CHAPTER II

LITERATURE REVIEW

Honey has been used for long time as medicine, the ancient Sumerians Babylonians, and Egyptians all recorded its use. Honey was used as a drug more than a nutrient. Avicenna (Ebne Sina), from about 1,000 years ago, mentioned the therapeutic effect of honey in *Ghanoon of medicine* (Yaghoobi et al., 2008). The color, flavor, mineral and vitamin contents of honey depend on the flower from which bees gather the nectar. Honey has been used to treat infected disease such as leg ulcer, earache, measles, eye disease, and gastric ulcers (Molan, 2001; Blair & Carter, 2005). Honey has been used for the treatment of respiratory, urinary disease, gastrointestinal disease, skin ulcers, wounds, eczema, psoriasis, dandruff, diaper dermatitis, and radiation mucositis (Molan, 1999; Al-Waili, 2003; Al-Waili, 2005). The honey produced by *Apis mellifera* is used in traditional medicines to be important in the treatment of several human diseases (Lusby et al., 2005).

Honey collected from both the United States and New Zealand inhibited *Helicobacter pylori* at concentration of 10% (Osato et al., 1999). Asadi-Pooya et al. (2003) reported that mycobacteria growth was inhibited by 10% concentration of honey. Similar findings were reported by Al-Waili, (2004) who reported that honey prevented the growth of Gram positive and Gram negative bacteria as well as *C. albicans*. Noori et al. (2013) reported that five honey samples which are Talah, Dhahian, Sumra-1, Sidr, and Sumra-2 inhibited Gram positive pathogens and fungi such as *C. albicans* ATCC 10231 and *C. tropicalis*. Theunissen et al. (2001) reported that three samples of South African honey (Wasbessie, Bluegum and Fynbos) inhibited the growth of *C. albicans*

ATCC 3118. The Wasbessie honey at concentration of 25 % showed 29.4 % inhibition on the growth, while Fynbos and Bluegum honey produced partial inhibition.

2.1 Chemical Composition of honey

Chemically, honey is a complex substance that has been estimated to contain at least 600 components (Bogdanov et al., 2004). Essentially, it is a supersaturated solution of four main sugars – fructose 38.2%, glucose 31.3%, sucrose1.3% and maltose 7.3%. Water is second component of honey, the weather and humidity inside the hives can effect the water content of honey. The organic acids represent about 0.57% of honey which are responsible for the acidity of honey and include gluconic acid. Honey contains 0.17% minerals such as potassium, copper, calcium, iron, phosphorus and manganese (Moundoi et al., 2001).

Honey also contains other components such as glucose acids, protein enzymes, amino acid, minerals, vitamins and water (Weston & Brocklebank, 1999). In addition honey contains oligosaccharides which stimulates the growth of probiotic bacteria in the gut (Leite et al., 2000; Sanz et al., 2004). Honey and propolis are bee products that used for centuries in medicine. (Muli et al., 2008). Propolis is a complex resinous mixture collected by bees from plant exudates, and mixed with hypo-pharyngeal secretions, beeswax and pollen. The chemical composition of propolis varies depending on the diversity of plants and geographic locations from which bees collect it (Bankova et al., 2000). The biological activities of propolis (antibacterial, antiviral, antifungal) vary according to its source (Muli et al., 2008).

The composition and properties of honey depend on several factors, such as geographic origin, collection season, mode of storage, bee species, botanical origin and

interactions between chemical compounds and enzymes in the honey. In addition, honey vary from country to country due to the different compositions of pollen or nectar (Oddo et al., 2004; Kaškonienė & Venskutonis, 2010). Blossom honey is honey that comes from nectars of plants while honeydew honey the sweet material that exudes from the leaves of certain plants in hot weather, honeydew honey contains higher amounts of the oligosaccharides melezitose and raffinose compared to the Blossom honey (Table 1).

TABLE 1: Composition of honey (Data in g/100 g).

