Oral Candida Carriage and Antifungal Susceptibility in Patients Receiving Antipsychotic Medication

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PREFACE

This thesis is presented as an icon of all the hard work started in the year 2012 until the year 2015. This thesis was undertaken as part of the requirements for the Doctor of Clinical Dentistry (DClinDent) Special Needs Dentistry degree from the University of Otago, New Zealand. Nine antipsychotic medications users were recruited as participants in this study, who answered 11-item Xerostomia Inventory (XI), had oral examinations, mucosal smears and saliva collection. The saliva was further tested for the presence of Candida hyphae, Candida species identification and quantification and antifungal susceptibility of Candida albicans.

This thesis contains five chapters including the literature review, materials and methods, results chapter (study participants and microbiological analysis) and discussion. The ‘study participants’ chapter contains results from the clinical aspect including details on participant’s sociodemographic characteristics, medical history and medications; and the cytological analysis of the thesis. The microbiological analysis contains detailed findings obtained from the microbiological aspect of the thesis including the identification and quantification of the Candida species obtained from participants, the susceptibility of C. albicans against fluconazole and fluphenazine based on two methods; the Etest™ and the liquid microtitre assay. The antagonism/synergism of the combination of fluconazole and fluphenazine was also tested using two methods; the disc diffusion assay and the liquid microtitre assay. The discussion chapter elaborates the analysis of the findings, conclusions, limitations of the study and future research recommendations.
ABSTRACT

Objective: The overall objective of this project was to investigate oral Candida carriage and antifungal susceptibility of Candida albicans isolates in patients receiving antipsychotic medications. Specific objectives were: to determine the level of colonisation with Candida of the oral mucosa in individuals taking antipsychotic medications relative to healthy controls and to xerostomic individuals not prescribed with antipsychotics; to determine the Candida species present; to measure the azole resistance of these isolates; and to determine whether the antipsychotic medication fluphenazine affected azole resistance of C. albicans isolates.

Methods: Nine participants aged between 20 and 70, who were currently on antipsychotic medications were recruited into this study with informed consent. Xerostomia symptoms were determined from the Xerostomia Inventory (XI) and clinical examinations. Saliva rinses were collected by asking each participant to rinse their mouth with 10 mL water for 30 s and then expectorated into specimen bottles. Smears were taken from the buccal mucosa (both sides), tongue, and any other mucosal site with signs of infection. Smears were sent to Medlab for Candida hyphae and yeast identification. Saliva samples were diluted and cultured on CHROMagar Candida™ plates. The colony-forming units (CFU) and species (presumptively assigned from the colony colour) were recorded. The susceptibility of the C. albicans isolates to fluconazole was measured using the E-test and liquid microdilution assay. The interaction between fluconazole and fluphenazine was investigated using a disc diffusion assay and checkerboard liquid microdilution assay.

Results: The majority of the participants (78%) presented with dry mouth. However, the degree of dry mouth symptoms they experienced was not severe based on their XI scores. Four of nine participants (44%) were diagnosed with oral candidosis. The infection coincided with antipsychotic medication intake, although may not have been caused by the antipsychotic because three of the four participants were on other medications as well. Higher numbers of participants were colonised with Candida spp. (7 of 9) compared to an age-matched group of healthy individuals (2 of 9). The
numbers of yeast detected per participant was also significantly higher than for the control group. The Candida species most frequently presumptively identified by colony colour on CHROMagar Candida™ was C. albicans. Fluphenazine showed low antifungal activity. However, fluphenazine was found to produce antagonistic effects towards fluconazole, both in disc diffusion assays and the more quantitative checkerboard MIC assays.

**Conclusion:** Many antipsychotic medications are known to cause xerostomia and predispose to Candida infections. Investigating C. albicans infections and their resistance to fluconazole will potentially lead to more appropriate treatment. The discovery that antagonism between the antipsychotic fluphenazine and fluconazole occurs with C. albicans clinical isolates indicates that careful consideration is necessary when prescribing fluconazole to patients currently taking fluphenazine or medications of a similar class. Other antifungal agents such as nystatin lozenges (not readily available in New Zealand) and amphotericin B lozenges might be better for individuals on such antipsychotic medications.
ACKNOWLEDGEMENTS

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54th Annual Scientific Meeting of the Australian & New Zealand Division of the IADR International Association of Dental Research (IADR) Conference

29th September 2014 until 1st October 2014
Brisbane, Australia

Title: Fluconazole Resistance in Individuals Taking Antipsychotic Drugs
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<tr>
<td>3D-MR</td>
<td>Three Dimensional Magnetic Resonance</td>
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<tr>
<td>ABC</td>
<td>ATP-Binding Cassette</td>
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<tr>
<td>AIDS</td>
<td>Acquired Immune Deficiency Disease</td>
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<td>ALS</td>
<td>Agglutinin-Like Sequence</td>
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<td>AMP B</td>
<td>Amphotericin B</td>
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<tr>
<td>ANAs</td>
<td>Anti-Nuclear Antibodies</td>
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<td>CFU</td>
<td>Colony-Forming Unit</td>
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<td>CLSI</td>
<td>Clinical and Laboratory Standard Institute</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<td>CO₂</td>
<td>Carbon Dioxide</td>
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<tr>
<td>DMFT</td>
<td>Decay-Missing-Filled Teeth</td>
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<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
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<td>DSM-IV</td>
<td>Diagnostic and Statistical Manual of Mental Disorders-fourth edition</td>
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<td>ECG</td>
<td>Electrocardiography</td>
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<td>ECM</td>
<td>Extracellular Matrix</td>
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<tr>
<td>EEG</td>
<td>Electroencephalography</td>
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<td>FDA</td>
<td>U.S. Food and Drug Administration</td>
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<tr>
<td>FIC</td>
<td>Fractional Inhibitory Concentration</td>
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<tr>
<td>FICI</td>
<td>Fractional Inhibitory Concentration Index</td>
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<tr>
<td>FLC</td>
<td>Fluconazole</td>
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<tr>
<td>FLUP</td>
<td>Fluphenazine</td>
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<tr>
<td>FPZ-D</td>
<td>Fluphenazine Decanoate</td>
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<td>FPZ-H</td>
<td>Fluphenazine Hydrochloride</td>
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<tr>
<td>GI</td>
<td>Gastrointestinal</td>
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<tr>
<td>HADS</td>
<td>Hospital Anxiety and Depression Scale</td>
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<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
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<tr>
<td>ICD-10</td>
<td>International Statistical Classification of Diseases and Related Health</td>
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<tr>
<td>INR</td>
<td>International Normalised Ratio</td>
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<tr>
<td>MIC</td>
<td>Minimum Inhibitory Concentration</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>MLST</td>
<td>Multi-Locus Sequence Typing</td>
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<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<tr>
<td>NaOH</td>
<td>Sodium Hydroxide</td>
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<tr>
<td>NCCLS</td>
<td>National Committee for Clinical Laboratory Standards</td>
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<td>NMS</td>
<td>Neuroleptic Malignant Syndrome</td>
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<td>NSAIDs</td>
<td>Non-Steroidal Anti-Inflammatory Medications</td>
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<td>OD</td>
<td>Optical Density</td>
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<td>PAS</td>
<td>Periodic Acid-Schiff</td>
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<tr>
<td>PC2 MBL</td>
<td>PC2 Molecular Biosciences Laboratory</td>
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<tr>
<td>PLs</td>
<td>Phospholipases</td>
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<td>RF</td>
<td>Rheumatoid Factor</td>
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<td>RPMI</td>
<td>Roswell Park Memorial Institute</td>
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<tr>
<td>SAPs</td>
<td>Secreted Aspartyl-Proteinases</td>
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<tr>
<td>SDA</td>
<td>Sabouraud Dextrose Agar</td>
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<tr>
<td>USA</td>
<td>United States of America</td>
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<tr>
<td>VAS</td>
<td>Visual Analogue Scale</td>
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<td>WHO</td>
<td>World Health Organisation</td>
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<td>XI</td>
<td>Xerostomia Inventory</td>
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<tr>
<td>YPD</td>
<td>Yeast-Peptone-Dextrose</td>
</tr>
<tr>
<td>mg/mL</td>
<td>Miligram/ Mililiter</td>
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<tr>
<td>µg/µL</td>
<td>Microgram/ Microgram</td>
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<td>ng</td>
<td>Nanogram</td>
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<td>IU</td>
<td>International Unit</td>
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CHAPTER 1:

LITERATURE REVIEW
CHAPTER 1: LITERATURE REVIEW

1.1 Introduction

The New Zealand Mental Health Survey, 2012/13, concluded that one in six adults (16%) of the New Zealand population experienced mental disorders at some time in their life. Some will have been prescribed with antipsychotic medications. The use of antipsychotic medications has helped reduce the number of hospitalisations due to psychiatric disorders and more patients are integrated into the society. However, these medications have several adverse effects, including reducing salivary gland function. The World Health Organisation (WHO) defined the term adverse effect as “a response to a drug that is noxious and unintended and occurs at doses normally used in man for the prophylaxis, diagnosis or therapy of disease, or for modification of physiological function” (WHO, 1972 as cited in Edwards and Aronson, 2000). All unwanted effects from medications could be termed as adverse effects.

One of the consequences of reduced salivary flow is a dry mouth. Xerostomia is a subjective feeling of dry mouth, almost always accompanied by reduced unstimulated (resting) salivary flow (Sreebny and Schwartz, 1987). Causes of dry mouth could be classified as irreversible or permanent and transient or reversible. Examples of irreversible damage are irradiation to the salivary gland and autoimmune diseases such as sicca syndrome or Sjögren’s syndrome. Dry mouth due to the effects of medications is usually transient or reversible. The diagnosis of dry mouth is based on the patient’s history, clinical oral examination and sialometry (a simple procedure for measuring salivary flow). It is accepted that people with xerostomia of any aetiology are at higher risk of developing oral fungal infections, especially those caused by Candida species (Torres et al. 2003). While there have been many reports of the association between dry mouth and use of antidepressant medications, there is little known about the effects of antipsychotic medication on salivary flow. In addition, the antipsychotic fluphenazine is known to induce expression of efflux pumps in Candida albicans that cause resistance to the antifungal azole medications (de Micheli et al., 2002, Coste et al., 2004, Manoharlal et al., 2011). Therefore, not only may patients
taking antipsychotics be more susceptible to candidosis, their infections may not respond to azole therapy.

1.2 Antipsychotic Medications

Clinicians often use the Diagnostic and Statistical Manual of Mental Disorders-fourth edition (DSM-IV) (American Psychiatric Association, 1994) for the diagnosis and classifications of psychiatric disorders. Another method commonly used for diagnosis and classification of mental health problems is the International Statistical Classification of Diseases and Related Health Problems of the WHO, version 10 (ICD-10). The common mental health problems include anxiety, depression, phobias, obsessive compulsive and panic disorders. A less common psychiatric disorder is psychosis that manifests with symptoms that interfere with the person’s perception of reality with loss of insights. It includes hallucinations and other mental health problems such as schizophrenia and bipolar affective disorder (manic depression). Mental disorders are difficult to categorise due to the difficulty in diagnosis, thus they may result in inconsistent treatment outcomes (Hyman, 2010).

Antipsychotic medications (also known as psychotropic or psychotherapeutic or neuroleptic medications) were first introduced in the 1950s. They were mainly used as treatment and maintenance therapy for people with severe and persistent mental illnesses. These conditions include dementia, delirium (acute state of confusion), psychosis, agitation and affective disorders (Wang et al., 2005). The principal action of antipsychotics is on the dopamine system (Miyamoto et al., 2005). Pharmacologically, there are two classes of antipsychotic medications: first-generation (typical) and the newer, second-generation (atypical) antipsychotics. Figure 1.1 illustrates the classification of antipsychotic medications based on their chemical structures and provides examples of typical and atypical antipsychotic medications. Highlighted in orange colour are the typical antipsychotic medications and highlighted in green are the atypical antipsychotic medications.
Figure 1.1 Groups of antipsychotic medications with examples. Boxes highlighted in orange colour are the typical antipsychotics, green boxes indicate atypical antipsychotics (Howard et al., 2011).
1.2.1 Typical Antipsychotic Medications

The pharmacological action of typical (conventional) antipsychotic medications, also known as first-generation antipsychotic medications or neuroleptics, is primarily by blocking the dopamine D₂ receptors in the dopamine pathways. These medications are effective in controlling active symptoms of psychosis, reducing assaultive behaviour, managing severe agitation and reducing the risk of relapse in psychotic disorders during maintenance period (Jibson and Tandon, 1998). Typical antipsychotic medications are grouped into phenothiazines including chlorpromazine, levomepromazine (methotrimeprazine), perphenazine, prochlorperazine, promazine, fluphenazine and trifluoperazine; and the butyrophenones group such as haloperidol. Medications that belong in the phenothiazine group are chlorpromazine, fluphenazine, mesoridazine, perphenazine, prochlorperazine, thiethylperazine, thioridazine, triavil (perphenazine/amitriptyline) and trifluoperazine. Haloperidol belongs to the butyrophenone group, loxapine belongs to the dibenzoxapine group, molindone belongs to the dihydroindolone group and thioxetene in the thioxanthen group. Fluphenazine decanoate (brand name Modecate) belongs to the piperazine class of phenothiazines and haloperidol decanoate (brand name Haldol) are typical antipsychotic medications commonly used as depot medication (long-acting), which are administered by injection every two to four weeks. Fluphenazine enanthate (Moditen) is also given orally. They are often prescribed when patients are non-compliant with oral medications especially in schizophrenia (Valenstein et al., 2001).

Typical antipsychotic medications can also be divided into groups based on their sedative effects. There are three main groups. Chlorpromazine (50-1000 mg daily), for example belongs to a group characterised by profound sedative and moderate antimuscarinic and extrapyramidal effects. The other two groups are characterised by moderate sedative effects with marked antimuscarinic effects and fewer extrapyramidal effects, such as thioridazine (25-250 mg daily), and a group characterised by fewer sedative and antimuscarinic effects but more pronounced extrapyramidal effects, for example trifluoperazine (2030 mg daily), haloperidol (2-80 mg daily) and flupentixol (2-100 mg daily).
1.2.2 Atypical Antipsychotic Medications

The atypical antipsychotic medications are classified as phenothiazines, butyrophenone, dibenzoazepine, dihydroindolone and thioxantene (Williams et al., 1999). Atypical antipsychotic medications provide therapeutic effects, more effective for the treatment of negative symptoms of schizophrenia and tend to produce less extrapyramidal side effects. Pharmacologically, they are also antagonists of dopamine D$_2$ receptors with additional antagonistic effects on muscarinic, cholinergic, histaminergic, noradrenergic and serotonic receptors. These medications produce less extrapyramidal side effects due to antagonistic actions on 5-hydroxytriptamine (serotonin) receptor 2A (5HT$_{2A}$) receptors, making them different from the typical antipsychotic medications. Examples of atypical antipsychotic medications are aripiprazole, clozapine, olanzapine, quetiapine, tiapride, risperidone and pipamperone dihydrochloride. Clozapine was approved for use in the United States in 1989 for treatment of positive and negative symptoms of schizophrenia. Positive symptoms (florid) in schizophrenia are psychotic behaviours not seen in healthy individuals delusions and hallucinations, while negative symptoms (defect) are symptoms associated with disruptions in normal behaviour and emotions such as attentional impairment (Andreasen and Olsen, 1982). It is effective in treating schizophrenia with a relatively low frequency of extrapyramidal and endocrine side effects due to its selectiveness in blocking mesolimbic and mesocortical dopaminergic pathways (Jibson and Tandon, 1998).

Prescription of typical antipsychotic medications is reducing and being replaced by the use of atypical antipsychotic medications resulting in fewer extrapyramidal side effects and lower risk of tardive dyskinesia (Meltzer, 2004). In Australia in 2001, 67.3% of prescribed antipsychotic medications were atypical versus 16.0% typical antipsychotics, whilst 16.7% were depot medications (Mond et al., 2003).
1.2.3 Side Effects Associated with Antipsychotic Medications

There are several adverse effects associated with antipsychotic medications. These include extrapyramidal side effects, anti-cholinergic effects including dry mouth, anti-adrenergic effects, electrocardiography (ECG) and electroencephalography (EEG) alterations, neuroleptic malignant syndrome, dermatological effects, haematological effects, ophthalmological effects, weight gain and seizures (Whitworth and Fleischhacker, 1995). They also induce hyperglycaemia and dyslipidaemia as additional side effects (Miyamoto et al., 2005). Adverse effects are dependent on dosage, patient characteristics, age and gender (Haddad and Sharma, 2007). The side effects of antipsychotic medications can appear as acute or chronic, and may be very detrimental to patients although the difficulties with side effects have to be weighed up against management of the primary pathology. The side effects vary according to the class of antipsychotic drug. Table 1.1 summarises the side effects of antipsychotic medications.

1.2.3.1 Adverse effects associated with typical antipsychotic medications

Conventional (typical) antipsychotic medications produce various adverse reactions. These reactions have been divided into physiologic changes, sexual and reproductive side effects and central nervous system (CNS) side effects. Physiologic changes are associated with changes in the autonomic and cardiovascular system; endocrinologic and metabolic; haematologic and hepatic; allergic, dermatologic and ophthalmologic. Potentially lethal adverse effects are the cardiac changes such as tachycardia and ECG changes that may lead to cardiac arrhythmia. Other effects include postural hypotension, seizures and dry mouth (Arana, 2000). Zelickson and Rogers (1986) concluded that conventional (typical) antipsychotic medications such as chlorpromazine, thioridazine, haloperidol and prochlorperazine cause xerostomia in patients. Endocrinologic effects of the typical antipsychotics include gynaecomastia, amenorrhea in women and impotence in men associated with hyperprolactinaemia. Typical antipsychotics may cause hyperprolactinaemia via the blockade of dopamine receptors in the pituitary prolactin-secreting cells. Other metabolic effects are weight
gain, hyperpyrexia and hypopyrexia (Arana, 2000). In the haematological system, leukocytosis as well as leukopaenia can be also associated with typical antipsychotics; these changes occur within three to six weeks after commencement of treatment. Photosensitivity and other skin reactions associated with allergy may occur with the use of typical antipsychotics. Skin hyperpigmentation was also reported in patients with high-dose long-term chlorpromazine. Ophthalmologic side effects associated with typical antipsychotics may lead to blindness. They also may have teratogenicity effects when taken by pregnant mothers and may cause neurological defects to breastfed babies (Arana, 2000).

Movement disorders associated with typical antipsychotic medications, particularly phenothiazines and butyrophenones are the extrapyramidal disorders consisting of tremor, akathisia, dystonia, bradykinesia and dyskinesia. Patients may develop acute dystonia, neuroleptic drug-induced Parkinsonism, bradykinesia, akathisia, rabbit syndrome and tardive dyskinesia (Casey, 1991). A tremor caused by antipsychotic medications occurs with a certain duration of exposure to the antipsychotic drug characterised by rhythmic, involuntary muscle contractions particularly intention tremor and resting tremor (Collins et al., 1979). Intention tremor is defined by increased amplitude during a visually guided movement towards a target at the termination of movement, while resting tremor occurs when a body is at complete rest against gravity for example while seating (Deuschl et al., 1998). Akathisia is sometimes described as anxiety with restlessness and resembles psychotic agitation (Arana, 2000). Akathisia literally means “unable to sit” (derived from a Greek word). Neuroleptic-induced akathisia is a side effect of antipsychotic medications characterised by a sense of restlessness especially in the legs, inability to remain seated and shuffling from foot to foot or pacing (Adler et al., 1989). Dystonia is characterised by sustained or intermittent muscle contractions causing abnormal movements (usually repetitive), postures or both (Albanese et al., 2013). It is characterised by involuntary muscle spasm that results in abnormal postures including, oculogryric crisis, tongue protrusion, trismus, torticollis, laryngeal-pharyngeal constriction, or bizarre positions of the limbs and trunk. Patients may have a reduction in spontaneous activity (Arana, 2000) or slowness in executing movements (Sheridan and Flowers, 1990) known as bradykinesia. Akinesia is a
condition that mimics the negative symptoms of schizophrenia or the psychomotor retardation of depression (Arana, 2000). Tardive dyskinesia is a debilitating neurologic disorder characterised by repetitive, involuntary and purposeless movements that usually involves the tongue, jaw, lips, face, trunk, limbs and respiratory system (Margolese et al., 2005). Rabbit syndrome is characterised by rhythmical tremors in the lips and perioral area, and usually responds to anti-extrapyramidal syndrome medications (Casey, 1991). It causes rapid and regular orofacial movement disorder with tongue sparing (Deshmukh et al., 1990).

