in high concentrations of glucose and to produce biofilm that allow it to remain attached to surfaces of plastic intravascular devices, *C. parapsilosis* is uniquely associated to

CRBSI (Thomaz *et al.*, 2018). In other words, the relatedness between invasive devices and *C. parapsilosis* infections has to be due to the organism ability to produce slime and consequently to adhere to plastic devices (Tóth *et al.*, 2019). Slime production may be an indicator for pathogenesis in both blood and catheter isolates. Isolates from blood and catheters were significantly more likely to produce slime than isolates from other sites (Cavalheiro and Teixeira, 2018; Shin *et al.*, 2002) thus formation of biofilm is strain dependent (Pannanusorn *et al.*, 2014). For evaluation purposes, the growth of the organisms in Sabouraud broth with 10% glucose were examined for the presence of an adherent slime layer on the walls of the tubes using electron microscope for scanning and quantified spectrophotometrically or biochemically (Pannanusorn *et al.*, 2014).

Many *Candida* species causing infections due to formation of biofilms on implanted devices such as indwelling catheters and prosthetic heart valves including *C. parapsilosis*. Biofilms are structured microbial communities that attached to surfaces; their structures might depend on the expression of highly specific, surface-induced genes. Microorganisms with biofilms are embedded in –often slimy- extracellular matrix of polymers, and of importance, are significantly less susceptible to antifungal agents, exhibit complex three dimensional architecture affecting nutrients and wastes flux, decrease growth rate, up-regulate genes responsible for antifungal resistance, and prevent complete eradication of pathogen (Mamtani *et al.*, 2020).

However, it has been reported recently that some new antifungal agents have activity against biofilms of *Candida* such as caspofungin and micafungin (Shirazi and Kontoyiannis, 2015).

Central venous catheter, which is used for administration of fluids, nutrients and cytotoxic drugs, is the most common surgically implanted devices. Contamination

of infusion fluid itself or catheter hub lead more often to the introduction of microbes from patients skin or from HCW hands (de Paula Menezes *et al.*, 2020). Some correlation between the ability to form biofilms and pathogenicity is observed when different *Candida* species were isolated from catheters (Modiri *et al.*, 2019), with higher mortality (Tumbarello *et al.*, 2007), revealing *C. parapsilosis* is more pathogenic than *C. orthopsilosis* and consequently than *C. metapsilosis* (Ataides *et al.*, 2015). Regarding *C. parapsilosis*, a biofilm appears quite readily when cultured in a medium with high glucose concentration, and because of that, patients receiving parenteral nutrition, where the solution being administered has high glucose concentration, are under high risk of acquiring candidemia (Herek *et al.*, 2019).

Biofilm formation takes place in three developmental phases: firstly, early phase; yeast cells adhere to device surface, secondly, intermediate phase; dimorphic switching from yeast to hyphal form forming a matrix, and finally, maturation phase; formation of three-dimensional architecture. Thus the mature biofilm contains mixture of yeast, hyphae and pseudohyphae (forming different layers in biofilm) in a matrix of polysaccharide, carbohydrates and protein, that are influenced by contact surface nature (chemical nature; increased in latex compared to polyvinyl chloride, while decreased on polyurethane and silicone, and increased in high- glucose concentration that correlates positively with hydrophobicity of the surface) and environmental factors (Kojic and Darouiche, 2004; Tóth *et al.*, 2019).

Candida parapsilosis and *C. orthopsilosis* can produce pseudohyphae, which are associated with phagocytosis and evasion, but they are not able to form true hyphae like those produced by *C. albicans*. Unipolar cell divisions are used in pseudohyphae formation, although pseudohyphae lack cytoplasm connections between individual cells and are more brittle than true hyphae (Tóth *et al.*, 2019). Among the complex

only *C. parapsilosis* species produces secreted lipases. Lipase activity has been linked to improved survival and greater cellular damage in the host (Toth *et al.*, 2017). Aspartyl proteinases are secreted by all three species of the *C. parapsilosis* complex which, in addition to their food acquisition role, impact the immunological response by destroying host immune cells and enhance adhesion and invasion by damaging host cells and tissues (Dostál *et al.*, 2021). The presence and absence profiles of these three virulence variables exhibit a strong correlation with reported pathogenicity levels: *C. parapsilosis* is more resistant than *C. orthopsilosis* and *C. metapsilosis* to human primary macrophages. *C. parapsilosis* causes the most severe damage to human primary macrophages, whereas *C. metapsilosis* causes the least harm. *C. parapsilosis* has the highest mortality (Tóth *et al.*, 2017). Although the three species of the *C. parapsilosis* complex are closely related, their pathogenicity is markedly different. To date, little is known about the genetic characteristics that contribute to variation in virulence within the *C. parapsilosis* complex. Thus, this group establishes an elegant framework for studying the evolution of opportunistic human pathogens through comparative genomics. Unfortunately, until recently, only one genome from this group was accessible (Butler *et al.*, 2009) in addition to sequencing and analysis of three additional strains of *C. parapsilosis* (Pryszcz, 2015) preventing such analyses.