	Blossom honey		**Honeydew honey**	
	Average	**min-max**	**Average**	**min-max**
Water	17.2	15- 20	16.3	15 – 20
Monosaccharides				
Fructose	38.2	30 – 45	31.8	28 – 40
Glucose	31.3	24 – 40	26.1	19 – 32
Disaccharides				
Sucrose	0.7	0.1 - 4.8	0.5	0.1 - 4.7
Others	5.0	2- 8	4.0	1- 6
Trisaccharides				
Melezitose	0.1	4.0	0.3	22.0
Erlose	0.8	0.5 – 6	1	0.1 – 6
Others	0.5	0.5 – 1	3	0.1 – 6
Oligosaccharides	3.1		10.1	
Total sugars	79.7		80.5	
Minerals	0.2	0.1 - 0.5	0.9	0.6 – 2
Amino acids, proteins	0.3	0.2 - 0.4	0.6	0.4 - 0.7
Acids	0.5	0.2 - 0.8	1.1	0.8 - 1.5
pH – value	3.9	3.5 - 4.5	5.2	4.5 - 6.5

Source: Bogdanov et al. (2008)

The antimicrobial factors in honey are summarized as follows:

1- Acidity the pH of honey is between 3.2 and 4.5 due to the presence of gluconic acid (White, 1975).
2- Osmotic effect high osmatic and low water activity of honey is inhibitory to the growth of bacteria and fungi (Molan, 1992a).
3- Hydrogen peroxide produced by glucose oxidase system has the antimicrobial activity against most pathogens (Snowdon & Cliver, 1996).

2.2 Microorganisms found in honey

There are many of microorganisms in honey which may influence the quality and safety of honey like bacteria, yeast and molds (Table 2). They come from the bees, pollen, nectar, honey bee intestine, human, containers, winds, and dust. The intestine of bees contains 1% yeast 27% Gram-positive bacteria and 70% Gram-negative bacteria (El-Leithy & El-Sibael, 1992). Spores of *Clostridium botulinum* type F were found in different containers of honey which can cause infant botulism (King et al., 2010). The clinical manifestations of infant botulism varies from mild constipation and poor feeding to severe neurological deterioration such as hypotonia, poor head control and apnea. To prevent the infant botulism, the raw honey should not be fed to infant younger than one year of age (Tanzi & Gabay, 2002). Honey can be sterilized by gamma radiation to reduce the risk of botulinum spores (Molan & Allen 1996; Al-Waili et al., 2012).

TABLE 2: Microorganisms found in honey

Bacteria	Yeasts	Moulds
Alcaligenes	*Ascosphaera*	*Aspergillus*
Achromobacter	*Debaromyces*	*Atichia*
Bacillus	*Hansenula*	*Bettsia alvei*
Bacteridium (sic)	*Lipomyces*	*Cephalosporium*
Brevibacterium	*Nematospora*	*Chaetomium*
Citrobacter	*Oosporidium*	*Coniothecium*
Clostridium	*Pichia*	*Hormiscium*
Enterobacter	*Saccharomyces*	*Peronsporoceae*
Escherichia coli	*Schizosaccharomyces*	*Peyronelia*
Erwinia	*Trichosporium*	*Tripsporium*
Flavobacterium	*Torula*	*Uredianceae*
Klebsiella	*Torulopsis*	*Ustilaginaceae*
Micrococcus	*Zygasaccharomyces*	
Neisseria		
Pseudomonas		
Xanthomonas		

Source: Shimanuki & Knox (1991).

2.3 Medical properties of honey

2.3.1 Oral health

In a study carried out on oral health by Molan reported that honey can promote the oral health by decreasing the risk of dental caries, dental plaque and gingivitis (English et al., 2004). Altun et al. (2015) showed that consumption of honey does not cause corrosion of tooth enamel because of protective role of honey contents such as calcium, phosphorous and fluoride.