The adverse effects of antipsychotic medications may appear as acute or chronic depending on the duration of exposure to the medications. Akathisia is an early, usually intolerable side effect of typical antipsychotic drug. Acute dystonia often occurs during the first week of therapy, usually within four days or shortly after a dose increase involving involuntary muscle spasms. Sometimes it can be misdiagnosed as hysteria or malingering (Arana, 2000). Prolonged contraction of the muscles due to dystonia may become detrimental or life-threatening especially the laryngeal spasms (Arana, 2000). Tardive dyskinesia is an adverse effect associated with long-term use of typical antipsychotic medications. It is often irreversible causing disfigurement to the patients and may interfere with normal daily activities (Arana, 2000; Margolese et al., 2005). Bradykinesia is an adverse effect often misdiagnosed by failure to recognise this condition and the patient is thought to have depression, psychological withdrawal or negative symptoms of schizophrenia (Arana, 2000). A rare but potentially fatal side effect of antipsychotic medications is neuroleptic malignant syndrome (NMS), characterised by hyperthermia, confusion, muscular rigidity and autonomic disturbances such as tachycardia, labile blood pressure and sweating. Other movement disorder includes neuroleptic drug-induced Parkinsonism that manifests similarly to idiopathic Parkinsonism. Careful prescriptions of typical antipsychotics are necessary to reduce the risk of seizures especially in high-risk patients, such as patients with history of seizures. A rare condition that may appear following antipsychotic medications is rabbit syndrome that manifests as a mild Parkinsonism especially around the lip and perioral region (Casey, 1991). Almost all the adverse effects of typical antipsychotic medications not only affect the level of compliance in patients but also the quality of life of the patients (Arana, 2000).
1.2.3.2 Adverse effects associated with atypical antipsychotic medications

The risk of developing extrapyramidal side effects (Parkinsonism, akathisia and acute dystonia) due to atypical antipsychotics including amisulpiride, clozapine, olanzapine, quetiapine and risperidone was lower when compared to haloperidol (Haddad and Sharma, 2007). Similarly, the risk of tardive dyskinesia was lower with atypical antipsychotic medications, especially clozapine and quetiapine, when compared to haloperidol. In regards to antipsychotic drug-induced hyperprolactinaemia, risperidone and amisulpiride may cause an increase in prolactin hormone; clozapine and quetiapine do not increase the prolactin level; olanzapine and ziprasidone may cause increased prolactin level at higher dose (Haddad and Sharma, 2007). However, all atypical antipsychotic medications cause significant weight gains (>7%) especially clozapine and olanzapine (Haddad and Sharma, 2007). The anticholinergic side effects including dry mouth, blurred vision, urinary retention, constipation and cognitive impairment due to atypical antipsychotic medications were dose-dependent. Olanzapine was found to cause dry mouth as a side effect (Tollefson et al., 2001). Dry mouth is also reported in users of quetiapine (Bagnall et al., 2003; Srisurapanont et al., 2004), risperidone (Mullen et al., 2001), tiapride and pipamperone dihydrochloride (Scully, 2003). The incidence of dry mouth is higher when olanzapine is combined with lithium when compared to olanzapine therapy alone (Tohen et al., 2002).

Clozapine was introduced in the U.S.A in early 1970s but was withdrawn from the market due the risk of agranulocytosis. However, it was approved and released back into the market in January 1990 by the U.S Food and Drug Administration (FDA) with careful dispensing and monitoring procedures. Clozapine is the only antipsychotic drug shown in clinical trials to effectively reduce positive and negative symptoms in schizophrenia patients non-responsive to typical antipsychotic medications. The risk of agranulocytosis from clozapine use is about 1% to 2% in the first six months, whilst the risk of seizures is dose-dependent within a range of 2% to 6%. Agranulocytosis (granulocyte count <500/mm$^3$) is a life-threatening condition, with the mortality rate of 3% to 4% (Iqbal et al., 2003). Iqbal and colleagues listed
the various adverse effects of clozapine in their article. Clozapine produces multiple adverse effects associated with the haematologic system, CNS, neuromuscular system, cardiovascular system, autonomic nervous system and gastrointestinal system. It also produces metabolic effects for example weight gain and diabetes mellitus and may be associated with urinary incontinence. The incidence of seizures increases with clozapine use, and is dose-dependent. Hypersalivation, drooling and choking sensation due to excessive saliva are common complaints by patients taking clozapine. They are dose-dependent and adjunctive treatment may be needed to reduce hypersalivation (Iqbal et al., 2003). It was unclear whether it truly causes hypersalivation via activation of muscarinic M₄ receptors or blockade of α₂-adrenoceptors or by causing impairment in the swallowing mechanisms causing saliva pooling in the mouth (Davydov and Botts, 2000; Haddad and Sharma, 2007). However, animal studies show that it induces saliva secretion via direct action on the muscarinic receptor (M₁) of the acinar cells (Ekström et al., 2010).

<table>
<thead>
<tr>
<th>Antipsychotic medications</th>
<th>Side-effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpromazine</td>
<td>Xerostomia, hyperprolactinaemia, weight gain, hyper/hypopyrexia, leukocytosis, leukopaenia, skin reaction, blindness, teratogenic, neurological defects, movement disorders</td>
</tr>
<tr>
<td>Thioridazine</td>
<td></td>
</tr>
<tr>
<td>Haloperidol</td>
<td></td>
</tr>
<tr>
<td>Prochlorperazine</td>
<td></td>
</tr>
<tr>
<td>Amisulpiride</td>
<td>Hyperprolactinaemia, weight gain, dry mouth, blurred vision, urinary retention, constipation</td>
</tr>
<tr>
<td>Clozapine</td>
<td>Movement disorders, weight gain, hypersalivation, agranulocytosis, diabetes mellitus, blurred vision, urinary retention, constipation, seizures</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>Hyperprolactinaemia (higher dose), weight gain, dry mouth, blurred vision, urinary retention, constipation</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>Movement disorders, weight gain, dry mouth, blurred vision, urinary retention, constipation</td>
</tr>
</tbody>
</table>
### Table 1.1 Summary of the side effects of antipsychotic medications

<table>
<thead>
<tr>
<th>Antipsychotic medications</th>
<th>Side-effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risperidone</td>
<td>Hyperprolactinaemia, weight gain, dry mouth, blurred vision, urinary retention, constipation</td>
</tr>
<tr>
<td>Ziprasidone</td>
<td>Hyperprolactinaemia (higher dose), weight gain, dry mouth, blurred vision, urinary retention, constipation</td>
</tr>
</tbody>
</table>

1.3 Xerostomia

1.3.1 Definitions

The terminology xerostomia, dry mouth, salivary gland hypofunction and hyposalivation often cause confusion between clinicians and researchers. Most authors agree that xerostomia is not a diagnosis but a symptom. In a review by Nederfors (2000), xerostomia was recognised as the subjective feeling of oral dryness, while hyposalivation was a sign of decreased salivary flow rate. Salivary gland hypofunction was a condition with subjective feeling of dry mouth combined with signs of decreased salivary flow rate. However, there is no global consensus on the term ‘dry mouth’. In this particular study, xerostomia, the subjective feeling of oral dryness, is the main area of interest.

1.3.2 Causes of Xerostomia

There are multiple possible causes of xerostomia including salivary and non-salivary causes. Salivary causes of xerostomia include hyposalivation and/or alteration in salivary composition. Examples of salivary causes of xerostomia due to salivary gland hypofunction or hyposalivation are salivary gland trauma or tumour, nutritional deficiencies and/or eating disorders (anorexia, bulimia), systemic diseases such as Sjögren’s syndrome or from side effects of medications and radiation therapy to the head and neck (Napeñas et al., 2009). Non-salivary causes of complaints of xerostomia are oral sensory dysfunction, neurological dysfunction, central cognitive
changes, psychological causes, mouth breathing and dehydration. Medical interventions such as radiotherapy in the head and neck region, prescription of oral radionuclides such as iodine 131 ($I^{131}$) for thyroid disease treatment and bone marrow transplantation with graft-versus-host disease may cause xerostomia (Fox, 2008; Guggenheimer and Moore, 2003). Systemic diseases that may cause xerostomia include diabetes mellitus, depression, anxiety and Sjogren’s syndrome. Other possible causes for xerostomia are tobacco smoking, alcohol use (including mouthwashes) and caffeine (coffee, tea and soft drinks) consumption (Scully, 2003). The mechanism of action for drug-related xerostomia is anticholinergic activity.

There are a great number of medications that have xerostomia as one of their side effects. Medications that are known to cause xerostomia include anticholinergic agents, antidepressant and antipsychotic agents, diuretics, antihypertensive agents, sedatives and anxiolytics, muscle relaxants, analgesics (opioids and non-steroidal anti-inflammatory medications (NSAIDs)), antihistamines, anti-acne medications, anti-convulsants, anti-dysrhythmics, anti-incontinence agents, anti-Parkinsonian agents, bronchodilators, ophthalmic formulations and smoking cessation agents (Guggenheimer and Moore, 2003). In a review by Scully (2003), the most common oral adverse drug reaction in the USA was found to be dry mouth (80.5%) and the use of psychiatric medications was the main cause for dry mouth (Pajukoski et al., 2001).

Subjective oral dryness was also associated with age, female gender, and intake of antipsychotic medications, anti-asthmatics and diuretics (Bergdahl and Bergdahl, 2000).

1.3.3 Clinical Manifestations of Xerostomia

People suffering from reduced salivary flow (hyposalivation) may present with oral complaints such as dry mouth, burning mouth or altered taste sensation. They may complain of difficulty in eating, speaking and swallowing certain foods or they may find the increased need to drink water. Halitosis and intolerance to spicy food may also be found (Cho et al., 2010). Clinical manifestations of xerostomia are tongue changes such as atrophy of the filiform papillae, fissured tongue, and erythematous mucosa with a parched appearance (Guggenheimer and Moore, 2003). Other
manifestations of xerostomia are dried and cracked lips, glossy or desiccated oral mucosa, frothy saliva and lack of saliva pooling in the floor of the mouth (Napeñas et al., 2009).

### 1.3.4 Diagnosis of Hyposalivation and Xerostomia

Longman et al. (2000) studied the diagnosis of salivary hypofunction based on three aspects; patient reported subjective feeling of xerostomia, clinical presentation and sialometry (of unstimulated whole saliva). They underlined three categories for oral dryness; moist mouth, ‘parchment appearance’ and dry mouth with small amount of frothy saliva. The ‘parchment appearance’ was defined as when there was no saliva coating on the dorsum of the tongue, on the buccal mucosa and no saliva pooling at the floor of the mouth. They found that these diagnostic criteria could predict salivary gland hypofunction with statistical significance.

#### 1.3.4.1 Patient-reported xerostomia-related symptoms

A number of tests can be undertaken to measure the subjective feeling of xerostomia. The use of a visual analogue ruler, single-item questionnaire and multiple-item questionnaire were developed in order to help clinicians and researchers to identify and classify xerostomia. Some of the questions were limited to a ‘yes’ or ‘no’ answer.

Thomson et al. (1999) developed a multi-dimensional questionnaire approach, the Xerostomia Inventory (XI) to classify the severity of xerostomia based on respondents’ experience of, and behaviour towards, dry mouth. The XI was based on a continuous scale for the measurement of xerostomia unlike the other previous measurement methods. They compared the multiple approaches that were available during that time. They found that the single-item approach was limited to only measuring dry mouth without exploring the other symptoms associated with the experience of xerostomia. The XI uses the question “how often does your mouth feel dry?” followed by 11-item questions with the answering options of “never”, “hardly ever”, “occasionally”, “fairly often” and “very often”. Each item was given a score
between 1 and 5 and the scores were then summated. The score of 1 was given to the answer “never”, score 2 for “hardly ever”, score 3 for “occasionally”, score 4 for “fairly often” and score 5 for “very often”. The sum of scores ranges from 11 to 55. This approach has subsequently been validated (Thomson et al., 2000; Thomson, 2007; Hopcraft et al., 2010). The mean XI scores based on individual answers of “never”, “occasionally”, “often” and “always” provide the investigator with information about the level of severity of the patients’ xerostomia symptoms.

The original XI was produced in 1999 with the purpose of measuring xerostomia in a continuous scale method to reduce the misclassification of xerostomia based on an arbitrary cut-off point (Thomson et al., 1999). The XI was shortened in 2011, when a Dutch version of the shortened XI was used (Thomson et al., 2011). Instead of using the 11-item questionnaire, they used 5-item questionnaire with three answering options. The items that were used were “my mouth feels dry when eating a meal; my mouth feels dry; I have difficulty in eating dry foods; I have difficulty in swallowing certain foods and my lips feel dry”. The answering options provided were “never” giving the score of 1, “occasionally” scores 2 and “often” scores 3. However, the shortened version of XI was limited to studying participants’ level of dry mouth. The purpose of the shortened XI was to develop a day-to-day clinical measure; and for researchers to retain the most important properties and characteristics of xerostomia. Respondents were found to be confused with the five answering options given, thus the shortened version provided only three. This measure was proven valid with the original version of XI, although the sample number used was not representative of population. Thomson and colleagues (2011) proposed that researchers should use the XI with the standard question “How often does your mouth feel dry?” followed by 4 answering options (“Never”, “Occasionally”, “Frequently” and “Always”) to provide a validity check. Thus this form of the XI was used in the present study with four answering options provided to the respondents (“Never”, “Occasionally”, “Frequently” and “Always”). The scoring system was 1 for “Never”, 2 for “Occasionally”, 3 for “Frequently” and 4 for “Always” giving a range of total score between 11 and 44.

Interviews can be also be used to gain information about xerostomia and the use of a visual analogue scale (VAS) or a categorical scale can provide quantitative data (Cho
et al., 2010). One example of a visual analogue scale is the Likert scale (Figure 1.2). The Likert scale is a psychometric scale developed by Rensis Likert (Esterman, 2003) which uses a 5-point scale of 1, 2, 3, 4 and 5. (1=mouth feels moist to 5=mouth extremely dry, tongue sticks to palate, difficulties with speaking).

<table>
<thead>
<tr>
<th>Please rate how dry your mouth feels (Circle one most appropriate answer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>mouth feels moist</td>
</tr>
</tbody>
</table>

Figure 1.2 Likert scale

**1.3.4.1 Measurement of salivary output**

Measuring the salivary output can determine salivary flow. Collection of unstimulated whole saliva at a rate of 0.12 to 0.16 ml per minute, or stimulated whole saliva at a rate of less than 0.5 ml per minute may indicate salivary hypofunction (Napeñas et al., 2009; Navazesh et al. 1992). Navazesh and Kumar (2008) outlined the methods for measuring salivary output. Output that can be measured includes whole unstimulated saliva, whole stimulated saliva or saliva from individual salivary glands. Several stimulants have been used in various studies including gum base, paraffin wax, rubber bands, citric acid, secretagogues such as cevimeline hydrochloride and pilocarpine and also mechanical stimulants such as transcutaneous electrical nerve stimulator and powered toothbrushes. Collection of whole saliva from the mouth can be performed by spitting, draining or by weighing cotton rolls inserted into the mouth. Stimulated salivary output collection is performed by using stimulants, such as applying 2% citric acid to the dorsum surface of the tongue or by mechanical stimulation for example by chewing. The collection of saliva from parotid glands is performed by using a catheter inserted into the parotid duct to drain the saliva or by using a suction cup. Saliva collection from submandibular or sublingual glands can be performed by selective suctioning technique from the
glands’ orifice at the floor of the mouth. Patients with Sjogren’s syndrome may need to undergo minor salivary gland output measurement from the lip. Although there is no standardised method for measuring salivary output, whole saliva measurement is considered acceptable for investigating the general level of xerostomia in patients or the relationship between symptoms and salivary output (Eisbruch et al., 2003). Collection of unstimulated whole saliva is easy, but it is sensitive to the hydration status of the patients and medications that may induce xerostomia. This is one of the reasons why the method of unstimulated whole saliva collection was chosen for this study.

The collection of whole unstimulated saliva is performed by advising the individuals to rinse their mouth with distilled water, minimise movement in their mouth, head tilting slightly forward, then collect remaining saliva in their mouth and expectorate into the collection tube after 1 minute. They should refrain from eating or drinking for one hour before the procedure. It is advisable to ask them to practice a few times before the actual collection. Stimulated whole saliva collection using chewing gum (or other agent) usually takes five minutes and is performed by asking the patient to swallow his or her saliva, followed by chewing the gum base (no flavours) and then spitting into a tube once every minute, without swallowing. For the first two minutes saliva is collected into a cup; for the remaining three minutes the saliva is collected in a tube. Collecting saliva from the parotid gland is performed via Stensen’s duct situated opposite the maxillary second molar using a special collecting cup. Saliva from submandibular and sublingual glands is collected via Wharton’s duct situated in the floor of the mouth. Hyposalivation is defined as an unstimulated salivary flow of less than 0.1ml per minute (Bergdahl and Bergdahl, 2000) or whole stimulated saliva of less than 0.7 ml per minute (Navazesh and Kumar, 2008).

**1.3.4.3 Observer’s assessment of the condition of the oral mucosa and dentition**

Clinical identification of dry mouth can be performed by oral examination, including examination of the salivary glands and the associated ducts. The salivary glands should be examined for enlargement or asymmetry, tenderness on palpation, lack of saliva on palpation, atrophic Stensen or Wharton duct and contaminated saliva (with
pus or blood). Lack of saliva pooling may indicate dry mouth, as may a dry, desiccated, atrophic, fissured, lobulated or discoloured mucosa. Examination of the teeth and gums may show evidence of carious lesions and/or gingivitis (Navazesh, 2003). Navazesh et al. (1992) identified the combination of four clinical measures that successfully predicted the presence of dry mouth, namely; dry lips, dry buccal mucosa, absence of saliva from salivary gland palpation and total DMFT (Decayed-Missing-Filled Teeth). However, they proposed that at least another diagnostic procedure should be done along with the clinical evaluations to confirm the diagnosis. They tested the salivary output against the other signs of dry mouth including lack of saliva pooling in the mouth, tongue mucosal changes, increased Candida count and higher gingival index. Although these signs were found more in participants with low salivary flow, they were not significant enough to be sole indicators of dry mouth.

### 1.3.3.4 Imaging procedures to determine salivary gland activity

Radiographic imaging such as scintigraphy or sialography may also help in determining salivary gland function or dysfunction. Scintigraphy is a minimally invasive diagnostic test performed by using the radionuclide $^{99m}$Tc-technetium pertechnetate (Tc-99). It has been used for the diagnosis of Sjogren’s syndrome, Bell’s palsy, sialolithiasis, gland aplasia and duct obstruction. A gamma scintillation camera visually records the uptake, concentration and excretion of the pertechnetate anion by major salivary glands and other body organs. Tc-99 is injected intravenously in the antecubital fossa and the camera immediately records at 60s/frame images with the exposure made every two minutes. Forty minutes after the injection, 0.5 mL of 2% citric acid is applied on the dorsum surface of the tongue and the study continued for additional 14 minutes. The complete study consists of 27 separate exposures which are then evaluated by the radiologists (Kohn et al., 1991). Sialography is radiographic imaging of the salivary gland ductal architecture after infusion of a contrast fluid through the salivary duct via a cannula. Sialogram images may be used to diagnose sialectasia in Sjogren’s syndrome (Kalk et al., 2002). Three-dimensional magnetic resonance (3D-MR) sialography has proven beneficial to capture the salivary duct anatomy especially after radiation therapy and has potential use in the future since it can record submandibular and sublingual duct systems.
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simultaneously and thus is more efficient than a two-dimensional (2D) sialogram (Astreinidou et al., 2007). Other imaging modalities such as salivary gland magnetic resonance imaging (MRI), plain radiographic films and ultrasonography are also used to detect salivary gland tumours or duct obstructions. Minor salivary gland biopsy, usually taken from the labial aspect of the lower lip may be useful if Sjogren’s syndrome is included in the differential diagnosis. The histopathological features of Sjogren’s syndrome are the presence of at least two foci (clusters of 50 lymphocytes in each foci) in a 4 mm x 4 mm tissue section obtained from the biopsy (Navazesh, 2003). Inflammatory changes may also be seen in the histopathological section, for example due to some medications and are usually reversible. Blood tests of individuals to investigate the presence of specific anti-nuclear antibodies (ANAs) such as anti-SSA/Ro and anti-SSB/La and positive rheumatoid factor (RF) are required if Sjogren’s syndrome or other autoimmune diseases are being considered.

1.3.5 Consequences of Hyposalivation and Xerostomia

Salivary hypofunction or hyposalivation is a condition with actual reduction in salivary flow due to medications, diseases or following radiation therapy to the head and neck (Nederfors, 2000; Tschoppe et al., 2010). A consequence of dry mouth is increased risk of dental caries especially on flat surfaces. Altered taste may cause patients to consume more sugar-containing food and beverages, thus contributing to the increased caries incidence (Guggenheimer and Moore, 2003). Increased caries incidence is also associated with the reduced buffering capacity of saliva due to reduced salivary flow (Turner et al., 2007). Oral candidosis and burning mouth syndrome are frequently seen in patients with hyposalivation (Jensena et al., 2006, Turner et al., 2007). Saliva is important for oral health and aids in swallowing, oral cleansing, digestion and taste. People with reduced salivary flow may have difficulty in chewing and swallowing food, especially dry foods. Hyposalivation is also associated with taste disturbances that may lead to poor eating, causing a lack of nutritional support, especially in elderly patients. They also have increased susceptibility to aspiration pneumonia (Turner and Ship, 2007). Individuals with hyposalivation may have difficulties with denture retention due to the lack of
lubrication and salivary film. They may complain of halitosis, burning mouth, intolerance to spicy and acidic food and speech and eating difficulties.

Xerostomia may result in an increased risk for clinical depression. In a study done in the United Kingdom, using the Hospital Anxiety and Depression Scale (HADS) (a self-administered questionnaire to evaluate the presence and degree of anxiety and depression), an increased risk for clinical depression was found in patients with Sjogren’s syndrome (Stevenson et al., 2004). A study done on radiotherapy patients with xerostomia showed a decrease in the quality of life, especially in female and younger patients that worsened with time (Jellema et al., 2007). Another study done on elderly patients living in a long-term care facility showed that xerostomia alone had a significant impact on the oral health-related quality of life of the patients (Locker, 2003).

1.4 Oral Candida

1.4.1 Candida Colonisation of the Oral Cavity

Candida species are normal oral commensals (Cannon and Chaffin, 1999). The most prevalent Candida species isolated from the oral cavity is Candida albicans (Cannon et al., 1995; Coronado-Castellote and Jiménez-Soriano, 2013). C. albicans is a polymorphic organism because it can grow in yeast, hyphal and pseudohyphal modes and may produce chlamydomospores in certain growth conditions (Odds, 1988 reviewed by Cannon and Chaffin, 1999). In most individuals, the presence of C. albicans in the oral cavity does not indicate a disease. C. albicans may cause two major types of infection; superficial infections such as oral or vaginal infections and life-threatening systemic infections (Mayer et al., 2013). Commensal colonisation occurs when the population of C. albicans cells remains stable in the oral tissues without giving rise to clinical disease.