2.5 Epidemiology

The global epidemiology of fungal infections is prone to change. While overall, *C. albicans* remains the most common pathogen; several medical centers in Europe, Asia, Latin America and Malaysia have reported the rapid emergence of *C. parapsilosis* as predominant *Candida* species.

Clinically, fever is the most common symptom (Wu *et al.*, 2020). Fungemia, endocarditis, septic arthritis, endophthalmitis, and peritonitis and other infections usually take place during invasive procedures or prosthetic devices are clinical manifestations representing BSI due to *C. parapsilosis* (Shin *et al.*, 2020), rarely renal failure, septic shock and meningitis (King *et al.*, 2017).

In China, *C. parapsilosis* was responsible for about 37.15% of BSI (Sun *et al.*, 2019), whereas it was responsible for 23.2% as reported by Li *et al.* (2016). In Italy, among 220 non-albicans *Candida* including *C. parapsilosis* (137; 62.2%) were isolated from blood (Caggiano *et al.*, 2015), whereas in Spain about 27% of candidemia episodes were attributed to *C. parapsilosis* (Escribano *et al.*, 2018). In public hospitals South Africa *C. parapsilosis* account for 35% of BSI (Govender *et al.*, 2016).

In Latin America, Da Matta *et al.* (2017) reviewed the distribution of *Candida* species bloodstream isolates from (1997–2016), *C. parapsilosis* account for 20, 24.1, 17, 42, 37.9, 28.1, 49, 26 and 26.5% in Argentina, Brazil, Colombia, Costa Rica, Mexico, Peru, Puerto Rico, Venezuela and Latin America respectively.

From 23 hospitals in 10 European countries, *C. parapsilosis* (contributed 28% of all cases) was the predominant *Candida* species among infants (42%) (Warris *et al.*, 2020).

The incidence of *C. parapsilosis* in a major regional tertiary referral hospital in Singapore was 13.8% (Teo *et al.*, 2017), while in Taiwan the percentage was 22.6 (Wu *et al.*, 2018).

In Australia, *C. parapsilosis* BSI was reported as the third most common *Candida* species contributing 14.4%, following *C. albicans* and *C. glabrata* (Boan and Gardam, 2019).

In Malaysia, Tzar *et al.* (2015) reported *C. parapsilosis* as 20.4% of candidemia cases. While Mohamed *et al.* (2018) reported that 15.4% isolated from blood were *C. parapsilosis*. More recently, *C. parapsilosis* was the most common species contributing to 29.2% of candidemia as reported by Yamin *et al.* (2020).

2.6 *Candida parapsilosis* conventional laboratory diagnosis

Sabouraud Dextrose Agar (SDA) is a non-selective isolation medium for growing and maintaining both pathogenic and non-pathogenic fungi from clinical and nonclinical sources.

Principally, SDA is composed primarily of pancreatic digested casein and peptic digested animal tissue, which offer amino acids, nitrogen, carbon, vitamins, and minerals necessary for organism growth. Dextrose is a source of energy. Agar acts as a solidifying agent. The high quantity of dextrose and the medium's acidic pH enable fungus growth to be selective.

The SDA plates are usually incubated after inoculation with the yeast at 20-25°C for 5-7 days or at 30-35°C for 24-48 hours (ThermoScientific, 2019). When grown on SDA, colonies of *C. parapsilosis* (Figure 2.1) are white, creamy, shiny, and smooth or wrinkled (Sharma *et al.*, 2019).

Figure 2.1: *Candida parapsilosis* colonies on SDA.

On CHROMagar, *C. parapsilosis* colonies, is a species without a specific colour, growing white to pink (Figure 2.2). Plates are incubated at 37 °C and read at 24, 36, and 48 hours of incubation (Bayona *et al.*, 2020).