2.3.2 Haematology and immunity

For people suffering from anaemia, it was found that honey can be beneficial medicine. Honey enhances haemoglobin concentration, haematocrit, and erythrocyte count due to presence of iron in honey which has an important role in improvement of haemoglobin

concentration (Ajibola et al., 2007). Honey has immune-nutrient benefit, it can stimulate and increase the production of antibodies against the T-cells of the thymus dependent as well as the thymus independent antigens (Al-Waili & Haq, 2004).

2.3.3 Ophthalmology

Honey was used in the treatment of eye diseases by the ancient people. Indian use honey as eye drops to treat eye diseases (Mahawar & Jaroli, 2006). Apply the honey on the eye can prevent the scarring effect of the cornea which occurs after measles infections (Imperato & Traore, 1979). Moreover, honey was used in treatment of different ophthalmological diseases such as blepharitis, conjunctivitis and keratitis (Emarah, 1982). Likewise, Park et al. (1996) and Ajibola et al. (2012), observed that honey possess antifungal, antibacterial, and anti-inflammatory activity when applied under the lower eyelid. Honey has been used for treatment of burns to the eye caused by chemical and thermal agents.

2.3.4 Gastroenterology

Honey inhibits the activity of *Helicobacter pylori* that cause gastritis and peptic ulcers (Osato et al., 1999). Honey reduced gastrointestinal disorders caused by alcohol, ammonia and aspirin which may result from the antioxidant properties of honey that stimulates the sensory nerves in stomach (Nasuti et al., 2006). Clinical studies on infants and children demonstrated that honey can shorten the duration of diarrhea. This may be attributed to the antibacterial activities of honey. The effect of honey in treating diarrhea may be repair of the intestinal mucosa which is damaged by infections (Dupont, 2005).

2.4 Physiological properties of honey

2.4.1 Antioxidant effects

Honey contains a variety of antioxidant compounds such as glucose oxidase, ascorbic acids, phenolic acids, amino acids and proteins catalase (Pérez et al., 2007). Honey also contains various polyphenols like caffeic acid, chrysin, gelangin, quercetie, acacetin, kaempferol, apigenin, pinoembrin and pinobanksin that could be used for treatment of cancer and cardiovascular diseases (Jaganathan & Mandal, 2009). Other study in California observed that when buckwheat honey was given to 37 persons at the average 1.5g/kg body weight with corn syrup as control, demonstrated increased plasma total phenolic connect and plasma antioxidant. Therefore, phenolic antioxidants from honey increase antioxidant activity (Schramm et al., 2003).

2.4.2 Anti-inflammatory effects

Al-Waili and Boni (2003) reported that the ingestion of 70 g of honey decreased the plasma concentration of thromboxane B2, PGE 2 and PGF by 48%, 63% and 50%, respectively. Kassim et al. (2010) reported that Gelam honey reduced inflammatory mediators like COX-2 and TNF thus, inhibiting the activation of the NF-XB pathway. In another study, Vallianou et al. (2014) observed that honey reduce the activity of cyclooxygenase-1 and cyclooxygenase-2. Consequently, showing anti-inflammatory effects of honey. Furthermore, ingestion of diluted natural honey produced reductions on concentrations of prostaglandins such as PGE_2 (prostaglandin E_2), $PGF_{2\alpha}$ (prostaglandin F_{2a}) and thromboxane B_2 in plasma of normal individuals (Reyes-Gordillo et al., 2007). Recently, Yao et al. (2011) reported that several types of honey

from Malaysia including Tualang, Nenas, Coconut and Gelam honey possess anti-inflammatory effects.