Colonisation depends on the entry of cells into the oral cavity (acquisition), attachment and growth of the cells, tissue penetration and cells removal from the oral cavity (Cannon and Chaffin, 1999). The rate of yeast cell entry into the oral cavity
versus the rate of removal determines whether Candida colonisation occurs or not. When tissue damage is present, candidosis is more likely to occur. In order for Candida to colonise the oral cavity, a population must adhere to the host surface. This depends on the Candida cell wall and adhesins. The cell wall of C. albicans not only provides a biological support for the organism, but is also important in the interactions with the human host. Adhesins are the binding molecules that mediate the binding of C. albicans to other cells, inert polymers or proteins (Cannon and Chaffin, 1999). In the oral cavity, Candida can adhere to the oral epithelial cells, saliva molecules and teeth. They also adhere to the inert polymers of dental prostheses and other oral microorganisms. In the mouth, C. albicans is primarily isolated from the posterior half of the dorsum of the tongue and secondarily from saliva and other sites of the oral cavity (Arendorf and Walker, 1980). Adherence of Candida to the epithelial cells was higher in infants, children with oral infection or during antibiotic therapy, diabetic patients and in AIDS patients. Candida growth was increased in the presence of glucocorticoids, dexamethasone or triamcinolone acetonide and was also affected by a rise in temperature (Cannon and Chaffin, 1999).

Adherence to dental prostheses depends on hydrophobicity, the surface roughness of the prosthesis and the type of base material. C. albicans is less hydrophobic compared to other species such as Candida tropicalis, Candida glabrata and Candida krusei. The more hydrophobic species adhere more to inert polymers. Acrylic bases promote less adherence than tissue conditioners and soft liners (Okita et al., 1991 reviewed by Cannon and Chaffin, 1999). Co-adherence of C. albicans with several oral bacteria is also reviewed by Cannon and Chaffin (1999), such as Streptococcus gordonii, Streptococcus mutans, Streptococcus oralis, Streptococcus sanguis, Streptococcus salivarius and Actinomyces species. C. albicans adheres to adsorbed saliva molecules (saliva pellicles) using specific adhesins that recognise cryptitopes (cryptic receptors) to promote colonisation and prevent saliva-mediated aggregation and clearance from the oral cavity.

The other important factor in oral colonisation is the growth of the Candida populations. The cells need to grow and multiply to at least match the level of clearance. C. albicans secretes aspartyl proteinases and hydrolytic enzyme N-acetylglucosaminidase (hexosaminidase) that release carbon and nitrogen sources and
contribute to its growth and survival in the oral cavity. Antibiotic therapy increases the growth rate of Candida cells because it lessens the competition for nutrients with other oral microorganisms (Cannon and Chaffin, 1999).

1.4.2 Pathogenicity of Candida spp.

The pathogenicity of C. albicans depends on several factors including polymorphism, adhesins, tissue invasion, biofilm formation, contact sensing and thigmotropism, secreted hydrolases, pH sensing and regulation, metabolic adaptation, environmental stress response, heat shock proteins and metal acquisition (Mayer et al., 2013). C. albicans may grow as ovoid-shaped budding yeast, elongated ellipsoids with constrictions at the septa (pseudohyphae), or parallel walled true hyphae. They may also be present as ‘white’ and ‘opaque’ cells, or chlamydomspores. Dimorphism is the transition between the yeast form and hyphal form (Mayer et al., 2013), also known as morphogenesis (Calderone and Fonzi, 2001). Phenotypic switching is the change of the organism between the white and opaque form that is reversible and occurs rapidly (Calderone and Fonzi, 2001). However, the role of phenotypic switching in C. albicans virulence is still unclear. The hyphal form has been shown to be more invasive, but its presence is related to certain conditions such as high pH (pH>7), starvation, presence of serum or N-acetylglucosamine, physiological temperature and presence of carbon dioxide (CO$_2$). There are two mechanisms of invasion of C. albicans into the host cells; induced endocytosis and active penetration. Induced endocytosis is a passive process where the fungus expresses specific proteins on the cell surface (invasins), particularly Als3, which triggers engulfment into the epithelial cells. Active penetration is an active process by viable C. albicans hyphae. Adhesins, namely agglutinin-like sequence (ALS) proteins are upregulated during C. albicans infections (Mayer et al., 2013), in particular Als3 during hyphae formation (Gow and Hube, 2012). Tissue damage may also occur due to hyperactivity of the immune system, for example massive infiltration of neutrophils (Gow and Hube, 2012). Secreted enzymes are also found to be responsible for the pathogenicity of C. albicans; secreted aspartyl proteinases (SAP) and phospholipases (PL), including nine SAPs and four PLs (Calderone and Fonzi, 2001).
The process of biofilm production involves adherence of yeast cells to substrates, proliferation of yeasts, formation of hyphal cells, accumulation of extracellular matrix and dispersion of yeast cells from the biofilm complex. Mature biofilm are more resistant to antimicrobial agents and host defence factors. It is proposed that β-glucans in the extracellular matrix protect the *C. albicans* from being attacked by neutrophils (Mayer et al., 2013). When *C. albicans* cells come in contact with a solid surface, contact sensing occurs and triggers switching of the fungus into the hyphal form and induces biofilm production. Thigmotropism is when there is a specific structure on the surface (for example ridges) that causes directional hyphal growth. Hydrolytic enzymes are secreted when *C. albicans* cells adhere to host cells. These enzymes can facilitate the invasion of *C. albicans* into cells. Glucose is the main carbon source for *C. albicans*. It can metabolise glucose in the blood stream via glycolysis and gluconeogenesis, especially in systemic candidosis. *C. albicans* is able to utilise host lipids, proteins, amino acids and phospholipids. *C. albicans* is capable of adapting to the environmental stresses. There are several stress response pathways including heat-shock, osmotic, oxidative and nitrosative. Heat-shock proteins facilitate the adaptation of *C. albicans* to thermal stress via trehalose production and prevent the protein unfolding that induces cell death. The osmotic stress response induces the accumulation of glycerol to prevent loss of water due to the outward-directed chemical gradient. The oxidative stress response induces detoxification of reactive oxygen species such as peroxide, superoxide anions and hydroxyl radicals. Trace metals including iron, zinc, manganese and copper are important for the growth and survival of all living cells. *C. albicans* acquires trace metals from the host via siderophores and transporters (Mayer et al., 2013).

The cell wall composition of *C. albicans* is 90% carbohydrate and 10% protein. The fibrillar outer layer consists of mannoproteins, while the β-glucans/chitin layer lies underneath the mannoprotein, with both providing strong support to the cell wall (Gow and Hube, 2012). The outer cell wall consists of mannans that are less structured and permeable thus affecting the cell resistance against antifungal agents. The carbohydrate in the cell walls not only induces the immune response of the host, but also induces hyperactivity of the inflammatory response causing pathogenicity of the *C. albicans*. The transition of *C. albicans* from colonising organism into pathogenic organism involves multiple complex pathways (Gow and Hube, 2012).
1.4.3 Oral Colonisation by *Candida* in Individuals Reporting Dry Mouth

In general, the number of *Candida* cells in the oral cavity is inversely affected by salivary flow. One of the reasons for this is the antimicrobial property of saliva. The number of *C. albicans* and *Candida parapsilosis* colony forming units (CFU) isolated from individuals with decreased salivary flow is high compared to individuals with normal salivary flow (Torres et al., 2003; Torres et al., 2002). Table 1.2 summarises studies quantifying the median *Candida* CFU/mL in participants with decreased salivary flow (median *C. albicans* CFU/mL in individuals with normal salivary flow varies between 0 and 500).

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of enrolled participants</th>
<th>Underlying medical conditions</th>
<th>Saliva sample used</th>
<th>Median salivary flow (mL/min)</th>
<th>Median <em>Candida</em> CFU/mL</th>
<th>Candida CFU/mL range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torres et al., 2003</td>
<td>133</td>
<td>Multiple</td>
<td>CS</td>
<td>0.78</td>
<td><em>C. albicans</em> 2000</td>
<td>10-85 200</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>C. parapsilosis</em> 420</td>
<td>10-7200</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>C. tropicalis</em> 3200</td>
<td>20-17 800</td>
</tr>
<tr>
<td>Torres et al., 2002</td>
<td>112</td>
<td>Multiple</td>
<td>CS</td>
<td>0.72 (F)</td>
<td>Not specific 430</td>
<td>0-82 000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.10 (M)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Navazesh et al., 1995</td>
<td>71</td>
<td>Medically compromised and healthy</td>
<td>UW CS ASP CSP</td>
<td>0.023</td>
<td><em>C. albicans</em> &gt;500</td>
<td>Not stated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.478</td>
<td></td>
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<td></td>
<td></td>
<td>0.353</td>
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<td></td>
<td></td>
<td></td>
<td>0.495</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1.2 Studies of *Candida* cfu in participants with decreased salivary flow (CS = chewing-stimulated whole; UW = unstimulated whole; ASP = acid-stimulated parotid; CSP = candy-stimulated parotid, F = female, M = male)

1.4.4 Oral Colonisation by *Candida* in Individuals on Antipsychotic Medications

Patients on long-term phenothiazine therapy were found to have a higher frequency of *Candida* infection and higher mean number of *Candida* CFU than a control non-psychiatric group (Lucas, 1993). The first reported case of oral *Candida* infection in a patient receiving chlorpromazine was in 1962 (Kane, 1963) where a patient on a high dose of chlorpromazine was described as having a brown hairy tongue and severe pharyngitis and glossitis due to *C. albicans* infection. The condition improved
when the patient was prescribed with 100,000 units of Mycostatin suspension four times a day. Chlorpromazine was continued but at a reduced dose. There is a lack of evidence in the literature that directly correlates oral Candida infections to antipsychotic drug intake. One of the correlations of oral Candida infection in patients taking antipsychotic medications is increased susceptibility due to dry mouth as an adverse effect of the medications. Another factor that may contribute to oral fungal infections when an individual is treated with antipsychotic medications is hyperglycaemia. Some antipsychotic medications such as chlorpromazine, fluphenazine, clozapine, olanzapine and quetiapine increase blood glucose levels (reviewed by Arulmozhi et al., 2006). However, the correlation between oral candidosis and higher glucose levels such as in diabetes mellitus, depended on other local factors as well. This included glycaemic control level, denture-wearing, hyposalivation, defective candidacidal activity of neutrophils and tobacco smoking (Soysa et al., 2006).

While the natural host defence mechanisms against Candida infection are weakened by the xerostomia induced by antipsychotic medication, an in vitro study of phenothiazines, specifically chlorpromazine and trifluoperazine, showed an inhibition of C. albicans growth (Sharma et al., 2001) and other Candida species including C. tropicalis, C. parapsilosis and C. glabrata (Afeltra and Verweij, 2003) suggesting these medications have some antifungal activity.
Chapter 1: Literature Review

1.4.5 Factors that Promote Oral Candidosis

Factors predisposing to Candida carriage include drug therapy, especially broad-spectrum antibiotics, immunomodulatory and xerogenic medications, blood dyscrasias and malignancy, dietary factors, endocrine disorders, immunologic disorders and salivary changes (Cannon et al., 1995; LaFleur et al., 2010).

1.4.5.1 Systemic factors that promote oral candidosis

The systemic health of the individual affects C. albicans colonisation. It was found that 40% to 60% of healthy adults harbour Candida in the oral cavity without signs and symptoms of candidosis (Zegarelli et al., 1993 reviewed by Soysa et al., 2006). Clinically, there are some factors that predispose to oral candidosis including drug therapy, especially broad-spectrum antibiotics, immunomodulatory and xerogenic medications, blood dyscrasias and malignancy, dietary factors, endocrine disorders, immunologic disorders and salivary changes (Farah et al., 2000). Drug therapy, including broad-spectrum antibiotics, immunomodulatory medications and cytotoxic medications, alters the host susceptibility, resulting in oral candidosis (Cannon et al., 1995). Individuals with diabetes were found to have higher Candida carriage rate than the non-diabetics, presumably due to increased Candida growth with high glucose levels in saliva and decreased pH (Soysa et al., 2006). Reduced phagocytosis, intracellular killing, bactericidal activity and chemotaxis in poorly controlled diabetes might increase the susceptibility of diabetic patients to oral Candida infection (Soysa et al., 2006). Furthermore, denture wearers with diabetes had higher Candida carriage rate due to the dentures inducing biofilm formation, acting as an additional reservoir for the Candida colonisation and infection (Soysa et al., 2006).

Medications predisposing to oral candidosis were antimicrobials, including excessive use of antibacterial mouthwashes and corticosteroids (topical, systemic and aerosolised) (Farah et al., 2000). The number of yeasts was found to be higher in patients who had undergone radiation therapy to the head and neck with subsequent xerostomia (Ramirez-Amador et al., 1997). Individuals with an impaired immune system, such as patients with myeloproliferative diseases or malignant diseases were
found to have a higher incidence of oral fungal infections. Deficiency in certain nutrients such as iron, folic acid and vitamins may predispose to oral candidosis. Diseases that cause immunodeficiency such as acquired immune deficiency syndrome (AIDS), severe combined immunodeficiency syndrome (SCID), hereditary myeloperoxidase deficiency and Chediak-Higashi disease may predispose to oral candidal infections.

1.4.5.2 Local factors that promote oral candidosis

Local factors that predispose to oral candidosis include irritation from ill-fitting dentures and poor oral hygiene (Farah et al., 2000). Poor denture hygiene such as wearing the dentures continuously without taking them out at night and poor denture cleanliness predisposes the individuals to denture-related oral candidosis. The continuous irritation from ill-fitting dentures may cause invasion of the organisms through the microscopic breaks in the oral mucosa. The yeast counts in denture wearing persons were found to be about ten-fold higher when compared to healthy individuals (Samaranayake et al., 1990 reviewed by Farah et al., 2000). Candida biofilms play a role in denture-induced stomatitis especially on dentures rough surfaces due to better adhesion of the organisms and the resistance to cleaning activities (Ramage et al., 2003).

1.4.6 Clinical Presentations of Oral Candidosis

Clinically, oral candidosis may present as pseudomembranous candidosis, erythematous candidosis, hyperplastic candidosis, denture-associated erythematous candidosis, angular cheilitis, median rhomboid glossitis and chronic mucocutaneous candidosis (Napeñas et al., 2009; Farah et al., 2010). To aid in making a clinical diagnosis for oral candidosis, staining of cytological smears from affected areas with periodic acid-Schiff (PAS) may be used to detect hyphal and yeast cells. A tissue biopsy may be useful for suspected chronic hyperplastic candidosis (Jensena et al., 2006). Sabouraud’s medium can be used to determine CFU and commercial investigation kits such as Oricult N (produced by Orion Diagnostica) may be useful.
1.4.6.1 Pseudomembranous candidosis

Pseudomembranous candidosis or thrush is the most common presentation of oral candidosis (Cannon et al., 1995). It presents clinically as confluent whitish-yellow creamy or yellow velvety plaques on the surfaces of the oral mucosa (Reichart et al., 2000; Farah et al., 2010). When the superficial material is wiped off, it reveals an erythematous surface that may easily bleed. Pseudomembranous candidosis may become painful with a burning mouth sensation, sour taste or altered taste sensation and discomfort or bleeding when it is symptomatic (Sharon and Fazel, 2010). Treatment with antifungals is usually indicated especially in immunocompromised patients to prevent oesophageal involvement and recurrent infections (Reichart et al., 2000; Williams and Lewis, 2011). In a debilitated patient, it may spread oesophageally and/or involve angular cheilitis (McCullough and Savage, 2005).

A biopsy is not usually taken to make the diagnosis of pseudomembranous candidosis and the diagnosis is made on the basis of the characteristic clinical features and the presence of numerous Candida hyphae on a smear. The host epithelium is destroyed by proteinases, lipases and other enzymes contained at the tip of the penetrating hyphae. Microabscesses are produced when Candida hyphae destroy the desmosomal bridges, thus opening the intercellular space for invasion of inflammatory cells. Enzyme activity produces microcavities that become the space for focal aggregates of polymorphs, thus producing microabscesses (Fisker et al., 1982 reviewed by Reichart et al., 2000). The pseudomembrane comprises necrotic material, food debris, leukocytes, bacteria and fungi. Acanthosis in the deeper layers of the epithelium with lymphocytic infiltrate in the surrounding connective tissue may be present in some of the plaques (Farah et al., 2000). This feature is commonly found in immunocompromised patients, elderly people, poorly controlled diabetes mellitus patients, HIV patients, and patients receiving corticosteroid therapy, anti-proliferative therapy, psychotropic medications and long-term broad-spectrum antibiotic therapy (McCullough and Savage, 2005). In an immunocompetent individual, the inflammatory infiltrate may be reduced with a thicker pseudomembrane and rarely penetrate deeper to the spinous cell layer (Reichart et al., 2000).
1.4.6.1 Erythematous candidosis

The clinical presentation of erythematous candidosis (previously known as atrophic candidosis) is localised erythema of the oral mucosa. It is uncommon and may be associated with corticosteroids, broad-spectrum antibiotics (topical and systemic) or human immunodeficiency virus (HIV) infections (Scully et al., 1994). It may appear with or without symptoms. The common sites for this lesion are the dorsum of tongue, the palate and less commonly the buccal mucosa (Reichart et al., 2000). Previously, it was known as antibiotic sore mouth. It appears as a well-circumscribed erythematous lesion (Reichart et al., 2000) or depapillated area (Scully et al., 1994) on the dorsum surface of the tongue, sometimes concomitant with a palatal lesion. It may mimic denture stomatitis Type I and Type II (Newton’s classifications) where pin-point erythematous patches on palate or erythema on the mucosa of the denture bearing area may appear (Reichart et al., 2000). Antifungal therapy is always needed along with correction of the underlying predisposing factor (Reichart et al., 2000).

Histologically, it may appear with epithelial atrophy or lack of a keratinised surface layer (Eversole et al., 1997; Romagnoli et al., 1997; Reichart et al. 2000) although a biopsy is not usually required for diagnosis. It does not usually show hyphal infiltration of the epithelium. However, yeast may be found superficially on the epithelial surface. The erythema may be associated with increased mucosal vascularity or hyperaemia (Reichart et al., 2000).

1.4.6.2 Hyperplastic candidosis

Hyperplastic candidosis presents as a chronic, well-demarcated, slightly raised, adherent white lesion of the oral mucosa. The terms previously used for this lesion are hyperplastic candidosis, Candida-associated leukoplakia or Candida leukoplakia (van der Waal, 1997). It ranges from small, translucent lesions to large, dense opaque, hard and rough on palpation, plaque-like lesions (Scully et al., 1994). There are two variants of this condition; isolated (homogenous form) and multiple white nodules on an erythematous background (nodular or speckled form), often symptomatic. It is most commonly found on the post-commisural buccal mucosa (Scully et al., 1994). It is generally found in tobacco smokers (Reichart et al., 2000).
Other less common locations are the tongue and the palate just posterior of the upper denture margin. This type of infection has been associated with a risk for dysplasia and malignant change (McCullough and Savage, 2005; Williams et al., 2008).

Histological features include infiltration of Candida hyphae in the superficial strata of the epithelium. Inflammatory cells may be found in the hyperplastic and acanthotic epithelium with the appearance of microabscesses (Reichert et al., 2000). It is crucial that the presence or absence of tissue dysplasia be determined from the biopsy and the patient managed accordingly.

1.4.6.3 Denture-associated erythematous candidosis

Denture-associated erythematous candidosis (chronic atrophic candidosis, denture sore mouth, Candida-associated denture stomatitis) is a common inflammatory oral mucosal lesion that appears on the mucosa in contact with the fitting surface of a denture. Clinically, it presents as erythematous and oedematous mucosa confined to the area of the fitting surface of the denture (Lynch, 1994; Samaranayake et al., 2002). The severity ranges from petechiae to generalised inflammation with papillary hyperplasia (Barbeau et al., 2003). It is frequently asymptomatic, but patients may experience slight soreness or a burning sensation (Scully et al., 1994). The primary aetiology of this lesion is the overgrowth of Candida due to the restricted salivary flow underneath the denture surface, especially on the palate (Samaranayake et al., 2002). Other contributing factors include poor oral and denture hygiene, wearing dentures at night, ill-fitting dentures (Farah et al., 2010), bacterial infections, mechanical irritation and allergic reaction to the denture base material (Samaranayake et al., 2002). Newton AV, 1962 reported three subtypes of Candida-associated denture stomatitis depending on the severity of the lesion;

Type I: localised simple inflammation or pin-point hyperemia
Type II: a diffused presentation of erythematous or generalised simple type involving part of or entire denture bearing mucosa
Type III: granular or papillary type commonly involving the midline of hard palate and alveolar ridge
1.4.6.4 Angular cheilitis

Angular cheilitis (angular stomatitis, angular cheilosis) is a chronic inflammatory lesion that affects the angles of the mouth. Clinically, it appears as erythematous, fissured lesions at the corners of the mouth, usually asymptomatic and bilateral (Samaranayake et al., 2002; Farah et al., 2010). One of the contributing factors is lack of soft tissue support or reduced vertical facial height especially in edentulous patients or denture wearing patients (Scully et al., 1994). The folded and wrinkled tissue at the corners of the mouth allows saliva accumulation thus a chronic moist environment for harbouring Candida and bacteria for example Staphylococcus and Streptococci is produced. Angular cheilitis may be associated with anaemia, vitamin B12 deficiency and can be seen in patients with denture-induced stomatitis (Scully et al., 1994).

1.4.6.5 Median rhomboid glossitis

The clinical presentation of this lesion is an area that is symmetrical, erythematous, elliptical or rhomboid-like on the posterior dorsal surface of the tongue, just anterior to the circumvallate papillae (Samaranayake et al., 2002). The atrophy is due to atrophic filiform papillae. Under the microscope, the hyperplastic rete pegs of the fungal hyphae extend into the corium, thus invading the superficial layers of parakeratotic epithelium (Farah et al., 2010).