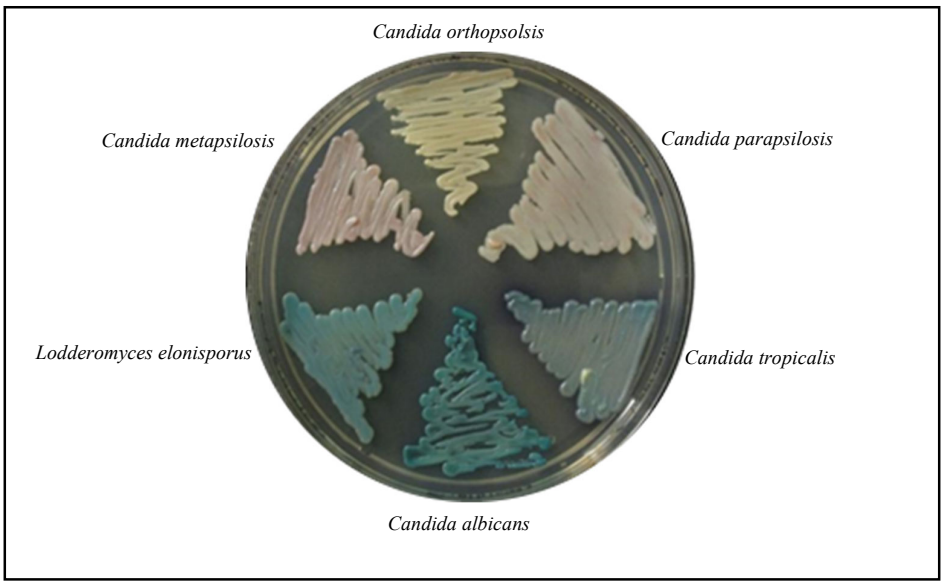


Figure 2.2: CHROMagar *Candida* colony colours. Adapted from (Döğen *et al.*, 2017).

Corn Meal Agar is a well-established mycological medium that is well-suited for the formation of chlamydospores by *C. albicans* as well as the preservation of fungal stock cultures. When *C. albicans* is cultivated on this medium, microscopic analysis reveals the distinctive chlamydospore formation that is an established criterion for this species identification (ThermoScientific, 2021).

For cultivation on Corn Meal Tween 80 agar, plates are incubated at 25°C ± 2°C for 72 hours, where *C. parapsilosis* colonies (Figure 2.3) appear as Blastoconidia along curved pseudohyphae; giant mycelial cells (Bharathi, 2018).

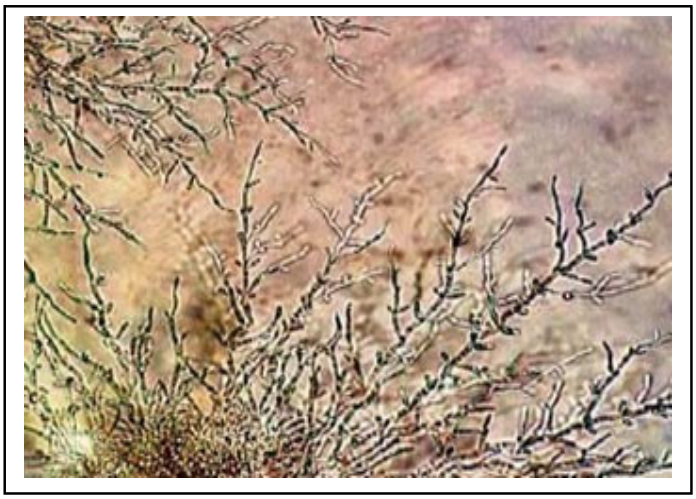


Figure 2.3: *Candida parapsilosis* growth on cornmeal agar. Adapted from (Prasanna *et al.*, 2016)

Germ tube test is a rapid method for the identification of *C. albicans* and *C. dubliniensis* by its capability to produce short, slender, tube-like structure called germ tubes when incubated in human blood serum at 37ºC for 2-3 hours. This structure is distinguished from pseudohyphae by the fact that it results from the extension of daughter cells from the mother cell without constriction at the origin (Aldossary *et al.*, 2016). *C. parapsilosis* is germ tube negative (Walsh *et al.*, 2018).

Gram staining was performed by staining the dried and heat fixed light smeared colony with distilled water on a clean grease-free slide; crystal violet was used as the primary stain, Lugol's iodine as the mordant, acid alcohol as the decolourizer, and safranin as the secondary stain; each step was washed briefly with distilled water as described by Gram (Boyanova, 2018). After Gram staining, the *C. parapsilosis* yeast cells (Figure 2.4) exhibited purple-blue, round to oval, big budding cells, showing a Gram positive reaction, a feature of yeast cells (Hassan *et al.*, 2018).