2.4.3 Antimicrobial activity of honey

Honey has antimicrobial activity against several pathogens which are resistant to antibiotics and cause many infectious diseases (Table 3). The antimicrobial activity of honey is dependent on a verity of factors such as hydrogen peroxide (H_2O_2), phenolic compounds, low pH and high osmatic pressure (Wilkinson & Cavanagh, 2005). Honey produced hydrogen peroxide when diluted because activation of glucose oxidase to gluconic acid, and H_2O_2 which is the main compound responsible for antimicrobial activity of honey. The presence of catalase and heat destroyed peroxide activity of honey (Simon et al., 2009). The antimicrobial activity of manuka honey from New Zealand in the presence catalase is due to non-peroxide compounds like methylglyoxal and methylsyringate (Mavric et al., 2008). In another study Khalil et al. (2010) reported that the antibacterial activity of five samples of Tualang honey from Malaysia showed strong antibacterial activity especially, against. *E. coli*, *S. typhimurium* and *S. pyogenes*. Tan et al. (2009) reported that Tualang honey from Malaysia possess high antibacterial activity against most bacteria pathogenic when compared with manuka honey. However, Tualang and manuka honeys showed good antimicrobial activity against *Stenotrophomonas maltophilia* that is resistance to many antibiotics and causes infections such as pneumonia, urinary tract infection and bloodstream infection specially, in immune compromised patients thus, led to nosocomial infections (Al-Jasser, 2006). Recently, Aween et al. (2012b) also reported that strains of *Lactobacillus acidophilus*1 isolated from different sources of honey samples showed high

antibacterial activity against three Gram negative pathogenic bacteria *S. typhimurium*, *E. coli* and *E. aerogenes*.

TABLE 3: Infections caused by bacteria that have found to be sensitive to honey

Pathogen	Infection caused
Bacillus anthracis	Anthrax
Corynebacterium diphtheria	Diphtheria
Escherichia coli	Diarrhoea, septicaemia, urinary infections, wound infections
Haemophilus influenza	Ear infections, meningitis, respiratory infections, sinusitis
Klebsiella pneumonia	Pneumonia
Mycobacterium tuberculosis	Tuberculosis
Proteus sp.	Septicaemia, urinary infections
Pseudomonas aeruginosa	Septicaemia, urinary infections
Salmonella sp.	Diarrhoea
Salmonella cholerae-suis	Septicaemia
Salmonella typhi	Typhoid
Salmonella typhimurium	Wound infections
Serrata marcescens	Septicaemia, wound infections
Vibrio choleriae	Cholera
Shigella sp.	Dysentery
Staphylococcus aureus	Abscesses, boils, carbuncles, wound infection
Streptococcus faecalis	Urinary infections
Streptococcus mutans	Dental carries
Streptococcus pneumonia	Ear infections, meningitis, Sinusitis
Streptococcus pyogenes	Ear infections, impetigo, puerperal , rheumatic fever, scarlet fever, wound infections
E. coli, salmonella, shigella, Hel. Pylori	Peptic ulcer

Source: Cooper et al. (2002).

2.5 Lactic acid bacteria

Lactic acid bacteria (LAB) are Gram-positive, non-sporulating, low-GC (< 55 mol% guanine cytosine base pairs in DNA), fastidious, acid-tolerant and catalase-negative cocci, coccobacilli or rods. The lack of a respiratory chain causes them to exhibit a fermentative metabolism, some species are aerotolerant, and others are strictly anaerobic (Stiles & Holzapfel, 1997; Axelsson, 2009). *Lactobacilli* are divided into

three groups based on their phenotypic characteristics. Group 1 includes the obligatory homo-fermentative *Lactobacilli* that ferment glucose to lactic acid. Group 2 includes facultative hetero-fermentative lactobacilli that ferment hexoses to lactic acid. Also pentose can be fermented into lactic and acetic acids. Group 3 includes the obligatory hetero-fermentative lactobacilli that ferment hexoses to lactic acid, acetic acid, ethanol and carbon dioxide (Stiles & Holzapfel, 1997). Lactic acid bacteria are microorganisms which are used in food preservation and responsible for the production of yogurt, cheese, sour cream, sausage, olives, fermented meats, wine, beer and sourdough bread (Asmahan, 2010).