1.4.6.6 Chronic mucocutaneous candidosis

When there is a persistent or recurrent Candida infection on the skin, nails and extraoral mucosa, about 90% of these cases also have oral candidosis and this syndrome is known as chronic mucocutaneous candidosis (Odds, 1988). It is associated with a defect in cell-mediated immunity and a variety of primary immunodeficiencies such as Nezelof syndrome (thymic alymphoplasia), DiGeorge syndrome (congenital thymic aplasia), hyperimmunoglobulin E syndrome, myeloperoxidase deficiency and endocrinopathies may be associated (Tarcin, 2011).
1.4.7 Diagnosis of Oral Candidosis

The diagnosis of oral candidosis should be made from comprehensive medical and history taking. The patients’ complaints should be investigated accordingly. Proper examinations of the signs that presented with the symptoms are crucial in order to make differential diagnosis, followed by additional investigations either non-surgical or surgical. Clinical laboratory procedures including PAS staining for investigations of the presence of *Candida* hyphae from cytological smears can usually confirm the diagnosis of pseudomembranous candidosis (Farah et al., 2000). However, in the denture-induced erythematous candidosis, *Candida* hyphae are not usually present (Farah et al., 2000). Swabs taken from the mucosal tissues followed by culturing are more useful in this condition. Investigating the oral *Candida* carriage rate may also be useful, where 50% of the population have less than 1,000 CFU/mL *Candida* compared to infected individuals with 4,000 to 20,000 CFU/mL of *Candida* population (Farah et al., 2000). Immunohistochemical techniques may identify the *Candida* species present, whilst media such as CHROMagar™ may identify the types of *Candida* based on their colony colour. This procedure may become necessary when the routine treatment of oral candidosis failed or when the patient is immunocompromised. Biopsy provides the most accurate diagnosis for chronic hyperplastic candidosis or median rhomboid glossitis (Farah et al., 2000).

Blood tests for patients with oral *Candida* infections may reveal abnormal haematological or immunological results. For example, patients with chronic mucocutaneous candidosis may have endocrinopathies or defects in the immune system, patients with angular cheilitis may have iron or vitamin deficiencies. When making a diagnosis of oral candidosis, the clinicians should exclude other oral mucosal lesions such as chemical burns, traumatic ulcers, mucous patches of syphilis, white keratotic lesions, thermal burns, drug reactions, erosive lichen planus, discoid lupus erythematosus and early erythema multiforme (Farah et al., 2000).

1.4.8 Treatment of Oral Candidosis

Oral candidosis can be treated with a range of antifungal medications (Ellepola and Samaranayake, 2000). Topical treatment with antifungal agents should be the first
line of treatment for oral candidosis. However, if the oral candidosis is complicated with systemic involvement, systemic treatment with antifungal agents should be considered. There are two main groups of antifungal agents; the polyene macrolides (for example nystatin and amphotericin B) and azole derivatives (examples are clotrimazole, ketoconazole, miconazole and fluconazole). In order to successfully treat oral candidosis, oral hygiene measures, denture hygiene (when applicable) and adjunct use of antiseptic mouthwashes such as chlorhexidine should be considered (Jørgensen, 1990). Patients with severe Candida infections, may need hospitalisation and treatment with systemic antifungals such as fluconazole, itraconazole and amphotericin B. Fungal infections from the oral cavity may spread along the gastrointestinal tract; isolates from the oral environment were found similar to the isolates from faeces in individuals with denture stomatitis (Bergendahl et al., 1979).

Antifungal medications may act by reducing the adherence of Candida to epithelial cells, changing the cell-surface charge or affecting the wall and membrane biosynthesis and structure (Cannon and Chaffin, 1999). There are several chemical classes of antifungal medications with different cellular targets. Polyenes such as amphotericin B and azoles such as fluconazole, itraconazole, and ketoconazole are the two main classes of antifungals. Other classes of antifungals not effective in treating Candida albicans infections are allylamines (terbinafine) and morpholines (amorolfine).

**1.4.8.1 Polyenes**

The mechanism of action of polyenes is mainly by binding tightly to the ergosterol molecules (the principal sterol in fungal membranes) and forming annuli thus damaging the cell plasma membrane resulting in a leakage of intracellular ions (Sanglard et al., 2003; Odds et al., 2003). However, the definite mechanism of action is still unclear (Odds et al., 2003). Polyenes are toxic to mammalian cells because of low selective affinity towards ergosterol and interactions with cholesterol. Amphotericin B is a polyene antifungal agent administered systemically to treat visceral infections (Odds et al., 2003). Nephrotoxicity is one of the toxic effects of amphotericin B, but newer formulations are being developed to overcome this (Odds et al., 2003). In some countries, Amphotericin B is used in lozenge form to treat
candidosis. Nystatin is a polyene derivative produced by *Streptomyces albidus* and *Streptomyces noursei*. It is only used as a topical agent due to severe toxicity when administered intravenously and is ineffective when taken orally. It is available in ointment, cream and powder form for cutaneous use; and in liquid, suspension and pastille form for the treatment of oral candidosis (Zhang et al., 2007). Nystatin ointment is useful for application to denture surfaces to treat denture associated erythematous candidosis.

### 1.4.8.2 Azoles

Azole antifungals are synthetic compounds with one or more 5-membered ring (each ring contains either two or three nitrogen atoms). Imidazole rings contain two nitrogen atoms, while triazole rings contain three nitrogen atoms (Lyman and Walsh, 1992). The mode of action is via the inhibition of cytochrome P450 enzymes, thus blocking the biosynthesis of ergosterol. They interact with the fungal enzyme lanosterol 14-α demethylase causing reduction in the ergosterol content of membranes resulting in the inhibition of fungal growth and replication. Azoles are mainly fungistatic, except in higher concentrations when they become fungicidal (Zhang et al., 2007). Examples of imidazole antifungals are clotrimazole, miconazole and ketoconazole. Itraconazole and fluconazole are triazoles. The most commonly usedazole is fluconazole. Fluconazole belongs to the newer category, bis-triazole antifungal drug and is only used systemically (Jørgensen, 1990). Fluconazole has advantages such as excellent oral bioavailability, stable parenteral formulation and fewer drug interactions when compared to other azoles (Marchetti et al., 2000). The choice of treatment depends on the patient’s medical history, oral symptoms, severity of infection and the level of compliance expected. Cautious use of fluconazole in patients with impaired mental state should be practised because these patients may deteriorate further (Lyman and Walsh, 1992). An oral suspension of fluconazole (5 mg/mL) is a topical alternative to the traditional nystatin oral suspension (100 000 IU/mL) for the treatment of oral candidosis (Tarcin 2011). Clotrimazole may also be used twice daily for the treatment of oral candidosis; available in spray or lozenge forms (Zhang et al., 2007).
Recent investigations found a risk for hospitalisation due to gastrointestinal (GI) bleeding when oral azoles are prescribed to warfarin users (Purkins et al., 2003; Holbrook et al., 2005; Schelleman et al., 2008). Fluconazole for example increases the international normalised ratio (INR) up to 38% in previously stable patients, while itraconazole and variconazole significantly prolonged the prothrombin time in patients taking warfarin (Shakeri-Nejad and Stahlmann, 2006). Topically applied miconazole oral gel and nystatin oral gel were found to interact equally with warfarin by impairing the metabolism of warfarin and causing an elevation in the INR in previously stable patients, predisposing the patients to major bleeding complications (Kovac et al., 2012). In order to avoid these complications, other alternatives to azoles such as amphotericin B should be considered.

1.4.9 Azole Resistant Oral Candida

Treatment failure can arise, however, if the yeast responsible for the candidosis develops antifungal drug resistance. Resistance to imidazoles and triazoles, particularly fluconazole, is more common than to polyenes, allylamines and echinocandins (Cannon et al., 2009). Host factors that predispose toazole resistance are immunocompromised, including HIV infection or in bone marrow transplant patients undergoing immunosuppressive therapy and repeated or long term use of azole antifungals (White et al., 2002; Sanglard et al., 2003).

There are multiple mechanisms of azole resistance in C. albicans including mutations in the gene ERG11 involved in ergosterol biosynthesis and the overexpression of drug efflux pumps (White et al., 2002). ATP-binding cassette (ABC) pumps and the major facilitator superfamily (MFS) transporters are the two main families of the efflux protein. Azoles target the 14α-demethylase enzyme encoded by ERG11, blocking ergosterol biosynthesis. This leads to depletion of ergosterol, thus inducing the accumulation of intermediates from the toxic sterol pathways, which inhibit growth (reviewed by Ramage et al., 2012). High-level azole resistance is caused by the overexpression of ABC efflux pumps in the plasma membrane (White et al., 2002; Cannon et al., 2009). The transmembrane ABC efflux proteins Cdr1p and Cdr2p from C. albicans expel hydrophobic molecules out of the cells using energy from
ATP hydrolysis. The overexpression of these transporters plays a major role in developing antifungal drug resistance, mainly to azoles (Akins, 2005) by reducing the intracellularazole concentration. Interestingly, expression of these ABC efflux pumps is induced in *C. albicans* cells by the antipsychotic fluphenazine (de Micheli et al., 2002; Coste et al., 2004; Manoharlal et al., 2011).

Another important factor in azole resistance is biofilm production on host tissues by *C. albicans* (Cannon et al., 2009). These biofilms are resistant toazole antifungal agents. Biofilms are defined as an enclosed extracellular matrix (ECM) containing highly structured communities of microorganisms, either attached to the surface or attached to one another (review by Ramage et al., 2012). Microorganisms that produce biofilms have advantages such as protection from the environment, resistance of physical and chemical stress, metabolic cooperation and community-based regulation of gene expression. ABC efflux pumps were thought to express at early stages of biofilm formation.

Although still somewhat controversial, particularly from a clinical standpoint, *in vitro* experiments using phenothiazines such aschlorpromazine and trifluoperazine resulted in some growth inhibition of *C. albicans* (Buchan et al., 1993; Sharma et al., 2001). Phenothiazines and also fluphenazine are calmodulin inhibitors. Calmodulin is a calcium binding protein involved in fungal proliferation. Buchan et al, (1993) concluded that although there was growth inhibition of *C. albicans* in mice treated with trifluoperazine, the dose was too large (more than 25 mg/kg per dose) to be considered of therapeutic value. Stylianou et al. (2014) compared the minimum inhibitory concentration (MIC) of haloperidol (MIC, 0.38 µg/mL) and trifluperidol (0.4 µg/mL) and found that they exhibited a comparable antifungal activity towards *C. albicans* as fluconazole (MIC value of 0.3 µg/mL).

Thus the prescription of certain antipsychotics for mental illness may have consequences for oral health. They may have an antifungal effect, or they may induceazole resistance in *C. albicans* due to induction of ABC pump expression. These are important considerations that warrant further investigation.
1.5 Aims and Objectives

The particular interest of this study is to investigate oral Candida carriage and antifungal susceptibility of Candida albicans isolates in patients receiving antipsychotic medications. Specific objectives of this study is to determine the level of colonisation with Candida of the oral mucosae in people taking antipsychotic medications when compared to healthy controls and to xerostomic individuals not prescribed with antipsychotics. The other objectives are to determine the Candida species present in their saliva samples and also to measure the azole resistance of these isolates. The final aim of this study is to determine whether the antipsychotic drug fluphenazine affected azole resistance of C. albicans isolates.
CHAPTER 2:

MATERIALS AND METHODS
CHAPTER 2: MATERIALS AND METHODS

2.1 Ethical Approval and Māori Consultation

Ethical approval was obtained from the University of Otago Human Ethics Committee (committee reference number 12/207) prior to the recruitment of participants. Māori consultation was undertaken through the Ngāi Tahu research consultation committee.

2.2 Participants

2.2.1 Selection of Participants

A convenience sampling of patients attending the Special Care Unit, of the University of Otago, Faculty of Dentistry was undertaken. Patients were approached to consider participating in this research based on specific criteria. The inclusion criteria for selecting participants for this study were:

a) Patients aged between 20 and 70 years old
b) Currently taking antipsychotic medications
c) Known smoking status
d) Living independently or with minimal supervision/support

A list of potential participants was prepared from the list of patients registered in the Special Care Unit. The patients’ recall cards in the Special Care Unit were examined. Some of the recall cards had a summary of the patient’s medical condition or disability written on them. Fifty names were obtained from the recall cards, where their cards had “psych problems” written on them. The clinical files of potential participants were obtained from the records office and carefully examined. Patients with a history of intellectual disability or an inability to cooperate in the dental chair were excluded from the list of potential participants. Other exclusion criteria included:

a) Medically compromised individuals with conditions known to affect Candida carriage such as diabetes mellitus
b) Taking medications other than antipsychotics, such as antibiotics and antifungals

### 2.2.2 Study Participants

By considering the inclusion and exclusion criteria, fifteen patients were found to be suitable for the research. The patients were contacted by phone with a brief explanation about the study and invited to participate. Nine patients agreed to participate in this study.

Patients were provided with an information sheet (Appendix III) to read either before they came to the research appointments or on the appointment day itself. They were given opportunities to ask questions if they needed to. Information about this research was explained carefully to them either by the author or by their accompanying caregiver. They understood that they could withdraw anytime from this study. Signed consent forms were obtained from the participants. Current smoking status was recorded. The ethnicity of each participant and their age at enrolment into the study were recorded.

### 2.2.3 Control Participants

Samples from two matched control groups (patients with xerostomia taking no antipsychotic medication, n=9 and healthy individuals with no history of xerostomia, n=9) had already been obtained for a different study performed by Leanne Xiao Li Hou for her Doctor of Clinical Dentistry degree titled “Oral yeast carriage and salivary protein profiles in xerostomia subjects and in age- and gender-matched controls” and the results from Dr Hou’s study were used in the current project.

### 2.3 Xerostomia Inventory

A validated measure, the Xerostomia Inventory (XI) was used in this research to quantify xerostomia symptoms in study participants (Thomson et al., 1999). All participants answered eleven questions about symptoms of dry mouth. The main question was “how often does your mouth feels dry?”, followed by eleven more
specific questions. Four answering options were given for each question; ‘never’, ‘occasionally’, ‘frequently’, ‘always’ (Table 2.1).

<table>
<thead>
<tr>
<th>How often does your mouth feels dry?</th>
<th>Never</th>
<th>Occasionally</th>
<th>Frequently</th>
<th>Always</th>
</tr>
</thead>
<tbody>
<tr>
<td>My mouth feels dry</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I have difficulty in eating dry food</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I get up at night to drink</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>My mouth feels dry when eating a meal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I sip liquids to aid in swallowing food</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I suck sweets or cough lollies to relieve a dry mouth</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I have difficulties in swallowing certain foods</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The skin of my face feels dry</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>My eyes feel dry</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>My lips feel dry</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The inside of my nose feels dry</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.1 Xerostomia Inventory (XI)

Each answer was given a score: 1 for ‘never’, 2 for ‘occasionally’, 3 for ‘frequently’ and 4 for ‘always’. The total score for the answers to all the questions was calculated. The total theoretical score ranged from 11 to 44. This method gives an overview of the individuals’ experience of, and behaviour with, xerostomia. The answering patterns of the participants were analysed to evaluate the severity of their xerostomia condition. The frequency of xerostomia was analysed by comparing the mean XI score that each participant obtained.

**2.4 Clinical Procedures**

**2.4.1 Clinical Examination**

All participants underwent a thorough clinical examination. Oral soft tissues were inspected. Any clinical evidence of candidosis in the mouth, for example
Chapter 2: Materials and Methods

pseudomembranous candidosis, erythematous candidosis, median rhomboid glossitis or angular cheilitis (Napenas et al., 2009) either on the buccal mucosa, tongue or palate was recorded. Signs of dry mouth were recorded, including: frothy saliva; dry shiny mucosa; depapillated or red, fissured, tongue; lack of saliva pooling in the floor of the mouth; and the mouth mirror sticking to the buccal mucosa. The denture status of all participants was recorded. Denture fitting surfaces were examined for evidence of debris and potential yeast colonisation in participants wearing dentures.

2.4.2 Obtaining Intra-oral Smears

Three smears were taken from each participant by gently rubbing a sterile stainless steel flat plastic instrument against the oral tissues. The smears were taken from the right and left buccal mucosa and the tongue. Whenever there was evidence of Candida infection elsewhere in the mouth, further smears were taken. The material obtained on the instrument was smeared onto glass slides. All slides were labeled with the participant’s name, date of birth and the site from which the smears were taken. They were placed in a transporting laboratory bottle containing 95% ethanol, and sent to Medlab Dental Oral Pathology laboratory (Faculty of Dentistry, University of Otago). Each participant was assigned with a laboratory registration number.

2.4.3 Oral Rinse Sampling

In this study, oral rinse samples were collected from participants by asking them to pour 10 mL of water from a sterile pathology pot in their mouths, swish it around for 30 s and then to expectorate the water mixed with saliva back into the specimen bottles. The oral rinse samples were taken to the PC2 Molecular Biosciences Laboratory (PC2 MBL), Faculty of Dentistry, University of Otago for processing.
2.5 Laboratory Procedures

2.5.1 Cytology

Procedures for rapid periodic acid-Schiff (PAS) staining were performed on the smears to determine whether *C. albicans* was present. The smears were fixed upon delivery to the laboratory because they had been soaked in 95% ethanol for about 1 h. Immediately upon receipt at the laboratory, the smears were rinsed with distilled water and then flooded with 1% fresh aqueous periodic acid for 3 min. They were then washed under running tap water for another 3 min. The smears were stained in Schiff's reagent for 10 min. After that, the smears were washed with hot running tap water for 5 min. They were then lightly counterstained with Gill’s haematoxylin for 30 s followed by dipping them 10 times in Scott’s tap water for blue stain. Finally, the smears were washed in tap water for 1 min and dehydrated before they were mounted. Under the light microscope, fungi appeared magenta and nuclei were blue.

The smears were examined using a light microscope where all material on the slide was viewed in a systematic manner from one end of the slide to the other, under low magnification (4x and 10x lens) initially, and with higher power (20x and 40x lens) for examination of possible hyphae and yeast cells. In this study, PAS staining was used to detect the presence of *Candida*. If hyphae were observed the smear was recorded as being positive for *C. albicans*. Other *Candida* species such as *C. glabrata* will be PAS positive but they will not show the hyphal form, and appear in the budding yeast form (Jayatilake et al., 2006).

2.5.2 Oral yeast identification and quantification

CHROMagar™ *Candida* (Figure 2.1) agar plates were used as the primary isolation medium due to the ease of recognising *Candida* species via their colony colour on the agar plates. CHROMagar™ *Candida* medium contains chromogenic chemicals that allow the rapid presumptive identification of *Candida* species. Different *Candida* species form colonies with different colours on this agar. For example, *C. albicans* colonies are green, *Candida tropicalis* colonies are blue with a pink halo or blue-grey colony with a dark halo of brownish purple (Odds et al., 1994) and *Candida krusei*
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colonies are pink with a downy appearance (Barasch et al., 2004; Birinci et al., 2004). *Candida glabrata* colonies vary from purple to pale pink (Beighton et al., 1995).

The oral rinse samples were gently mixed before volumes of 100 µL and 200 µL were plated onto separate CHROMagar™ *Candida* plates. The rest of the oral rinse sample was poured into a 15 mL tube and then centrifuged at 3000 × g for 5 min. The supernatant was poured back into the specimen bottle and the pellet was resuspended in the remaining liquid using a vortex mixer and then plated onto a third CHROMagar™ *Candida* plate. After the plates had been incubated at 37°C for 48 h they were removed from the incubator and examined for any *Candida* growth. The species of the *Candida* colonies was determined from the colony colour, based on the manufacturer’s instructions.

![Figure 2.1 Image taken from product leaflet on the website (www.chromagar.com)](image)

The number of colony-forming units (CFU) per oral rinse for each species was calculated from the number of colonies on the plates and the volume of the oral rinse sample plated.
2.5.3 Antifungal Susceptibility Testing

The yeasts used in the antifungal susceptibility assays were fresh clinical isolates of *C. albicans* grown from the patients’ saliva samples. Two yeast colonies were chosen for each *Candida* species present on each patient’s CHROMagar™ *Candida* plates (that showed *Candida* colonies) and subcultured onto Sabouraud dextrose agar (SDA) (Fort Richard Laboratories Ltd., New Zealand). The plates were incubated at 37°C for 48 h. Isolates were stored in yeast extract, peptone and dextrose (YPD) medium with 15% (w/v) glycerol at -80°C until further use.

RPMI 1640 (Sigma-Aldrich Corporation, St. Louis, Missouri, USA) buffered with MOPS (3-(N-morpholino) propanesulfonic acid, Sigma-Aldrich Corporation) was used as the culture medium for yeast susceptibility testing. RPMI liquid medium was prepared by dissolving 5.15 g RPMI 1640 powder and 17.3 g MOPS powder in distilled water, then the mixture was adjusted to pH 7 by adding sodium hydroxide (NaOH). The volume was then made up to 300 mL with distilled water. The RPMI 1640/MOPS mixture is referred to as RPMI in this thesis. RPMI agar plates were prepared by adding 7.5 g agar (Oxoid Ltd., Wade Road, Basingstoke, Hants, UK) to 300 mL RPMI liquid. The solution was then autoclaved at 121°C for 15 min. After cooling the autoclaved agar for 30 min in a 50-52°C water bath, it was poured into sterile petri dishes in a laminar flow cabinet. The agar plates were left at room temperature to set and then kept in the cold room, at 4°C until needed. RPMI was prepared fresh every month.

2.5.3.1 Agar diffusion susceptibility assay (Etest®)

The initial determination of the susceptibility of representative *Candida* isolates from subjects to fluconazole was measured by agar diffusion with the Etest® (BioMérieux Inc., USA) system (Holmes et al., 2002). *C. albicans* isolates from each patient were taken from the glycerol stocks and inoculated onto SDA plates. The plates were incubated at 37°C for 48 h. *C. albicans* colonies from the SDA plates were suspended in sterile saline (0.9% w/v NaCl) to give an optical density (OD) of 0.25 at a wavelength of 540 nm (OD$_{540}$) using a spectrophotometer (Ultrospec 6300pro
Amersham Biosciences, NJ, USA). This was in order to achieve a turbidity of 0.5 McFarland units. A sterile cotton swab was dipped into the yeast suspension, excess liquid squeezed out, and then the swab was spread evenly over an RPMI agar plate to make a lawn of C. albicans. An Ettest® strip was placed in the centre of the agar plate which was incubated at 37°C for 48 h.

The plates were examined at 24 h and at 48 h. The fluconazole diffused from the strip and inhibited the growth of the yeast when at high concentration, giving a clear zone of no growth around the strip (Figure 2.2). The inhibition ellipse intersected the graduated strip at the MIC value (Holmes et al., 2002). The size of the zone of growth inhibition around the strip gave a quantification of the susceptibility of the C. albicans isolate to fluconazole. The plates were photographed in the PC2 MBL.

![Image](image_url)

Figure 2.2 Photograph of Ettest® performed on one of the C. albicans isolates (a = the lowest concentration, b = the highest concentration).

**2.5.3.2 Liquid MIC susceptibility assay (fluconazole)**

Broth microdilution MIC determination was performed using RPMI liquid medium in sterile 96-well flat-bottom microtitre plates (Nunc, Thermo Fisher Scientific Inc., Waltham, USA) to confirm the values obtained using the fluconazole Ettest®. The fluconazole used in this study was purchased as Diflucan from Pfizer (New York, USA) and kept as a stock solution (2 mg/mL) dissolved in sterile water.

The diagram below shows the microtitre plate layout (Figure 2.3). All the wells in rows A and G and columns 1 and 12 were filled with 200 µL RPMI to act as a
negative no-drug, no-inoculum control and to prevent desiccation of the inner microtitre plate wells during incubation.

![Figure 2.3 Liquid MIC microtitre plate layout](image)

All the inner wells were filled with 100 µL of RPMI except column number 11, which contained 200 µL fluconazole at the highest concentration (128 µg/mL). The fluconazole in column 11 was diluted two-fold from right to left by taking 100 µL from column 11 and adding it to column 10, mixing and then taking 100 µL from column 10 and adding it to column 9 and so on until fluconazole was added to column number 3. The contents of column 3 were mixed and then 100 µL removed and discarded. Column 2 contained only RPMI medium without fluconazole and was the positive cell growth (zero drug) control. To each of the inner wells (i.e. not rows A or G nor columns 1 or 12) 100 µL of inoculum (prepared as described below) was added.

*C. albicans* cells from glycerol stocks were subcultured onto SDA plates, and incubated at 35°C for 24 h. Cells from single colonies were suspended in 1 mL RPMI growth medium to form a *C. albicans* pre-culture. The pre-culture was incubated in a Multitron II shaker at 35°C with agitation at 200 rpm for 18 h. The OD_{600} of the pre-culture was measured and then the pre-culture was diluted 100-fold into 1 mL of fresh
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RPMI solution and the culture was incubated at 35°C for 8 h. The OD$_{600}$ of the culture was measured and it was diluted with fresh RPMI to get a standardised concentration of $4 \times 10^3$ cells/mL according to the CLSI (Clinical and Laboratory Standard Institute) recommended inoculum for *C. albicans* susceptibility assays. This is to get the cell growth at exponential, pre-plateau phase at the time of testing in the MIC assay.

This *C. albicans* cell suspension (100 µL of $4 \times 10^3$ cells/mL) was added to the inner wells of the microtitre plate in duplicate rows such that the plate tested 3 strains of *C. albicans*. The plate was incubated at 35°C with agitation at 200 rpm for 48 h. The amount of *C. albicans* growth in each well was measured by reading the OD$_{600}$ using a Synergy 2 (Biotek Instruments Inc., Vermont, USA) microtitre plate reader at 24 h and at 48 h. The MIC optical densities were recorded using Gen 5 Microtitre Data Collection Analysis software (Biotek Instruments Inc., Vermont, USA) and transferred into Microsoft Excel. The minimum growth inhibitory concentration was defined as the minimum concentration of drug resulting in more than 80% growth inhibition relative to the no-drug control (MIC$_{80}$).

### 2.5.3.3 Liquid MIC susceptibility assay (fluphenazine)

Fluphenazine dihydrochloride was purchased from Sigma-Aldrich Corporation, St. Louis, Missouri, USA. The susceptibility of *C. albicans* to fluphenazine was measured by standard liquid MIC assays as described above (section 2.5.3.2) except the top final concentration of fluphenazine in the assay was 100 µg/mL. Readings of the microtitre plate were undertaken at 24 h and at 48 h. The results were interpreted as for section 2.5.3.2.

### 2.5.3.4 Statistical Analysis

Statistical analysis was undertaken using Microsoft Excel with StatPlus: mac LE (AnalystSoft.Inc, CA, USA). The data obtained did not show normally distributed samples, thus the non-parametric statistical analysis comparing the mean concentration of *C. albicans* in the saliva rinses was done using Kruskal-Wallis test
whilst the individual comparison was done between the participants and healthy control using the Mann-Whitney U test.

2.5.3.5 Agar diffusion synergism/antagonism assay for fluconazole and fluphenazine

An agar diffusion synergism/antagonism assay was used to determine whether interactions between fluconazole and fluphenazine affected the growth of *C. albicans*. Amphotericin B (Sigma-Aldrich Corporation, St. Louis, Missouri, USA) was used as a negative control. RPMI agar plates were inoculated with *C. albicans* cells as in the Etest® method (section 2.5.3.1). The antifungal medications fluconazole (5 µL of 2 mg/mL), fluphenazine (40 µL of 2 mg/mL), and amphotericin B (2 µL of 8 mg/mL) were put on sterile filter paper discs. Thus the amounts of the antifungal medications placed on the discs were: fluconazole, 10 µg; fluphenazine, 80 µg; and amphotericin B, 16 µg. The discs were placed on the seeded agar plates as illustrated in Figure 2.4.

![Figure 2.4 Agar diffusion synergism/antagonism disc layout](image)

The zones of growth inhibition around the discs were recorded at 24 h and photographed using the camera in the PC2 MBL. Antagonism between the medications on two discs would result in yeast growth between the two discs. Synergism between the medications on two discs would result in increased growth inhibition between the two discs.
2.5.3.6 Checkerboard liquid MIC susceptibility assay

A checkerboard liquid MIC assay was undertaken to measure the susceptibility of *C. albicans* strains to combinations of fluconazole and fluphenazine (Figure 2.5) and to get a quantitative measure of synergy or antagonism.

The method was based on the normal liquid MIC assay (section 2.5.3.2). Fresh *C. albicans* inoculum cell suspensions were prepared as described in section 2.5.3.2 but with a double concentration of $8 \times 10^3$ cells/mL. The peripheral wells (rows A and G and columns 1 and 12) in a sterile 96-well, flat-bottom microtitre plate, termed the receiver plate, were filled with 200 µL RPMI to act as a negative no-drug, no-inoculum control and to prevent desiccation of the inner microtitre plate wells during incubation. A transfer plate was prepared by adding 100 µL RPMI in the inner wells of the microtitre plate except row B. Then, 200 µL fluconazole (FLC) was added in the top row (row B, columns 2 to 11). A two-fold dilution of the fluconazole was performed by taking 100 µL from the wells in row B and mixing it with the RPMI in row C before 100 µL was taken from row C and added to row D. This process was continued down to the bottom row (row E) where after mixing 100 µL solution was removed and discarded. The top concentration of fluconazole was 64 µg/mL, this is four-times the required final concentration (16 µg/mL) as 50 µL was to be transferred from this plate to the receiver plate in a final volume of 200 µL. The bottom row (row F) acted as a no-fluconazole control.

The receiver plate was prepared by adding 100 µL RPMI to all inner wells apart from column 11, and putting 200 µL fluphenazine (FLUP) in column 11. The top concentration of fluphenazine was 100 µg/mL. A two-fold dilution of fluphenazine was carried out by transferring 100 µL fluphenazine from column 11 from right to the left, as described for fluconazole in section 2.5.3.2 (Figure 2.3). Column 2 acted as a no-fluphenazine control. Then, 50 µL fluconazole from each inner well of the transfer plate was added to the corresponding well on the receiver plate. The final top concentration of fluconazole in the plate is 16 µg/mL. Finally, 50 µL inoculum cell suspension was added to all the inner wells. The microtitre plate was incubated at 35°C with agitation at 200 rpm for 48 h. The OD$_{600}$ of the microtitre plate wells were recorded at 24 h and at 48 h.
Antagonism between the two medications in the checkerboard MIC would result in more yeast growth for combinations of the medications than expected. Synergism between the two medications in the checkerboard MIC would result in less yeast growth for combinations of the medications than expected.
CHAPTER 3:

STUDY PARTICIPANTS
CHAPTER 3: STUDY PARTICIPANTS

3.1 Overview

Recruiting the required number of participants for this study was a challenging task. When dealing with individuals with mental health issues, their co-operation is often unpredictable. Some of the patients agreed to participate when invited, but suddenly changed their minds when they were booked for the actual appointment. Finally, nine participants were recruited in this study. Their medical history was recorded, including the regular medications they were taking during this study. Some participants needed to have some assistance understanding instructions and when answering the questions. A general data for study participants can be viewed in the Table 3.1.

There were two matched control groups, nine participants with xerostomia taking no antipsychotic medication and nine healthy individuals. These patients were not seen as part of the current study, but their information was gathered from a related project performed by Leanne Xiao Li Hou for her Doctor of Clinical Dentistry degree titled “Oral yeast carriage and salivary protein profiles in xerostomia subjects and in age- and gender-matched controls”.

# Chapter 3: Study Participants

<table>
<thead>
<tr>
<th>Participant Identification (ID)</th>
<th>Age</th>
<th>Gender (M/F)</th>
<th>Medical conditions</th>
<th>Antipsychotic medications</th>
<th>Other medications</th>
<th>Smoker (Y/N)</th>
<th>Denture wearer (Y/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>69</td>
<td>M</td>
<td>Psychiatric problem, hypertension</td>
<td>Lithium, risperidone</td>
<td>Felodipine, metoprolol</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>P2</td>
<td>51</td>
<td>M</td>
<td>Schizophrenia</td>
<td>Quetiapine, flupentixol (depilcor) injection</td>
<td>Nil</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>P3</td>
<td>47</td>
<td>M</td>
<td>Schizophrenia, thyroid problems</td>
<td>Clozapine</td>
<td>Benztropine, sodium valproate, zopiclone, citalopram, carbimazole</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>P4</td>
<td>50</td>
<td>M</td>
<td>Schizophrenia</td>
<td>Olanzapine</td>
<td>Nil</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>P5</td>
<td>54</td>
<td>M</td>
<td>Schizophrenia</td>
<td>Risperidone</td>
<td>Nil</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>P6</td>
<td>52</td>
<td>F</td>
<td>Psychiatric problem</td>
<td>Flupentixol, quetiapine</td>
<td>Benztropine, docusate, calcium, metoclopramide, multivitamin</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>P7</td>
<td>46</td>
<td>M</td>
<td>Schizophrenia</td>
<td>Clozapine, lithium, haloperidol</td>
<td>Sodium valproate</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>P8</td>
<td>64</td>
<td>F</td>
<td>Psychiatric problem, hypertension</td>
<td>Olanzapine</td>
<td>Citalopram, propranolol, simvastatin, carbamazepine</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>P9</td>
<td>58</td>
<td>M</td>
<td>Psychiatric problem</td>
<td>Quetiapine, buspirone</td>
<td>Lamotrigine, clonazepam, zopiclone, omeprazole</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

Table 3.1 General data for study participants
3.2 Socio-Demographic Characteristics

The majority of the participants in the study group were males (7 of 9, 78%). Their age ranged from 47 to 64 years with most (56%) belonging to the age group of 50 to 59 years old. Table 3.2 summarises the age group and gender of the participants. The oldest participant in this study was aged 69 and the youngest was aged 46 years at the time of recruitment. The ethnicity of all the participants was European.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age group (years)/number of participants</th>
<th>Total (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20-29</td>
<td>30-39</td>
</tr>
<tr>
<td>Male</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Female</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3.2 Age and gender of participants

The participants were age-matched as closely as possible with individuals from the control groups (Figure 3.1). The age difference between the study participants and the control groups ranged from 0 to 12 years. This was accepted to avoid large age differences between all the groups. However, the gender for the study participants and the control groups could not be matched because the majority of the control participants were female (90%). Xerostomia is more commonly reported in women, with an average of 8% higher prevalence than men (Orellana et al., 2006).
3.3 General Medical Conditions

The dental files of the participants were obtained from the records office of the Faculty of Dentistry, University of Otago. Their updated medical conditions and list of current medications were recorded. Participants or carers were advised to bring along an updated list of medications when they came for data collection. Table 3.3 summarises the medical conditions of the recruited participants. Most of the participants suffered from schizophrenia (55.6%). Some participants suffered from other psychiatric problems that were not specified in their dental files. Two participants reported hypertension along with psychiatric problems. One participant suffered from depression, anxiety and eating disorders (bulimia and anorexia). Of the seven male participants, five (71.4%) had schizophrenia, whilst the other two had unspecified psychiatric problems. One participant reported having thyroid problems.
### Medical conditions

<table>
<thead>
<tr>
<th>Medical conditions</th>
<th>Number of participants (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schizophrenia</td>
<td>4</td>
</tr>
<tr>
<td>Schizophrenia and hyperthyroidism</td>
<td>1</td>
</tr>
<tr>
<td>Depression, anxiety, bulimia and anorexia</td>
<td>1</td>
</tr>
<tr>
<td>Psychiatric problem (not specified)</td>
<td>1</td>
</tr>
<tr>
<td>Psychiatric problem (not specified) and hypertension</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 3.3 Summary of participants’ medical conditions

### 3.4 Medications

Most participants were on a combination of medications. It was very uncommon for participants to be taking antipsychotic medications exclusively as their regular medications. Only three (33%) of the participants were exclusively taking antipsychotic medications, namely quetiapine, olanzapine, risperidone and flupentixol (depixol) injection as their regular medications. One (11%) of the participants was on a combined therapy of quetiapine and flupentixol injection. Another participant was on a combined therapy of clozapine, lithium, haloperidol and sodium valproate (epilim). Sodium valproate is an anticonvulsant, frequently used as an adjunct for the treatment of psychiatric disorders as a mood stabiliser. Other anticonvulsants that were prescribed to the participants were lamotrigine, clonazepam and carbamazepine (tegretol). Three (33%) of the participants had a combined therapy of typical antipsychotic medications and atypical antipsychotic medications. Figure 3.2 displays the antipsychotic medications taken by all the participants.
Chapter 3: Study Participants

Two participants (22%) were on antipsychotic therapy combined with beta-blockers (metoprolol and propranolol) and statins (simvastatin) for their hypertension. One participant was also prescribed a calcium-channel blocker (felodipine) for the treatment of hypertension. Two (22.2%) of the participants were taking citalopram, a selective serotonin reuptake inhibitor antidepressant, along with their antipsychotic medications. An anticholinergic agent, benztropine was also added into the medications list for another two (22%) of the participants to reduce the side effects of antipsychotic medications. Hypnotic agents such as zopiclone were prescribed for two (22%) of the participants. One of the participants was taking carbimazole for the treatment of his thyroid problems. Other medications included docusate (laxative), metoclopramide (anti-emetic) and omeprazole (proton-pump inhibitor).

A large number of medications, including antipsychotic medications, may cause dry mouth as a side effect. Several participants in this study were found to be taking xerogenic medications as their regular medications. Table 3.4 shows the group of xerogenic medications and the number of participants taking those medications.
Chapter 3: Study Participants

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Drug group</th>
<th>Number of participants (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metoprolol, propranolol</td>
<td>Beta-blocker</td>
<td>2 (22)</td>
</tr>
<tr>
<td>Citalopram</td>
<td>Selective serotonin reuptake inhibitor (SSRI)</td>
<td>1 (11)</td>
</tr>
<tr>
<td>Zopiclone</td>
<td>Hypnotic</td>
<td>2 (22)</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>Proton-pump inhibitor</td>
<td>1 (11)</td>
</tr>
</tbody>
</table>

Table 3.4 Other medications associated with dry mouth being taken by participants (n = 9)

The medical conditions of the xerostomia control group included cardiovascular diseases and autoimmune connective tissue diseases. Participants with self-reported Sjogren’s syndrome were not included in the control group for this study. Some of the healthy control group from the Hou study were taking medications including tricyclic antidepressant, antihypertensive, anticholinergic, medication for arthritis, cholesterol lowering medication, cardio medication, diuretics and antihistamines. However, the medications being taken by particular control subjects were not specified (Hou, 2012), thus the medication status of the matched control group for this study is unknown.

3.5 Smoking status

The current smoking status of the participants was recorded after they were recruited. Three (33%) of the participants were current smokers and the rest were non-smokers. No ex-smokers or quitters were identified during the data collection. All three smokers were males, with two of them diagnosed with schizophrenia.

3.6 Denture status

The denture status of the participants was also recorded during recruitment into this study. The majority of the participants (67%) were non-denture wearers. Two of the participants had complete upper and lower dentures and one of them had an upper partial denture. Two of the denture wearers were male participants (aged 46 and 69 years old) while the female participant was 64 years old.
3.7 Xerostomia Inventory (XI)

The Xerostomia Inventory (XI) was used in this study to evaluate the participants’ subjective feeling of dry mouth. The highest total XI score obtained from participants in this study was 32. This was obtained from a 52-year old female participant (P6). This participant was on multiple medications including flupentixol, quetiapine, benztropine, calcium, docusate, multivitamins and metoclopramide. The lowest total score was 14, provided by two participants (P2 and P4), both male, belonged to the age group 50-59. One of them (P2) was on quetiapine and flupentixol injection while the other one (P4) was on olanzapine alone. Both were non-denture wearers, but one of them was a smoker (P4). Table 3.5 summarises the answering pattern of participants based on the four answering options (“never”, “occasionally”, “frequently” and “always”). Numbers in the answering options column indicate the number of participants that had chosen that answer. The highest number of participants choosing the same answer option was six (67%), where they occasionally sip liquids to aid in swallowing food and felt their lips were dry. This shows that most participants respond to the feeling of dry mouth by sipping liquids to help them swallow food. Interestingly, all the participants felt their mouth were dry with different frequency level between each participant, with most of them (55.6%) answering “frequently”.
Chapter 3: Study Participants

<table>
<thead>
<tr>
<th>Items in XI</th>
<th>Never</th>
<th>Occasionally</th>
<th>Frequently</th>
<th>Always</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>How often does your mouth feel dry?</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>My mouth feels dry</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>I have difficulty in eating dry food</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>I get up at night to drink</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>My mouth feels dry when eating a meal</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>I sip liquids to aid in swallowing food</td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>I suck sweets or cough lollies to relieve dry mouth</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>I have difficulties in swallowing certain foods</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>The skin of my face feels dry</td>
<td>5</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>My eyes feel dry</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>My lips feel dry</td>
<td>2</td>
<td>6</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>The inside of my nose feels dry</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3.5 Number of participants with their answer options (n=9)

Five participants (56%) who “frequently” found that their mouth was dry were on a combination of medications. One participant (P6) always felt that her eyes, lips and the inside of her nose were dry. This participant scored the highest total in XI score in this study. About half of the participants never sucked sweets or cough lollies to relieve their dry mouth, had difficulty in swallowing certain foods or felt the skin of their face and eyes were dry. Two of the participants (P1 and P9) answered “always” sucking sweets or cough lollies to relieve dry mouth, which might contribute to teeth loss (one of them wore complete upper and lower dentures). This participant (P1) was also a smoker, whose medications were lithium, risperidone, simvastatin and metoprolol. Figure 3.3 illustrates the mean XI score based on the frequency of dry mouth for the main question “How often does your mouth feel dry?”
3.8 Clinical Examination

All participants had a clinical examination by the candidate after recruitment into this study. A standard sterile mouth mirror and dental operating light were used in every examination. Any clinical evidence of dry mouth and oral *Candida* infection were recorded.

3.8.1 Evidence of dry mouth

Evidence of dry mouth such as lack of saliva pooling, depapillated tongue, mouth mirror sticking to the buccal mucosa and frothy saliva was recorded for each participant. Figure 3.4 shows an example of lack of saliva pooling from one of the participants after they had completed the XI questionnaire and an interval of 10 minutes.
Chapter 3: Study Participants

Figure 3.4 Lack of saliva pooling in one of the participants

Figure 3.5 summarises the number of participants with clinical evidence of dry mouth. Only two (22%) of the participants (P2 and P5) had no clinical evidence of dry mouth. Almost all (78%) of the participants showed lack of saliva pooling during the clinical examination. Five (56%) had a combination of two or more signs of dry mouth during the clinical examination.

Figure 3.5 Number of participants (n = 9) with clinical evidence of dry mouth (FS – frothy saliva, DT – depapillated tongue, SP – lack of saliva pooling, MM – mouth mirror sticks to the buccal mucosa, COMB – combination of 2 or more evidence and NONE – no clinical evidence)
One of the two participants who had no evidence of dry mouth was taking quetiapine and flupentixol injection (P2) and the other one was taking risperidone only (P5). Table 3.6 lists the medications for each participant with their XI scores and clinical findings.

### 3.8.2 Evidence of oral candidal infection

Only one (11%) of the participants had clinical evidence of oral candidal infection. This was confirmed by wiping off whitish plaques on the participant’s hard palate and right buccal mucosa using a piece of sterile gauze, leaving a raw area on the mucosa. The participant (P1) was a 69-year old man wearing complete upper and lower dentures constantly without removing them at night. The other participants did not show any signs of oral *Candida* infection during the examination.
Table 3.6 Individuals’ Xerostomia Inventory (XI) scores and their clinical presentations.