Lactobacilli play an important role in prevention of infection, it can interfere with pathogens by different mechanisms including competitively inhibit pathogen-adherence to epithelial cells congregate with pathogens and produce a variety of antimicrobial compounds like hydrogen peroxide, lactic acid, bacteriocins and biosurfactants to limit growth of pathogen (Reid & Burton, 2002; Hatakka et al., 2007).

Many studies assessed the antibacterial activity of honey compared to studies on antifungal activity of honey (Theunissen et al., 2001; Irish et al., 2006; Boukraa et al., 2008; Koc et al., 2009). Recently, Estevinho et al. (2011) showed that Portuguese lavender honey inhibited the growth of *C. albicans, C. krusei* and *Crptococcus neoformans* by 31.0%, 16% and 23.0%, respectively.

2.5.1 Antifungal activity of LAB

Most of the antifungal activity of LAB studied are due to production of diverse compounds such as organic acids, diacetyl, hydrogen peroxide, nisin, bacteriocins and biosurfactants that have antifungal activity against several pathogenic microorganisms

(Guerra et al., 2005; Kaewsrichan et al., 2006; Mijač et al., 2006; Martinez et al., 2008). Okkers et al. (1999) reported that *L. pentosus* inhibits the growth of *C. albicans*. While Strus et al. (2005) reported that *L. debrueckii* showed strong inhibitory activity against *C. albicans*.

LAB play an important role that prevent the development *C. albicans* by producing lactic acid (Savadogo et al., 2004). Cabo et al. (2002) reported that lactic acid and acetic acid in the medium was responsible for the antifungal activity. Ronnqvist et al. (2007) also reported that *L. fermentum* Ess-1 showed strong inhibitory activity against both *C. albicans* and *C. glabrata*. Monthon, (2005) reported that the supernatant produced by *Lactococus lactis* showed inhibitory activity against *C. albicans* DMST 5239. Askari et al. (2012) showed that cell free supernatant of *L. fermentum* isolated from dried fruits had high activity against *Streptococcus* spp, *Streptococcus* sanguins, *Staphylococcus epidermis*, *Staphylococcus aures*, *Proteus mirabilis*, *Hafnia alvei* and *Yersinia* spp. Sungsri et al. (2012) reported that *L. paracasei* inhibited the growth of *C. albicans* BCC6120 using dual agar overly method. Oluwafemi and Adetunji (2011) add that *L. plantarum* isolated from Oqi had inhibitory activity against *C. albicans*. Recently, Adeniyi and Damsa (2013) reported that the supernatant of LAB isolates produced by *Lactobacillus plantarum* showed higher antifungal activity against *C. albicans* ATCC 90029 with inhibition zone of 25mm.

2.5.2 Anti-adhesion activity of LAB

LABs from different sources are documented to have anti-adhesion activity. Ouwehand et al. (2001) reported that *L. brevis* PEL1, *L. reuteri* ING1, *L. rhamnosus* VTT E-800 and *L. rhamnosus* LC-705 have anti-adhesion activity against *Salmonella typhimurium* and *E. coli*. Fracchia et al. (2010) reported that *Lactobacillus* CV8LAC

isolated from cabbage showed higher anti-adhesion activity against two *C. albicans* CA- 2894 and DSMZ 11225 in pre-coating and co-incubation experiments and adhesion was reduced by 82% and 70% respectively.

Matijasic et al. (2006) observed that *L. gasseri* had anti-adhesion activity against *E. coli*, also Gudina *et al*. (2010b) reported that *L. acidophilus* and *L. paracasei* ssp. *paracasei* A20 had lower anti-adhesion activity against *C. albicans*. Recently, Gomaa (2013) reported that *L. fermentum* showed the highest anti-adhesion activity against *C. albicans* ATCC 70014 by 84.69%.