<table>
<thead>
<tr>
<th>Participant ID</th>
<th>Antipsychotic medication</th>
<th>Other medications</th>
<th>XI score</th>
<th>Clinical evidence of dry mouth</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Lithium, risperidone</td>
<td>Felodipine, metoprolol</td>
<td>22</td>
<td>✓    ✓   ✓</td>
</tr>
<tr>
<td>P2</td>
<td>Quetiapine, flupentixol injection</td>
<td>Nil</td>
<td>14</td>
<td>✓</td>
</tr>
<tr>
<td>P3</td>
<td>Clozapine</td>
<td>Benztrapine, sodium valproate, zopiclone, citalopram, carbimazole</td>
<td>26</td>
<td>✓    ✓</td>
</tr>
<tr>
<td>P4</td>
<td>Olanzapine</td>
<td>Nil</td>
<td>14</td>
<td>✓</td>
</tr>
<tr>
<td>P5</td>
<td>Risperidone</td>
<td>Nil</td>
<td>18</td>
<td>✓</td>
</tr>
<tr>
<td>P6</td>
<td>Flupentixol, quetiapine</td>
<td>Benztrapine, docusate, calcium, metoclopramide, multivitamin</td>
<td>32</td>
<td>✓    ✓</td>
</tr>
<tr>
<td>P7</td>
<td>Clozapine, lithium, haloperidol</td>
<td>Sodium valproate</td>
<td>15</td>
<td>✓    ✓</td>
</tr>
<tr>
<td>P8</td>
<td>Olanzapine</td>
<td>Citalopram, propranolol, simvastatin, carbamazepine</td>
<td>22</td>
<td>✓</td>
</tr>
<tr>
<td>P9</td>
<td>Quetiapine, buspirone</td>
<td>Lamotrigine, clonazepam, zopiclone, omeprazole</td>
<td>26</td>
<td>✓    ✓   ✓</td>
</tr>
</tbody>
</table>
3.9 Cytological Examination

Three (33%) of the participants showed PAS-positive *Candida* hyphae on all the smears that were taken as part of this study. One participant (P5) had negative results on both buccal mucosal smears but had occasional PAS positive *Candida* hyphae on the tongue smear. One participant (P9) showed positive PAS staining of *Candida* hyphae only on the tongue smear but not the smears from the right and left buccal mucosa. Four (44%) had negative PAS staining of the candidal hyphae. Two (P1 and P7) of the four participants diagnosed with oral candidosis by the Medlab Dental laboratory had upper dentures, either complete or partial. Figure 3.7 illustrates the PAS staining of *Candida* hyphae results for each participant. Participant P9 was not diagnosed with oral candidosis due to the absence of *Candida* hyphae in the smears. Participant P1 underwent a palatal smear due to the clinical evidence of oral *Candida* infection. This participant had a complete upper and lower denture.

Figure 3.6 Example of positive PAS staining of Candida hyphae at 10x magnification (left) and 40x magnification (right).
Chapter 3: Study Participants

3.10 Conclusions

This part of the investigation identified the sociodemographic characteristics of the participants in the present study and their medical background. The link between antipsychotic drug intake and the presence of dry mouth could be established especially when most of the participants (78%) presented with dry mouth. However, the condition of the dry mouth symptoms they experienced were not severe based on the XI scores they obtained. Most participants (6 of 9) felt that their mouth was dry, but the condition was not too severe for them to notice or to induce them to modify their behaviour towards dry mouth such as drinking water to aid in swallowing. The presence of oral candidosis in four of the participants could also be associated with the antipsychotic drug intake, although not directly because only one of these participants was exclusively taking an antipsychotic drug (risperidone). The other participants were on a combination of medications for their underlying medical condition.
CHAPTER 4:

MICROBIOLOGICAL ANALYSIS
CHAPTER 4: MICROBIOLOGICAL ANALYSIS

4.1 Introduction

*Candida* species are normally present in the oral cavity (Cannon and Chaffin, 1999) with *Candida albicans* being the most prevalent species (Cannon et al., 1995). It has been found that a decrease in salivary flow rate correlates with an increase in the number of *Candida* cells in the oral cavity (Torres et al., 2002). It is also known that xerostomia is a side effect of many antipsychotic medications. This part of the study was specifically designed to determine not only the species that are present in the oral cavity of individuals taking antipsychotic medications, but also the level of colonisation by *Candida* species and their susceptibility to antifungal medications. The level of colonisation of the oral mucosa with *Candida* in people taking antipsychotic medications will be compared to that in xerostomic patients and their healthy controls not prescribed with antipsychotics. These individuals may be taking medications with xerostomic potential or have systemic conditions resulting in xerostomia. Furthermore, the fluconazole susceptibility of the *C. albicans* strains isolated from the patients taking antipsychotic medications will be measured and the effect of fluphenazine (one of the older, highly potent first-generation piperazine class of phenothiazine antipsychotic medications, in the same class with perphenazine and prochlorperazine) on this susceptibility will be determined. *In vitro* studies have shown that fluphenazine is capable of inducing expression of ATP-binding cassette (ABC) efflux pumps, such as Cdr1p, in *C. albicans* cells (de Micheli et al., 2002; Coste et al., 2004; Manoharlal et al., 2011). This pump reduces the intracellular azole concentration in *C. albicans* cells grown in the presence of azoles resulting in resistance to azoles' growth inhibitory effect. ABC efflux pump activity plays a major role in clinical azole antifungal resistance (Cannon et al., 2009).
4.2 Candida Colonisation

The microbiological analysis of participants’ samples was carried out as described in Section 2.5.2 in the “Materials and Methods” chapter. Colonies growing on the CHROMagar™ Candida plates were presumptively identified, counted, and recorded as colony-forming units per mL saliva rinse (CFU/mL). Table 4.1 compares the colonisation levels of the different Candida species identified. In the present study, four different colony types were identified, and most participants (6 of 9) were colonised with C. albicans. The table shows that the lowest concentration of C. albicans was 9 CFU/mL and the highest was 3979 CFU/mL. The highest mean level of colonisation for all participants was by C. albicans (517 CFU/mL) and the lowest was by C. tropicalis with a mean value of 21.3 CFU/mL.

<table>
<thead>
<tr>
<th>Species present (colour on CHROMagar)</th>
<th>No. of positive participants</th>
<th>Range of colonisation (CFU/mL)</th>
<th>Mean level of colonisation in colonised participants (CFU/mL)</th>
<th>Mean level of colonisation in all participants (CFU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans (green)</td>
<td>6</td>
<td>9 - 3979</td>
<td>776</td>
<td>517</td>
</tr>
<tr>
<td>C. tropicalis (purple/blue)</td>
<td>1</td>
<td>0 - 192</td>
<td>192</td>
<td>21</td>
</tr>
<tr>
<td>C. krusei (pink)</td>
<td>2</td>
<td>1 - 596</td>
<td>299</td>
<td>261</td>
</tr>
<tr>
<td>Unidentified (white)</td>
<td>1</td>
<td>0 - 3200</td>
<td>3200</td>
<td>356</td>
</tr>
</tbody>
</table>

Table 4.1 Presence of Candida species in participants’ saliva rinses

The colonisation of individual participants is shown in Figure 4.1. Seven out of nine participants (77.8%) were yeast-positive and for two participants no yeasts were detected in their saliva rinse samples. Samples from four participants contained a single colony type, two contained two-colony types and one contained three-colony types. Colony type (presumptive species) details are summarised in the Table 4.1. Six out of seven positive saliva rinse samples (85.7%) had colonies presumptively identified as C. albicans and three participants (33.3% of all participants; 43% of yeast-positive participants) contained only C. albicans (green colonies) in their saliva rinse. The highest number of colony types was obtained from participant P9, where three types of colonies were identified including C. albicans, C. krusei (pink colonies)
and other species (white colonies). Three participants had \( C. \text{ krusei} \) in their saliva rinse samples, with participant P9 having the highest concentration. Only one participant (P6) grew \( C. \text{ tropicalis} \) from the saliva rinse. This participant also had the highest \( C. \text{ albicans} \) CFU/mL when compared to the other participants.

![Figure 4.1 Colonisation of individual participants by different yeast species (CFU/mL saliva rinse)](image)

Table 4.2 compares the oral candidosis diagnosis from the cytological study (Section 3.9) with the concentration of yeast in the saliva rinse. Four participants had a diagnosis of oral candidosis and all four were colonised with yeasts. Participant P6 presented with the highest colonisation by \( \text{Candida} \) species and was a 52 year-old female on multiple medications including flupentixol, quetiapine, benztropine, docusate, metoclopramide, calcium and multivitamin. All three of her oral smears gave positive PAS staining resulting in a clinical diagnosis of oral candidosis. Interestingly, participant P9 who had the second highest concentration of \( C. \text{ albicans} \) (561 CFU/mL) and \( C. \text{ krusei} \) (1755 CFU/mL) in his saliva rinse, did not have PAS positive smears except from the tongue. He had not been diagnosed with oral candidosis. The risk of oral candidosis has been reported to be higher with denture or prosthesis wearing, hyposalivation or dry mouth and in elderly individuals (Campisi et al., 2007). The colonisation of participant P1 is in accord with this observation; P1
was a 69 year-old man, wearing complete upper and lower dentures with clinical evidence of dry mouth and oral candidosis. Three of the four participants with a clinical diagnosis of oral candidosis presented with the clinical indication of dry mouth. Participants P2 and P6 were taking flupentixol, a typical antipsychotic drug in the thioxanthene class, which is closely related chemically to the phenothiazine class of antipsychotics which includes fluphenazine. Interestingly, participant P6 had the highest level of colonisation compared to other participants.

<table>
<thead>
<tr>
<th>Participant ID</th>
<th>Oral candidosis diagnosis (Yes/No)</th>
<th>Total CFU/mL</th>
<th>Denture wearer (Yes/No)</th>
<th>Clinical presentation of dry mouth (Yes/No)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Yes</td>
<td>48</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>P2</td>
<td>No</td>
<td>0</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>P3</td>
<td>No</td>
<td>0</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>P4</td>
<td>No</td>
<td>10</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>P5</td>
<td>Yes</td>
<td>9</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>P6</td>
<td>Yes</td>
<td>4171</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>P7</td>
<td>Yes</td>
<td>642</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>P8</td>
<td>No</td>
<td>1</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>P9</td>
<td>No</td>
<td>2316</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Table 4.2 Comparison of clinical oral candidosis diagnosis with concentration of yeasts in saliva rinses. Results highlighted in blue are for participants with a clinical diagnosis of oral candidosis.

The findings from the present study were compared to those obtained in a previous study for two other groups: individuals with xerostomia (n = 9) and age- and gender-matched controls without xerostomia and not on antipsychotic medications (n = 9) (Hou, 2012). Control participants from the Hou study were age-matched with the current study participants, but gender-matching was not possible because the Hou study had mostly female participants (90%). The yeast species present in the saliva rinses from healthy controls were *C. albicans* (1 of 9) and *C. glabrata* (1 of 9). The mean concentration of *C. albicans* in participants’ saliva rinses from the present study was slightly less than that for the xerostomia participants in the Hou study not taking
antipsychotic medications (Figure 4.2). The data obtained showed a skewed distribution, thus the statistical analysis comparing the mean concentration of *C. albicans* in the saliva rinses was done using the Kruskal-Wallis test. The test revealed a statistically significant difference (H = 10.38, df = 2, p<0.05) between the three groups. An individual comparison was done between the participants and healthy control using the Mann-Whitney U test that indicated a statistically significant difference (p<0.05). However, a Mann-Whitney U test between the participants and xerostomia subjects did not show statistical significance (p>0.05). This is probably due to the similarity of the conditions between the two groups, which both displayed xerostomia.

Figure 4.2 Mean concentrations (CFU/mL) of *C. albicans* in saliva rinse samples from study participants, individuals with xerostomia (without antipsychotics) and healthy controls.
4.3 Antifungal Susceptibility Testing

4.3.1 Agar Diffusion Susceptibility Assay (Etest®)

Glycerol stocks of yeast isolates were prepared from four colonies for each of the saliva rinse samples from yeast-positive participants. When a saliva rinse contains two species of *Candida*, two colonies from each species were inoculated. Six participants were *C. albicans* positive and two isolates from each of these samples (twelve isolates) were analysed for antifungal susceptibility. They were labeled W1a, W1b, W4a, W4b, through to W9a and W9b based on the origin of the isolates (the number refers to the *C. albicans*-positive participant).

The agar diffusion susceptibility assay using the Etest® method was described in Section 2.5.3.1. This method was used for the preliminary determination of the susceptibility of *C. albicans* isolates obtained in this study to fluconazole. Application of the Etest® strips containing fluconazole resulted in elliptical zones of growth inhibition that crossed the graduated strip, and the MIC was recorded as the concentration value at which the ellipse crossed the scale on the strip (Figure 4.3). This figure shows representative images of two types of growth pattern. For some isolates (e.g. W7b) a clear elliptical inhibition zone was observed (Figure 4.3; b and d) in this example giving an MIC of 0.125 µg/mL. For other isolates (e.g. W1a) the inner zone showed partial inhibition of growth, known as trailing growth (Lee et al., 2004), which, however, is not reflected in clinical resistance of strains showing this effect (Pfaller et al., 1998). In such examples, the MIC was taken as the point at which the inner zone of lesser growth crossed the strip (0.38 µg/mL for isolate W1a) as shown in Figure 4.3 (a and c).
Figure 4.3. Representative Etest® results for two apparently fluconazole-sensitive isolates. Examples shown are for the isolates W1a (a and c); and W7a (b and d). In the images (c) and (d), the green lines trace the elliptical zones of inhibition to indicate the MIC values (0.38 µg/mL for W1a; 0.125 µg/mL for W7a).

The Etest® results for the twelve *C. albicans* isolates obtained from saliva rinse samples of the six *C. albicans*-positive participants are given in the Table 4.3. A low MIC value indicates high susceptibility to fluconazole, for example isolate W1b was highly susceptible to fluconazole because it had the lowest MIC value (0.064 µg/mL) compared to the other isolates. In contrast, the isolates W4a, W4b, W6a, and W6b showed apparently uniform growth over the whole plate containing the Etest® strip, giving apparent MIC values greater than 256 µg/mL (Figure 4.4 shows the Etest® plate results for isolates W4a and W4b). Therefore, based on the use of this method, these isolates were apparently resistant to fluconazole. The interpretive susceptibility breakpoints for fluconazole and *Candida* spp. are considered to be as follows: sensitive: MIC ≤ 8 µg/mL; resistant: MIC ≥ 64 µg/mL (Pfaller et al., 2006). Interestingly, two pairs of the apparently resistant isolates were from the same participants (W4a and W4b; W6a and W6b) and showed similar patterns of growth in
the Etest®. In conclusion, seven (58.33%) of the twelve \textit{C. albicans} isolates tested were found to be susceptible to fluconazole using the Etest® method.

![Etest® plates](image)

Figure 4.4 Etest® plates for two apparently fluconazole-resistant isolates (W4a and W4b) showing MIC values of $>256 \mu g/mL$.

<table>
<thead>
<tr>
<th>Participant ID</th>
<th>\textit{C. albicans} isolate</th>
<th>Apparent MIC ($\mu g/mL$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>W1a</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>W1b</td>
<td>0.064</td>
</tr>
<tr>
<td>P4</td>
<td>W4a</td>
<td>$&gt;256$</td>
</tr>
<tr>
<td></td>
<td>W4b</td>
<td>$&gt;256$</td>
</tr>
<tr>
<td>P5</td>
<td>W5a</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>W5b</td>
<td>0.5</td>
</tr>
<tr>
<td>P6</td>
<td>W6a</td>
<td>$&gt;256$</td>
</tr>
<tr>
<td></td>
<td>W6b</td>
<td>$&gt;256$</td>
</tr>
<tr>
<td>P7</td>
<td>W7a</td>
<td>0.125</td>
</tr>
<tr>
<td></td>
<td>W7b</td>
<td>0.19</td>
</tr>
<tr>
<td>P9</td>
<td>W9a</td>
<td>$&gt;256$</td>
</tr>
<tr>
<td></td>
<td>W9b</td>
<td>0.125</td>
</tr>
</tbody>
</table>

Table 4.3 Apparent fluconazole susceptibilities of \textit{C. albicans} isolates determined by the Etest® method.

4.3.2 Liquid MIC Susceptibility Assay (Fluconazole)

The liquid microdilution method for measuring the susceptibility of \textit{C. albicans} to fluconazole was described in Section 2.5.3.2. The minimum inhibitory concentration (MIC$_{80}$) was the lowest concentration of fluconazole that resulted in at least 80%
growth inhibition of \textit{C. albicans}. The readings were taken at 24 h and 48 h, but values at 24 h were more reliable. If the amount of growth after 24 h was plotted as a proportion of growth in the no-drug control versus the fluconazole concentration, it can be seen that at higher drug concentrations, growth was below 20% of the control (Figure 4.5)

![Figure 4.5 Growth inhibition of C. albicans by fluconazole (t = 24 h). Typical growth inhibition curves for four C. albicans isolates (W4a, W4b, W7a, and W9a) from which MIC values were calculated.](image)

The growth inhibition pattern for fluconazole typically shows ‘trailing growth’ whereby a certain amount of growth persists even at high fluconazole concentrations (eg isolates W4b and W7a, Figure 4.5). This is why a cut-off of 80% growth inhibition (rather than 100% growth inhibition) was used to measure fluconazole MIC values. Only isolate W9a demonstrated total growth inhibition - at a fluconazole concentration of 0.5 µg/mL. The liquid MIC values for all \textit{C. albicans} isolates were determined (Figure 4.6).
A majority (66.7%) of the isolates had a fluconazole MIC$_{80}$ value of 0.5 µg/mL (Figure 4.6). Three of the isolates had a fluconazole MIC$_{80}$ value of 1 µg/mL. One of the isolates had a higher fluconazole MIC$_{80}$ value of 2 µg/mL. The breakpoint for fluconazole resistance as determined by liquid MIC is 64 µg/mL (Pfaller et al., 2006; Pfaller et al., 2010a) and so by this method, all the isolates were fluconazole sensitive.

4.3.3 Liquid MIC Susceptibility Assay (Fluphenazine)

The standard liquid MIC technique (Section 2.5.3.3) was also used to determine the fluphenazine MIC values for the *C. albicans* isolates. The results were recorded at 24 h and 48 h - the values at 48 h were found to be more reliable. Figure 4.7 shows an example of the growth pattern for the isolate W9a after 48 h. The fluphenazine MIC value for this isolate was 100 µg/mL. Unlike fluconazole, fluphenazine achieved a clear 100% growth inhibition at the MIC.
Figure 4.7 Growth inhibition of *C. albicans* isolate W9a by fluphenazine.

The fluphenazine MIC values for all the *C. albicans* isolates used in this study, taken at 48 h are shown in Figure 4.8. Fluphenazine does not have a strong antifungal activity. The majority of the isolates (75%) had the same fluphenazine MIC value of 50 µg/mL. Three of the isolates had a higher fluphenazine MIC value (100 µg/mL). The MIC value of 100 µg/mL was used as a reference for fluphenazine for conducting the checkerboard susceptibility experiments described in Section 2.5.3.6.

Figure 4.8 Fluphenazine MIC values (µg/mL) for *C. albicans* isolates after 48 h.
4.3.4 Agar Diffusion Synergism/Antagonism Assay For Fluconazole And Fluphenazine

The technique for measuring drug synergy/antagonism using the disc diffusion assay is described in Section 2.5.3.5. Figure 4.9 (showing plates inoculated with isolates W6a and W9b) displays two examples of the disc diffusion assay results obtained. The zones of growth inhibition around the fluconazole disc are flattened opposite the fluphenazine discs, and yeast growth is observed between the two discs, indicating an antagonistic interaction between fluconazole and fluphenazine. Similar results were observed for five other isolates, and no example of synergy (coalescing zones of inhibition) was observed for any isolate. Note that the zones of growth inhibition around the fluphenazine discs are small, because this compound has little antifungal activity, yet fluphenazine is clearly diffusing some distance from the fluphenazine discs in order to reduce the effect of fluconazole on growth of the yeast. For some isolates, antagonism was also observed between fluconazole and amphotericin B (see Figure 4.9, isolate W6a).

Figure 4.9 Disc diffusion analysis of drug synergy/antagonism. Isolate W6a (left), and isolate W9b (right). (FLC = fluconazole, FLUP = fluphenazine and AMP B = amphotericin B)
4.3.5 Checkerboard Liquid MIC Synergism/Antagonism Assay

The checkerboard liquid MIC susceptibility assay method was carried out as described in Section 2.5.3.6. This test was undertaken to investigate in more detail whether fluphenazine induces fluconazole resistance in *C. albicans* because fluphenazine is known to cause upregulation of drug efflux pump genes *CDR1* and *CDR2* mRNA expression by up to 500 fold (de Micheli et al., 2002; Zhu et al., 2014). An increase in fluconazole resistance in the presence of fluphenazine would be seen as antagonism in a checkerboard assay, confirming the disk assay results described above (Section 4.3.4). The results presented use MIC absorbance readings taken after 24 h of incubation. Figures 4.10 to 4.12 show typical checkerboard results - for the isolates W1a, W5b and W9b. The columns at the left of each graph show the growth inhibition by fluconazole in the absence of fluphenazine; the top rows show the effects of fluphenazine alone on growth.

![Checkerboard growth inhibition results](image)

Figure 4.10 Checkerboard growth inhibition results for isolate W1a.
The data show that growth of each of the isolates was substantially reduced (>80%) in the presence of 1 µg/mL fluconazole alone (the lowest concentration of fluconazole tested), therefore the fluconazole MIC$_{80}$ values for each of these three isolates is <1.0 µg/mL under these conditions. However the growth inhibition by fluconazole was
Chapter 4: Microbiological Analysis

reduced in the presence of fluphenazine at concentrations between 0.35 – 3.125 µg/mL, with an increase in the fluconazole MIC₈₀ values to between 4.0 µg/mL (isolates W1a and W9b) and 8.0 µg/mL (isolate W5b). Thus the fluphenazine was antagonising the antifungal effect of fluconazole. In contrast, the MICs of fluphenazine for these isolates were unaffected by the presence of fluconazole. Under these conditions (24 h growth), the fluphenazine MICs in the presence and absence of fluconazole were equal at 3.125 µg/mL; a lower value than that observed at 48h (Figure 4.8), possibly reflecting incomplete growth of the cultures at the time of observation. However, the data demonstrate that in contrast to the effect of fluphenazine on the fluconazole MIC, fluconazole was not having an effect on the cells’ susceptibilities to fluphenazine.

The interactions between fluconazole and fluphenazine were assessed by calculating the fractional inhibitory concentrations (FICs) for each drug, that were then combined to give the fractional inhibitory concentration index (FICI) for these drug combinations. FICs and FICIs were calculated as follows:

\[
\text{FICI} = \text{FIC of fluconazole} + \text{FIC of fluphenazine}
\]

\[
\text{FIC of fluconazole} = \frac{\text{MIC value of fluconazole} + \text{fluphenazine}}{\text{MIC value of fluconazole only}}
\]

\[
\text{FIC of fluphenazine} = \frac{\text{MIC value of fluphenazine} + \text{fluconazole}}{\text{MIC value of fluphenazine only}}
\]

The FICI values can be used to classify the interactions between two medications as being either synergistic or antagonistic. An FICI value of $\leq 0.5$ indicates a synergistic interaction, while an FICI value of $> 4.0$ indicates an antagonistic interaction (Odds, 2003). A value between 0.5 and 4.0 indicates no significant interaction between the two medications.

Table 4.5 shows the FIC and FICI values for the twelve C. albicans isolates studied (two from each of the participants who had C. albicans in their saliva samples). Five isolates (W1a, 5a, 5b, 7b and 9b) had FICIs of $>4.0$ indicating significant antagonism.
(Odds 2003). Other FICI values were between 1.0 and 3.0 suggesting a trend towards antagonism between fluconazole and fluphenazine in all isolates.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>FLC MIC&lt;sub&gt;50&lt;/sub&gt; (µg/mL)</th>
<th>FLUP MIC (µg/mL)</th>
<th>FICI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alone Combined* FIC</td>
<td>Alone Combined* FIC</td>
<td></td>
</tr>
<tr>
<td>W1a</td>
<td>&lt;1.0 4.0 (1.5) &gt;4.0</td>
<td>3.125 3.125 (1.0) 1.0</td>
<td>5.0</td>
</tr>
<tr>
<td>W1b</td>
<td>2.0 4.0 (0.75) 2.0</td>
<td>&gt;100.0 3.12 (2.0) &lt;0.03</td>
<td>2.0</td>
</tr>
<tr>
<td>W4a</td>
<td>8.0 8.0 (0.37) 1.0</td>
<td>&gt;100.0 6.25 (1.0) &lt;0.06</td>
<td>1.0</td>
</tr>
<tr>
<td>W4b</td>
<td>2.0 4.0 (0.37) 2.0</td>
<td>12.5 12.5 (1.0) 1.0</td>
<td>3.0</td>
</tr>
<tr>
<td>W5a</td>
<td>&lt;1.0 4.0 (0.37) &gt;4.0</td>
<td>6.3 6.25 (1.0) 1.0</td>
<td>5.0</td>
</tr>
<tr>
<td>W5b</td>
<td>&lt;1.0 8.0 (0.75) &gt;8.0</td>
<td>6.3 6.25 (1.0) 1.0</td>
<td>5.0</td>
</tr>
<tr>
<td>W6a</td>
<td>&lt;1.0 2.0 (0.37) &gt;2.0</td>
<td>6.3 3.12 (1.0) 0.5</td>
<td>2.5</td>
</tr>
<tr>
<td>W6b</td>
<td>2.0 2.0 (0.37) 1.0</td>
<td>&gt;100.0 3.12 (1.0) &lt;0.03</td>
<td>1.0</td>
</tr>
<tr>
<td>W7a</td>
<td>2.0 4.0 (0.75) 2.0</td>
<td>3.12 3.12 (1.0) 1.0</td>
<td>3.0</td>
</tr>
<tr>
<td>W7b</td>
<td>&lt;1.0 8.0 (0.37) &gt;8.0</td>
<td>3.12 3.12 (1.0) 1.0</td>
<td>9.0</td>
</tr>
<tr>
<td>W9a</td>
<td>&lt;1.0 2.0 (0.37) &gt;2.0</td>
<td>12.5 12.5 (1.0) 1.0</td>
<td>3.0</td>
</tr>
<tr>
<td>W9b</td>
<td>&lt;1.0 4.0 (0.75) &gt;4.0</td>
<td>3.12 3.12 (1.0) 1.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Table 4.5 Fractional inhibitory concentration index (FICI) value for twelve C. albicans isolates. Results highlighted in green show significant antagonistic interactions.
* The combined MIC was measured in the presence of the other compound (FLUP or FLC) at the concentration (µg/ml) indicated in parentheses.
4.4 Conclusions

The microbiological analysis of saliva samples in this study found a significantly higher number of participants were colonised with *Candida* spp. (7 of 9) when compared to an age-matched group of healthy individuals (2 of 9). The numbers of yeast detected per participant was also significantly higher than for the control group (Figure 4.1). Although a direct correlation between antipsychotic medications intake and the level of colonisation by *Candida* spp. could not be established, increased colonisation may have been because antipsychotic medications are known to have a side effect of reduced saliva production (Zelickson and Rogers, 1986; Scully, 2003; Haddad and Sharma, 2007). Seven of nine participants in the current study presented with symptoms of dry mouth (Table 4.2). This is in agreement with previous studies demonstrating a correlation between high levels of *Candida* colonisation and xerostomia (Navazesh et al., 1995; Torres et al., 2002; Torres et al., 2003; Hou, 2012). Clozapine is thought to induce hypersalivation (Haddad and Sharma, 2007). The present study found that the participants who were taking clozapine presented with dry mouth (P3 and P7). However, they were also on other medications that may contribute to dry mouth. The most frequently identified *Candida* species (presumptive identification by colony colour) in the present study was *C. albicans* (Table 4.1); although three other colony types were detected which were presumptively identified as *C. tropicalis* (purple/blue), *C. krusei* (pink) and an unknown species (white). It has been reported that some *Candida glabrata* isolates produce white colonies on CHROMagar™ *Candida* (Bishop et al., 2008). Confirmation of the species of the white colony would require nutritional utilisation tests (ability to grow on various carbon and nitrogen sources) or deoxyribonucleic acid (DNA) sequence analysis. The observation that *C. albicans* was the most common species detected is in agreement with other reports regarding oral *Candida* species (Coronado-Castellote and Jiménez-Soriano, 2013). Although *Candida* colonisation was found in 7 of 9 participants, the prevalence of a clinical diagnosis of oral candidosis in the participants was lower (4 of 9); however this might be expected as *Candida* species are commensal organisms and therefore detection of *C. albicans* does not necessarily indicate disease (Mayer et al., 2013).
In vitro measurement of the susceptibility of yeasts to fluconazole can be undertaken following the standardised CLSI (formerly National Committee for Clinical Laboratory Standards (NCCLS)) guidelines using the spectrophotometric MIC broth microdilution procedure, agar based methods including disc diffusion susceptibility assay, and using the commercially available Etest® strips (Pfaller et al., 1998; Holmes et al., 2002; Pfaller et al., 2006). However, getting a reliable measure of susceptibility with the Etest® was difficult due to the presence of a large number of microcolonies within the zone of inhibitions. For example, when the Etest® was used to measure the C. albicans isolates’ susceptibilities to fluconazole, five (W4a, W4b, W6a, W6b and W9a) (Table 4.3) were apparently resistant to fluconazole. It has been reported that the Etest® can be unreliable, particularly when a trailing pattern of growth is present (Alp et al., 2010). In contrast, when a liquid microtitre assay was used, based on the "gold standard" CLSI methodology (Rex et al., 2008), all twelve isolates were susceptible.

The effect of antipsychotic medications on fluconazole susceptibility has not been well studied. However, as it was discovered in this study that participants on antipsychotic medication had increased prevalence of Candida colonisation it was important to test the effects of antipsychotic medications on C. albicans fluconazole susceptibility. Importantly, this study confirmed the earlier molecular studies on efflux pump gene expression and demonstrated that fluphenazine antagonized the effect of fluconazole on C. albicans clinical isolates. All the participants in this study were on a single or combination of antipsychotic medications. It is not known whether their drug particular drug regimes would have affected resistance to the most commonly used antifungal, fluconazole, in the yeast strains isolated from participants. The antipsychotic drug, fluphenazine was used in the present study because of its use in previous in vitro experiments (de Micheli et al., 2002; Coste et al., 2004; Manoharlal et al., 2011).

Fluphenazine was one of the first medications classed as ‘antipsychotic drug’ and was approved by the FDA in 1959. It is still widely used in the treatment of schizophrenia, particularly when it is the only antipsychotic medication available, in developing countries for example. Although it is still available in Britain and North America, it is being replaced by newer generation of antipsychotic medications.
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(Matar et al., 2013). The present study found that fluphenazine resulted in weak antifungal activity against \textit{C. albicans}; it only affected the yeast growth at high concentrations (≥ 50µg/mL) (Figure 4.11). However, when both medications were combined, fluphenazine was found to produce antagonistic effects towards fluconazole, both in disc diffusion assays (Figure 4.9) and the more quantitative checkerboard MIC assays (Figure 4.10 and Table 4.5). It remains to be determined whether the concentrations of medications achieved in the oral cavity would result in \textit{in vivo} antagonism. It will also be important to determine whether other types of antipsychotic medications demonstrate antagonism of fluconazole antifungal activity.

Although some of the participants were on flupentixol, a drug of a similar class to fluphenazine, none of the isolates from those participants showed any indication of having developed fluconazole resistance. This is probably because the effect of fluphenazine is transient – causing up-regulation of the efflux pumps – and susceptibility to fluconazole may return to normal in the absence of fluphenazine. It would have been interesting to know whether any of the participants had ever been treated with both fluconazole and fluphenazine, and if so whether the candidosis had responded to treatment. The discovery that antagonism between fluphenazine and fluconazole occurs with \textit{C. albicans} clinical isolates indicates that careful consideration may be necessary when prescribing fluconazole to patients currently taking fluphenazine or medications of a similar class. Other antifungal agents such as nystatin lozenges (not readily available in New Zealand) or amphotericin B lozenges might be better for individuals on such antipsychotic medications.
CHAPTER 5:

DISCUSSION
CHAPTER 5: DISCUSSION

5.1 Overview

The present study was focused on the oral health of participants who were taking antipsychotic medications. This interest was due to the paucity of investigations of mucosal fungal infections in these individuals. The goals for the present study were to determine the level of colonisation of Candida spp. in the oral cavity of individuals taking antipsychotic medications when compared to healthy controls and xerostomic patients who were not taking antipsychotic medication. The Candida species that were present were identified and finally the antifungal susceptibility of the C. albicans detected was determined. In this chapter, the topics that are going to be discussed are the characteristics of participants in this study, the effects of antipsychotic medications on the oral environment, Candida colonisation and oral candidosis in individuals taking antipsychotic medications and the azole resistance of C. albicans in those individuals. This study has limitations that will also be discussed further in this chapter. At the end of the chapter, some recommendations will also be mentioned.

5.2 Characteristics of Participants

5.2.1 Socio-demographic characteristics of participants

Gender differences in the prevalence of mental health illnesses vary across age groups (Afifi, 2007). The majority of the participants in this study were males (78%). This was consistent with the commonly accepted distribution of schizophrenia between sexes, where a systematic review has shown men to have a higher incidence of schizophrenia than women (male:female ratio of 1.4: 1) (McGrath and Susser, 2009). Schizophrenia is one of the most severe psychiatric disorders with an estimated prevalence of 0.7% to 1.0% of the worldwide population (Santos et al., 2012). It is a mental disorder of thought, disorganised behaviour and can cause hallucinations,
Chapter 5: Discussion

delusions and illusions including cognitive impairment. It belongs to the psychosis group of mental disorders, along with bipolar disorder and mania.

5.2.2 General Medical Conditions

Most of the participants in the present study suffered from schizophrenia (55.6%). The other medical conditions that presented along with their psychiatric problem were hypertension, depression, anxiety, eating disorders (bulimia and anorexia) and thyroid problems. The presence of thyroid pathology is interesting since some studies reported antipsychotic medications and/or schizophrenia may cause imbalance in thyroid hormones levels (Santos et al., 2012). Hypothyroidism may cause negative symptoms (depressive symptoms) in schizophrenia and hyperthyroidism may cause positive symptoms (psychosis) (Santos et al., 2012). Long-term antipsychotic medications may elevate prolactin production via blocking dopamine receptors in the anterior pituitary thus inducing thyroid autoantibodies as well as reducing sex hormones causing sexual dysfunction and possibly accelerating osteoporosis in women (O’Keane, 2008).

5.2.3 Medications

The majority of participants were on a combination of medications (6 of 9, 67%) including antipsychotic medications and prescriptions for their underlying medical illnesses. According to a literature review study, there were 16 treatment recommendations in total available for the management of schizophrenia (Buchanan et al., 2010). There were 11 revised treatment recommendations and 5 new treatment recommendations, grouped either by intervention or outcome. The treatment recommendations were based on treatment for acute schizophrenia, maintenance therapy, treatment for residual symptoms, prophylactic antiparkinson treatment, treatment for acute agitation and medications for smoking cessation. They found that the practice of polypharmacy in the treatment of schizophrenia was common since many patients will also have symptoms of depression (Buchanan et al., 2010). Further, many people had failed to respond adequately to previous single-item antipsychotic treatment. Although there was no strong evidence of the efficacy of combination therapy, multiple medications were prescribed to address the issue of
persistent psychopathological symptoms or other symptoms such as anxiety and cognitive impairment and also for the treatment of medical disorders for example antipsychotic-induced prolactin elevation or hormonal disturbances. For example, the use of anticonvulsants for the treatment of treatment-resistant positive and negative symptoms, benzodiazepines for anxiety, antidepressants for depression and management of antipsychotic-related side effects such as tardive dyskinesia. Sodium valproate is an anticonvulsant, frequently used as an adjunct in the treatment of psychiatric disorders as a mood stabiliser. Other anticonvulsants that may be prescribed to patients are lamotrigine, clonazepam and carbamazepine (tegretol). Anticholinergic agents, such as benztropine or hypnotic agent such as zopiclone may also be added into the medications list to reduce the side effects of antipsychotic medications. Other medications that were prescribed to participants in the current study include laxatives, anti-emetics, anti-thyroid medication and proton-pump inhibitors.

5.2.4 Smoking status

In the present study, three participants were tobacco smokers. They were all males, with two of them diagnosed with schizophrenia. In other studies it was found that the prevalence of cigarette smokers was higher in schizophrenia patients when compared to the overall population and people with other psychiatric disorders (de Leon, 1996; de Leon and Diaz, 2005). The data in the current study did not show a high number of smokers probably due to limiting the category to smokers and non-smokers. The data may show differences if quitters were identified in this study. Smoking could be considered to be a form of self-medication for psychiatric patients since nicotine reduces the side effects of antipsychotic medications and helps eliminate cognitive impairment associated with schizophrenia (Kumari and Postma, 2005).

5.2.5 Denture status

The presence of dentures in the oral cavity may affect the oral Candida carriage. It has been shown that patients taking long-term antipsychotic medications had a higher prevalence of denture-related denture stomatitis and oral candidosis compared to
patients taking antidepressants (Lucas, 1993). The low number of denture wearers found in this study is in accord with a previous study done investigating the community dwelling individuals living with psychiatric disorders, where it was found that they were less likely to use dentures because they did not have adequate determination to seek dental treatment due to their mental health state (McCreddie et al., 2004). Not only do they not seek out denture construction for mental health and financial reasons but they also tend to get profound medication induced xerostomia. These resulted in poor denture retention, leading to ulcers, pain and ultimately them not being worn. They often lost the dentures or had dentures made but did not use them due to problems with their dentures such as pain, gagging and difficulty in getting used to them or defective, broken and non-retentive dentures.

5.3 Methods to Determine Xerostomia and Dry Mouth

Dry mouth is a condition where salivary flow or output is decreased or reduced, with changes in the salivary composition, whilst xerostomia is the subjective feeling of dry mouth experienced by individuals (Hopcraft et al., 2010). With changes in the salivary composition and output, multiple compounding effects usually follow. These factors not only affect the individual’s general oral condition but also their quality of life. The level of severity of dry mouth and xerostomia can be measured by using several investigation methods. These methods can be classified into special imaging procedures to determine salivary gland activity, measurement of salivary output, observers’ assessments of the condition of the oral mucosa and dentition and instruments used to record patient-reported evaluation of xerostomia-related symptoms.

5.3.1 Imaging procedures to determine salivary gland activity

Scintigraphy is a special imaging procedure using Tc-pertechnetate. This procedure captures information related to the movement of saliva from serum into the salivary glands (uptake), its concentration and then the movement and modification of saliva through ducts into the mouth (excretion). When combined with single-photon emission tomography, it can provide spatial information about the gland anatomical
volumes and their response to the variation of doses of the Tc-pertechnetate (Eisbruch et al., 2003). However, due to the technical complexity and the need for nuclear medicine specialists, scintigraphy was not feasible for this study.

5.3.2 Measurement of salivary output

Salivary output can be measured as an objective investigation for dry mouth. The collection of unstimulated saliva, stimulated saliva after chewing, whole saliva collection and selective saliva collection from specific salivary glands are methods used for measuring salivary output. Whole saliva measurement is considered acceptable for investigating the general level of xerostomia in patients or the relationship between symptoms and salivary output (Eisbruch et al., 2003). The measurement of the production of unstimulated whole saliva is easy, but it is sensitive to the hydration status of the patients and medications that may induce xerostomia. This is one of the reasons why the method of unstimulated whole saliva collection was chosen for this study – it allowed a measure of decreased salivary output.

5.3.3 Observer’s assessment of the condition of the oral mucosa and dentition

Clinical examination of the oral cavity provides useful information on the patient’s oral environment. The signs observed in a patient such as frothy saliva, dry shiny mucosa, depapillated or red, fissured tongue, lack of saliva pooling in the floor of the mouth, and the mouth mirror sticking to the buccal mucosa are signs of dry mouth. The present study identified the occurrence of dry mouth based on the presence of one or more sign of dry mouth (frothy saliva, dry shiny mucosa, depapillated or red, fissured tongue, lack of saliva pooling in the floor of the mouth, and the mouth mirror sticking to the buccal mucosa). The clinical assessment was undertaken after the participants completed their XI questionnaires, which took approximately 5 to 10 minutes, allowing sufficient time to assess whether the mouth was truly dry or moist.
5.3.4 Patient-reported xerostomia-related symptoms

In the present study, the majority of participants (6 of 9 scored ≤ 22 in the XI) reported less severe dry mouth symptoms. Although at the lower end of the spectrum, the oral changes described in the present study were still sufficient for the individual to be aware of the condition and often resulted in a reduced quality of life and behaviour modification such as always carrying a water bottle, waking up at night to drink water and avoiding certain foods.

5.4 The Effects of Antipsychotic Medications on the Oral Environment

Participants who are taking multiple medications, especially those with an anticholinergic action may have a more severe dry mouth due to the synergistic effects of these medications with antipsychotic medications. The participant with the highest XI score in the study (Table 3.6, Section 3.8.1) was on a combination of xerogenic medications, with clinical evidence of a dry mouth.

Antipsychotic medications may cause multiple side effects on the oral environment including hyposalivation or conversely increased salivation in patients. One of the two participants who had no evidence of dry mouth was taking quetiapine and flupentixol injection and the other one was taking risperidone only. This finding contradicts the usual side effects of quetiapine and risperidone, since they both usually cause dry mouth (Bagnall et al., 2003; Srisurapanont et al., 2004). One participant, on olanzapine alone, showed clinical signs of dry mouth, but did not have a high XI score suggesting this person experiences a less severe dry mouth. The presence of dry mouth and the severity of xerostomia found in the present study was not exclusively due to the effect of antipsychotic medications, but due to the cocktail of medications, in particular those with xerostomic potential taken by the participants.

Although clozapine may cause hypersalivation more often than dry mouth (Davydov and Botts, 2000), in the present study, participants taking clozapine were found to have clinical signs of dry mouth. However, this might be due to other medications
with xerostomic potential taken along with their antipsychotic prescriptions such as lithium, haloperidol and citalopram.

The present study did not investigate other side effects in the oral cavity that may appear in individuals taking antipsychotic medications. Although the effects of antipsychotic medications on the oral environment did not appear significant, the dry mouth effect due to antipsychotic drug intake may reduce the quality of life for individuals. Gerdin et al. (2005) studied the impact of dry mouth on the oral health-related quality of life of elderly people. They found dry mouth significantly affects the oral health-related quality of life of the elderly people. In a different study, the combination of clinical symptoms, adverse effects of medications and the interactions between distress factors (psychological distress) and protective factors (coping, social support) affect the quality of life of hospitalised patients living with schizophrenia (Ritsner et al., 2002).

5.5 Candida Colonisation and Oral Candidosis in Individuals Taking Antipsychotic Medications

There is little information currently available about the presence of oral candidal infection in patients taking antipsychotic medications. Antipsychotic medications may not be the main cause for oral candidosis, although a study of patients on long-term phenothiazine therapy participants were found to have a higher frequency of candidal infection and a higher mean number of Candida colony-forming units (CFU) than a control non-psychiatric group (Lucas, 1993). While the natural host defence mechanisms against candidal infection are weakened by the xerostomia induced by antipsychotic medication, an in vitro study of phenothiazines, specifically chlorpromazine and trifluoperazine, showed an inhibition of C. albicans growth (Sharma et al., 2001) and growth of other Candida spp. including Candida tropicalis, Candida parapsilosis and Candida glabrata (Afeltra and Verweij, 2003) suggesting these medications have some antifungal activity.

In the present study, four of nine participants were diagnosed with oral candidosis from smears. They were on a combination of multiple antipsychotic medications along with other medications for underlying medical conditions. One of the
participants with oral candidosis was on the combination of flupentixol (typical) and quetiapine (atypical). Studies have documented dry mouth as the side effect of these medications (Eberhard and Helbom, 1986; Mullen et al., 2001). Flupentixol is a very potent antipsychotic drug in the thioxantene category, introduced in 1965 for the treatment of various mental disorders (Jørgensen, 1980).

In the present study, the level of oral Candida colonisation was quantified from the number of colony forming units (CFU) obtained from the saliva samples. These findings were compared with healthy controls and participants with xerostomia not prescribed antipsychotic medications. The numbers of CFU/mL in each of the participant group in this study were significantly higher than the number in the control group (H = 10.38, df = 2, p<0.05). There was no statistical difference in CFU/mL between participants and xerostomia subjects (p>0.05), however there were statistically significant more CFU/mL in the participants compared with the healthy controls (p<0.05). The species presumptively recognized from the colony colour on the CHROMagar™ Candida, that was present at the highest concentration was C. albicans. Other species that were presumptively identified in the saliva samples were C. krusei and C. tropicalis.