2.5.3 Antifungal compounds produced by LAB

2.5.3.1 Organic acids

LAB produce a variety of antimicrobial compounds such as organic acids, lactic and acetic acid are main products of their metabolism. LAB have long been used in fermented foods for the preserve of various foods (Daeschel, 1989; Kim, 1993; Johan & Jesper, 2005).The organic acids produced by *L. sanfrancisco* CBI strains are low-molecular mass (Corsetti et al., 1998). The organic acids play a role in inhibiting fungal growth. Cabo et al. (2002) showed that acetic acid originates from the bacterial growth medium and the lactic acid produced are responsible for inhibiting the growth of several pathogenic microorganisms therefore, the production of organic acids helps to reduce the cytoplasmic pH and stop metabolic activities. Mechanisms other than cytoplasmic pH reduction have been associated with organic acids, leading to death of susceptible organisms (Batish et al., 1997).

Lavermicocca et al. (2000) showed that *L. plantarum* produce of the antifungal compounds like, phenyllactic acid and 4- hydroxy phenyllactic acid have antifungal

activity against several microorganisms. Ström et al. (2002) also reported that *Lactobacillus* produced various antifungal compounds, like cyclic dipeptides, pyroglutamic acid and lactones that have high inhibitory activity against *candida* species, *L. plantarum* Mi LAB 393 produced three antifungal compounds cyclo (L-Phe-L-Pro), cyclo (L-Phe-trans-4-OH-L-Pro) and phenyllactic acid which have high antifungal activities against moulds and yeasts.

2.5.3.2 Hydrogen peroxide

Hydrogen peroxide is produced by most LAB in the presence of oxygen (Kandler, 1983). LAB produce hydrogen peroxide which can decrease the growth of many undesirable microorganisms. (Schnürer & Magnusson, 2005; Zalan et al., 2005). Various studies show that LAB strains inhibit the growth bacteria and fungi species by the produced H_2O_2 (Falagas et al., 2007). Hydrogen peroxide change to reactive oxygen species such as super peroxide anions and hydroxyl free radicals which are highly toxic against several pathogenic microorganisms (Kullisaar et al., 2002).

Jaroni and Brashears (2000) reported that *L. delbrueckii* subsp. *lactis* produce H_2O_2 which has high effect against several pathogens microorganisms, and Ito et al. (2003) showed that cell free supernatant produced by *L. casei* subsp contain H_2O_2 that has high effect against food borne pathogens like, *Listeria*, *Yersinia* and *Aeromonas* spp. Recently, Hasslof et al. (2010) showed that *L. plantarum* strains and *L. reuteri* ATCC 55730 produce H_2O_2 which is responsible for inhibitory effect against *C. albicans*.

2.5.3.3 Bacteriocins

Some LAB produce different types of bacteriocins, several reports demonstrated that bacteriocins can be used as preservation of food, also have inhibitory effect against food borne and human pathogenic bacteria (Cleveland et al., 2001; Forouhandeh et al., 2010) and (Oh et al., 2000) showed that *L. acidophilus* produce bacteriocins as first antimicrobial compound. Also Zalan et al. (2005) added that bacteriocins produced by *L. plantarum* 2141 has inhibitory effect against *C. glabrata* and *L. sakei*. Recently, Maria and Janakiraman, (2012) demonstrated that *L. acidophilus* NCIM 5426 has high inhibitory effect against food borne and human pathogens.