5.6 Determining the fluconazole resistance of C. albicans isolates

5.6.1 Determining end-point for fluconazole resistance assays

In the present study fluconazole resistance was measured using the Etest® method and the broth microdilution technique (liquid MIC susceptibility assay). The Etest® plates were read according to the manufacturer’s instructions. However, it was noticed that the yeast growth (from standardised 0.5 McFarland turbidity C. albicans inoculum lawns on the agar plates) was insufficient at 24 h to measure the MIC, thus the author took the readings at 48 h. The presence of microcolonies within the entire, or almost the entire, elliptical zone of inhibition resulted in difficulties in determining the end-point when reading the Etest® values. The difficulties observed in the present study were similar to those reported previously. Similar patterns were observed more often with C. albicans and C. tropicalis compared to other Candida species (Colombo et al., 1995). Five of the 12 isolates in the present study were
apparently resistant to fluconazole by the Etest® method. Two pairs (W4a and W4b; W6a and W6b) showed a similar pattern of growth in the Etest® indicating that each pair might be isolates of the same strain of *C. albicans*. The strains of *C. albicans* isolated in the present study were not identified prior to the susceptibility testing. Ideally, the strains should be identified using multi-locus sequence typing (MLST), which uses a DNA sequencing method to type strains. The MIC values obtained from the broth microdilution technique were more consistent with previously reported susceptibilities of commensal isolates and more reliable with more reproducibility (Colombo et al., 1995). In the present study, none of the clinical isolates (at 24 h) were found to be resistant to fluconazole as determined by liquid MIC; the breakpoint for resistance being 64 µg/mL (Pfaller et al., 2006; Pfaller et al., 2010a). The CLSI guidelines allow readings to be obtained at 24 h or 48 h if there is insufficient growth. The MIC readings obtained from the Etest® for susceptible strains were in the lower range when compared to the broth microdilution method in the present study.

### 5.6.2 Clinical relevance of the Etest® in determining in vitro and in vivo fungal susceptibility

The Etest® is a simple yet reliable tool available to quantify antifungal susceptibility in terms of MIC values. The results obtained from the Etest® generally agree with the results obtained from the gold-standard broth microdilution technique (Colombo et al., 1995). However, in the present study, five of 12 isolates (41.7%) gave MIC values of >256 µg/mL with the Etest® method, indicating resistance, compared to none with the liquid MIC susceptibility assay. This is probably due to the difficulty in reading the Etest® MIC and the trailing growth of *C. albicans* in the liquid MIC. The Etest® is an instrument suitable for use for clinical isolates due to the simple and rapid process. Well-trained and experienced clinical microbiology personnel are required to provide correct judgement and consistent MIC readings.
5.7 Azole Antifungal Resistance of *C. albicans* Isolates from Individuals Taking Antipsychotic Medications

5.7.1 Interactions between fluphenazine and fluconazole

Potential interactions between fluconazole and fluphenazine are not well documented and yet it is realistic to expect that this combination of medications might be prescribed together reasonably often. The present study investigated the effect of this drug combination on *C. albicans* growth and viability using the disc diffusion method and the checkerboard liquid MIC synergism/antagonism assay. Antagonistic interactions between fluphenazine and fluconazole were observed in the disc diffusion assay for all the isolates in this study. However, in the checkerboard liquid MIC synergism/antagonism assay, antagonism was not seen for all isolates. The checkerboard assay results were analysed using fractional inhibitory concentration (FICI) calculations. A FICI value of $\leq 0.5$ indicates a synergistic interaction, while an FICI value of $> 4.0$ indicates an antagonistic interaction (Odds, 2003). A value between 0.5 and 4.0 indicates no significant interaction between the two medications. Although using the FICI value has been a popular choice amongst bacteriologists and mycologists (Odds, 2003; Meletiadis et al., 2009), it can be inconsistent. The FICI values differ with different cut-off MIC values pre-determined for each drug. For example, if the MIC$_0$ (total inhibition of yeasts) was used for all the medications, the end result of synergism/antagonism between all isolates differs from the MIC values taken at 80% of growth inhibition (MIC$_{80}$). The incubation period of the yeast growth also affects the results. In the study done by Meletiadis and colleagues, the best incubation period for determining the MIC for antifungal combinations was found to be 24 h. They proposed more research should be done in comparing the *in-vivo* and *in-vitro* results to correlate the FICI values with the pharmacodynamics in clinical situations.

In the present study, for five out of 12 isolates antagonism between fluphenazine and fluconazole was observed. Although the other isolates did not give a definite value of synergistic/antagonistic interactions, three of the isolates obtained a FICI value of 3.0 indicating potential antagonism. In several cases antagonism was observed at the lowest fluphenazine concentration tested, 0.375 µg/mL.
5.7.2 Clinical dosage of fluphenazine and plasma concentrations

Individuals receiving fluphenazine via intramuscular injection (as a depot medication) are prescribed either 25 or 50 mg fluphenazine decanoate (FPZ-D) every two weeks, while oral fluphenazine hydrochloride (FPZ-H) is prescribed as 2.5 to 10 mg every six or eight hours. Patients receiving 25 mg FPZ-D were found to have fluphenazine plasma concentrations of 0.17-0.61 ng/mL (Chang et al., 1985; Whelpton and Curry, 1976), and the plasma concentration of fluphenazine in patients receiving 50 mg FPZ-D ranges between 0.20 and 0.93 ng/mL (Chang et al., 1985). An earlier study found the fluphenazine plasma level was between 1.02 and 3.48 ng/mL in the patients receiving 25 mg dose (Wiles and Gelder, 1979). For patients receiving fluphenazine, the drug will enter saliva via the gingival crevicular fluid, but the concentration achieved in saliva has not been reported. It remains debatable as to whether antagonism between fluphenazine and fluconazole will be seen with oral C. albicans in vivo. Antagonism was seen in vitro at fluphenazine concentrations of 375 ng/mL. This was the lowest concentration tested. Further research is required to see if this effect is still seen at concentrations of fluphenazine and fluconazole achieved in saliva in vivo.

The fluphenazine MIC values for the C. albicans isolates were all between 50 and 100 µg/mL therefore the drug will not have an antifungal affect in vivo, as this concentration will not be achieved, but may reduce susceptibility of yeast to fluconazole through antagonism.

5.8 Limitations Of the Study

5.8.1 Study participants

One of the limitations of the present study was the low number of recruited participants. The study participants were limited to patients attending the Special Care Unit, Faculty of Dentistry, University of Otago. This was one of the reasons for the low number of participants recruited into this study. Individuals living with mental disorders or psychosis are not easy to deal with. They sometimes have mood swings causing them to change their mind about previous decisions such as whether
to attend dental appointment. Another factor contributing to the relatively low numbers of participants was the exclusion factor of certain other medical conditions being present along with their mental disorder. Patients with medical conditions such as diabetes mellitus were not included in this study because the presence of diabetes may have acted as a confounding factor in the perception of oral dryness and Candida colonisation. Patients with poor co-operation and impaired intellectual ability were excluded from this study. Most of the patients attending the Special Care Unit needed a relative or support worker to accompany them to attend the clinic appointments. Some of the patients’ relatives or caregivers decided they were not suitable to be recruited into this study. It was important to make sure the relatives or caregivers understood the purpose of this study and how it was performed to get their full support. The data collection period was completed in a manner that was comfortable for the participants to avoid unnecessary stress on them. They were provided with sufficient time to read, understand and complete the questions in the XI.

5.8.2 Determination of dry mouth

The method for determining dry mouth in the present study was limited to clinical assessment. The volumes of unstimulated saliva collected in this study were not measured. Results from the clinical assessment alone were insufficient to diagnose dry mouth. The volumes of saliva collected in this study should have been measured to properly distinguish dry mouth and moist mouth. The XI in the present study was used merely to determine the subjective feeling of xerostomia in participants taking antipsychotic medications. Individuals with clinical signs of dry mouth do not necessarily experience a severe level of xerostomia. This is probably due to the symptoms not being severe enough to be noticed by the individuals or perhaps they experienced a less severe form of xerostomia and thus they did not adopt xerostomia coping behaviours.

5.8.3 Combination of antipsychotic medications

Most participants recruited into the present study were on polypharmacy. Three participants were exclusively taking antipsychotic medications, while the other six
were on combinations of antipsychotic medications and medications for their underlying medical conditions. Three participants were on a combination of typical and atypical antipsychotic medications; flupentixol and quetiapine; and haloperidol, clozapine and lithium. The findings from the present study could not be directly correlated with the antipsychotic medications due to the intake of other medications with xerostomic potential that may contribute to the results. For example, other medications such as antihypertensives may contribute to the presence of dry mouth in the participants.

5.8.4 Azole antifungal resistant testing

The present study exclusively tested *C. albicans* for fluconazole antifungal resistance and fluphenazine susceptibility. The results were obtained from the broth microdilution technique and the synergism/antagonism discs diffusion assays. Fluconazole combined with the antipsychotic medication; fluphenazine was studied in the checkerboard synergism/antagonism susceptibility assay (broth microdilution technique). The susceptibility of fresh *C. albicans* isolates towards other, more frequently used, antipsychotic medications belonging in the same group as fluphenazine was not determined due to time constraints and limited resources. The present study was limited to studying fluconazole alone, without testing the other azoles, again due to time constraints.

5.9 Summary and recommendations

Findings from the present study indicated that azole antifungal resistance could be induced by fluphenazine in the *C. albicans* strains isolated from patients taking antipsychotic medication. This is an important clinically relevant finding and deserving of further study. I would recommend inclusion of more participants if similar studies were to be performed and a population-based study is recommended. Comparing findings from institutionalised individuals and uninstitutionalised individuals could also be performed in the future. Institutionalised patients taking antipsychotic medications are presumably less well and taking a more complex range of medications that may have a more severe impact on the oral mucosa and lead to a
lower quality of life compared to patients living independently. Recruiting participants exclusively taking typical antipsychotic medications or atypical antipsychotic medications should be considered to study the effect of antipsychotic medications on the oral environment without other confounding factors. Other effects of the antipsychotic medications on the oral environment such as incidence of oral mucosal lesions could be another area of investigation. The determination of dry mouth in the present study should have included measuring the salivary output either through whole unstimulated/stimulated saliva or collection from specific salivary glands. Findings from the clinical evaluation of dry mouth alone were insufficient to accurately categorise the person as having a dry mouth. Future studies on the impact of side effects of the antipsychotic medications on the oral health-related quality of life or overall quality of life of individuals should also be considered.

5.10 Conclusions

The present study has found that there was a tendency towards antagonism between the combination of fluconazole and fluphenazine towards *C. albicans*. This may induce fluconazole resistant oral candidal infections in individuals taking antipsychotic medications leading to treatment problems when treating these patients with fluconazole. The risk of developing resistance to other types of azoles and antipsychotic medications requires investigation. In the meantime treating oral candidosis with polyenes should be considered in patients taking antipsychotic medications in the same or similar class as the fluphenazines (phenothiazine derived from piperazines), such as perphenazine, prochlorperazine and trifluoperazine.


Reference List

Pathology, Oral Radiology and Endodontology, 98(1), 53–59.


doi:10.1046/j.1529-8019.2002.01533.x


APPENDICES
Appendices

APPENDIX I: Ethical Approval Letter

10 July 2014

Professor A Rich
Sir John Walsh Research Institute
Department of Oral Diagnostic and Surgical Sciences
Faculty of Dentistry

Dear Professor Rich,

I am again writing to you concerning your proposal entitled “Are patients on antipsychotic drugs at risk of developing azole-resistant oral candidal infections”, Ethics Committee reference number 12/207.

Thank you for your letter of 8th July 2014 addressing the issues raised by the Committee.

On the basis of this response, I am pleased to confirm that the proposal now has full ethical approval to proceed.

Approval is for up to three years from the date of this letter. If this project has not been completed within three years from the date of this letter, re-approval must be requested. If the nature, consent, location, procedures or personnel of your approved application change, please advise me in writing.

Yours sincerely,

[Signature]

Mr Gary Witte
Manager, Academic Committees
Tel: 479 8256
Email: gary.witte@otago.ac.nz

c.c. Professor R D Cannon Director Sir John Walsh Research Institute
APPENDIX II: Ngāi Tahu Maori Approval Letter

Ngāi Tahu Research Consultation Committee
Te Komiti Rakahau ki Kāi Tahu

15/05/2012 - 45
Tuesday, 15 May 2012

Ms MacFadyen
Sir John Walsh Research Institute Faculty of Dentistry
Dunedin

Tēnā koe Ms MacFadyen

Title: Are patients on antipsychotic drugs at risk of developing azole-resistant oral candidal infections?

The Ngāi Tahu Research Consultation Committee (The Committee) met on Tuesday, 15 May 2012 to discuss your research proposition.

By way of introduction, this response from the Committee is provided as part of the Memorandum of Understanding between Te Rūnanga o Ngāi Tahu and the University. In the statement of principles of the memorandum, it states "Ngāi Tahu acknowledges that the consultation process outlined in this policy provides no power of veto by Ngāi Tahu to research undertaken at the University of Otago". As such, this response is not "approval" or "mandate" for the research, rather it is a mandated response from a Ngāi Tahu appointed committee. This process is part of a number of requirements for researchers to undertake and does not cover other issues relating to ethics, including methodology; they are separate requirements with other committees, for example the Human Ethics Committee, etc.

Within the context of the Policy for Research Consultation with Māori, the Committee bases consultation on that defined by Justice McGechan:

"Consultation does not mean negotiation or agreement. It means: setting out a proposal not fully decided upon, adequately informing a party about relevant information upon which the proposal is based; listening to what the others have to say with an open mind (in that there is room to be persuaded against the proposal); undertaking that task in a genuine and not cosmetic manner. Reaching a decision that may or may not alter the original proposal."

The Committee considers the research to be of importance to Māori health.

As this study involves human participants, the Committee strongly encourage that ethnicity data be collected as part of the research project. That is the questions on self-identified ethnicity and descent, these questions are contained in the 2006 census.

Appendices

NGĀI TAHU RESEARCH CONSULTATION COMMITTEE

Publication, Hauora: Māori Standards of Health IV (2000-2005), has its own website, http://www.hauora.maori.nz/. These publications provide information on a range of Māori health issues and will assist in ensuring your research has an appropriate Māori health focus.

The Committee suggests dissemination of the findings to relevant Māori health organisations, for example the National Māori Organisation for Dental Health, Oranga Nīho and to Professor John Broughton, who is involved in Māori Dental Health, University of Otago.

We wish you every success in your research and the Committee also requests a copy of the research findings.

The recommendations and suggestions above are provided on your proposal submitted through the consultation website process. These recommendations and suggestions do not necessarily relate to ethical issues with the research, including methodology. Other committees may also provide feedback in these areas.

Nāhaku noa, nā

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Facilitator Research Māori
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Te Whare Wānanga o Otago
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Web: www.otago.ac.nz
APPENDIX III : Information Sheet For Participants

Are patients on antipsychotic medications at risk of developing azole-resistant oral candidal infections?

INFORMATION SHEET FOR PARTICIPANTS

Thank you for showing an interest in this project. Please read this information sheet carefully before deciding whether or not to participate. If you decide to participate we thank you. If you decide not to take part there will be no disadvantage to you and we thank you for considering our request.

What is the Aim of the Project?

This project is undertaken as part of the requirements for the Doctor of Clinical Dentistry in Special Needs Dentistry, University of Otago.

The New Zealand Mental Health Survey, 2006, concluded that 39.4% of the New Zealand population experienced mental disorders at some time in their life. A proportion of this group will have been prescribed antipsychotic medications. These medications have several adverse effects including reducing salivary gland function leading to a dry mouth. People with dry mouth have a higher risk of developing fungal infections. This study aims to determine the number and the type of fungal infections in people taking antipsychotic medications. Furthermore, people who are taking these medications may not respond to antifungal therapy. So, it would affect the choice of treatment for these patients if they develop fungal infections.

What Type of Participants are being sought?

• Recruitment method: All potential participants, who are on antipsychotic medications who come to the Special Care Unit, Faculty of Dentistry, University of Otago and the out-patient clinic, Psychiatric services of Wakari Hospital will be invited to take part in the study.

• Inclusion criteria:
  Potential participants currently taking antipsychotic medications, known smoking status
aged between 20 to 70 years old

• Exclusion criteria:
  Potential participants with medical conditions such as diabetes mellitus and
  Potential participants taking medications other than antipsychotics, such as antibiotics

• Number of participants to be involved : 20

What will Participants be Asked to Do?

Should the potential participants agree to take part in this project, they will be screened for dry mouth. They will be asked to complete a form with 11 questions marking their subjective symptoms of a dry mouth. All potential participants will have a standard oral mucosal examination to check for clinical evidence of xerostomia (dry shiny mucosa, frothy saliva) and for clinical evidence of *Candida* infection.

Then a smear will be taken from the buccal (cheek) mucosa and tongue using a wooden spatula. If there is evidence of *Candida* infection elsewhere in the mouth, further smears will be taken. They will also be asked to rinse their mouth with 10mL bottled water which will be collected in a sterile bottle. There is no discomfort during the smear and the whole procedure will take no longer than 15 minutes.

What Data or Information will be Collected and What Use will be made of it?

The smears will be checked to see if they show evidence of candidal infection and the candida will then be grown in the laboratory from the saliva rinse. The species, number of colonies present and their colour will be recorded. Candidal growth will then be tested against antifungal drug fluconazole.

The data collected will be securely stored in such a way that only those in the research team will be able to gain access to it. Data obtained as a result of the research will be retained for at least 5 years in secure storage.

The results of the project may be published and will be available in the University of Otago Library (Dunedin, New Zealand) but the published work will contain only pooled data and will be anonymised.

Can Participants Change their Mind and Withdraw from the Project?

You may withdraw from participation in the project at any time and without any disadvantage to yourself of any kind.
What if Participants have any Questions?
If you have any questions about our project, either now or in the future, please feel free to contact either:-

Wan Syasliza Mohamed Thani
Department of Oral Diagnostic and Surgical Sciences (Special Needs Unit)
University Telephone Number: - [03 479 7024]
Email Address :-[binwa316@student.otago.ac.nz]

and/or

Ms Eithne Mac Fadyen
Department of Oral Diagnostic and Surgical Sciences (Special Needs Unit)
University Telephone Number: - [03 479 7030]
Email Address :-[eithne.macfadyen@otago.ac.nz]

This study has been approved by the University of Otago, Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (ph 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.
Appendices

[Reference no. 12/07]

Are patients on antipsychotic medications at risk of developing azole-resistant oral candidal infections?

CONSENT FORM FOR PARTICIPANTS

I have read the Information Sheet concerning this project and understand what it is about. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage. I know that:-

1. My participation in the project is entirely voluntary;

2. I am free to withdraw from the project at any time without any disadvantage;

3. Personal identifying information on record book will be destroyed at the conclusion of the project but any raw data on which the results of the project depend will be retained in secure storage for at least five years;

4. I will not have any discomfort during the smear.

5. The results of the project may be published and will be available in the University of Otago Library (Dunedin, New Zealand) but the published work will contain only pooled data and will be anonymised.

6. At the end of the study, I consent to any remaining samples being disposed of using standard disposal methods.

I agree to take part in this project.

............................................................................
(Signature of participant)
(Date)
This study has been approved by the University of Otago Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (ph 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.
APPENDIX IV: Xerostomia Inventory

Please tick (✓) the most suitable answer for each question. Please answer ALL questions.

**How often does your mouth feel dry?**

<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>Occasionally</th>
<th>Frequently</th>
<th>Always</th>
<th>Office use only</th>
</tr>
</thead>
<tbody>
<tr>
<td>My mouth feels dry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I have difficulty in eating dry food</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I get up at night to drink</td>
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<td></td>
</tr>
<tr>
<td>My mouth feels dry when eating a meal</td>
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<tr>
<td>I sip liquids to aid in swallowing food</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I suck sweets or cough lollies to relieve dry mouth</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I have difficulties in swallowing certain foods</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>The skin of my face feels dry</td>
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<td></td>
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<tr>
<td>My eyes feel dry</td>
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<tr>
<td>My lips feel dry</td>
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<td></td>
</tr>
<tr>
<td>The inside of my nose feels dry</td>
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</tr>
</tbody>
</table>

Please answer ALL questions.
APPENDIX V: Abstract for the 54th Annual Scientific Meeting of the Australian & New Zealand Division of the IADR

Title: Fluconazole Resistance Against Oral Candida in Patients Taking Antipsychotic Medications

Authors: W.S. MOHAMED THANI, E. MACFADYEN, A.M. RICH, and R.D. CANNON
Department: Sir John Walsh Research Institute, University of Otago, Dunedin, New Zealand

Objective: To determine the Candida species and colonisation level of the oral mucosa in people taking antipsychotic medications relative to healthy individuals and other xerostomic patients. To investigate fluconazole resistance to other antipsychotics, as fluphenazine is known to cause resistance.

Method: Consented participants aged between 20 and 70, who were currently on antipsychotic medications were enrolled. Xerostomia symptoms were determined from the Xerostomia Inventory (XI) and clinical examinations. Saliva rinses were collected. Smears were taken from the buccal mucosae and tongue, and other suspicious mucosa. Smears were sent to Medlab for candida hyphae and yeast identification. Saliva samples were titrated and plated onto Chromagar, then kept at 37°C for 48 hours. The colony-forming units (CFU) and species (from the colony colour) were recorded. The susceptibility of the colonies towards fluconazole was measured using the E-test by making lawns of candida species with the optical density (OD) of 0.25 on the HDMI and MOPS agar. Plates were incubated at 37°C for 48 hours.

Result: Current findings show that although 75% of participants have evidence of dry mouth clinically, they may not have symptoms (only a third scored high XIs). More than 60% have positive oral candida hyphae and candida colonies. Susceptibility testing is currently undertaken.

Conclusion: Many antipsychotic medications were known to cause xerostomia and increased candida infections. Investigating candidal infection and their resistance to fluconazole will address and potentially lead to more appropriate treatment.