2.5.3.4 Biosurfactants

Biosurfactant is amphipathic compound produced by LAB which has antimicrobial and anti-adhesive activity against several pathogenic microorganisms (Das & Mukherjee, 2007; Pascual et al., 2008). Rodrigues et al. (2006a) showed that biosurfactant produced by *L. fermemtum* RC-14 had anti-adhesion activity against uropathogenic bacteria and reduce infection caused by *S. aureus*. Rodrigues et al. (2007) reported that biosurfactants produced by *L. acidophilus* prevent microbial biofilm formation on silicone rubber voice prostheses. Gudiña et al. (2010a) demonstrated that biosurfactants produced by *L. paracasei* ssp. A20 inhibited the growth of *C. albicans*. Tahmourespour et al. (2011) reported that biosurfactant secreted by *L. acidophilus* reduced the adhesion of *S. mutans* to glass side. Recently, Gomaa (2013) reported that *L. fermentum* has high anti-adhesion activity against *C. albicans* 70014 by 59.37%. Consequently, biosurfactant can be used as preventive strategy to pathogenic biofilm formation on

medical devices in biomedical field and also in the food industry (Falagas & Makris, 2009).

2.5.4 Mechanisms of the antifungal activity of compounds produced by LAB

The mechanisms of antifungal activity of compounds produced by LAB are not fully understood. The effect of organic acids such as lactic, acetic and propionic acids produced by LAB on the growth of many pathogenic microorganisms is by interfering with the maintenance of cell membrane, inhibiting active transport, reducing intracellular pH and inhibiting a variety of metabolic function (Lindgren & Dobrogosz, 1990). The effect of organic acids on inhibitory activity against microorganisms is related to the pH value (Kashket, 1987; Ljungh & Wadström, 2009). In addition, to acids LAB can produce other antimicrobial metabolites such as H_2O_2 which has strong effect on membrane lipids and cellular proteins and is produced by enzymes such as NADH peroxidase, NADH oxidase and x-glycero phosphate oxidase (Daeschel, 1989; Reid, 2008). The mode of action of bacteriocins produced by LAB is the formation of holes in the membrance of the target microorganisms by depleting the transmembrane potential and the pH gradient which cause the leakage of the cellular materials and the death of microorganism (Chung et al., 2000; Cleveland et al., 2001; Parada et al., 2007; Todorov, 2009).

2.6 Identification of lactic acid bacteria

The molecular biology has developed very fast and this leaves a high impact on the microbiology world. The identification of LAB using API 50 CHL Kit method (API system, BiomMerieux, France) is conventional method and it based on sugar fermentation which may not always offer sufficient basis for the reliable identification

of the LAB (Ahmed & Kanwal, 2004; Nair & Surendran, 2005; Ashmaig et al., 2009). Recently, the genetic techniques developed to identify LAB especially polymerase chain reaction (PCR) based methods such as 16S rDNA sequencing has become commonly used in microbiology and biotechnology for identification of the bacteria specially LAB. In this method two primers 16S.S: (5-AGAGTTTGATCCTGGCTC-3) and 16S.R. (5 CGGGAACGTA TTCACCG-3) are suitable for identification of LAB (Schleifer & Ludwig, 1995).

2.7 Pathogenic *Candida* species

Pathogenic *Candida* spp. are the most common cause of hospital- acquired infections especially, in cancer and transplant patients which use immunosuppressive therapy. *Candida* species cause many human infections such as urinary tract infection, vulvovaginal candidiasis (VVC), and nosocomial pneumonias (Carlson et al., 2000; Chandra et al., 2001; Douglas, 2003; Ramage et al., 2006). *Candida* species have potential resistance to several antifungal agents like fluconazole, voriconazole and amphotericin B (Odds, 2005; Panizo et al., 2008).

Candida spp. are able to adhere to surface medical devices such as pacemakers, joint replacement, prosthetic heart valve, silicone voice prostheses, end tracheal tubes, catheters and cerebrospinal fluid shunts. These devices can become colonized by *Candida* spp. which form biofilm and this biofilm leads to acute disseminated infection because of increased resistance to antifungal therapy (Ramage et al., 2006; Silva et al., 2011).

2.7.1 Biofilms

Biofilms are aggregates of microorganisms, which are formed due to the attachment of cells to host surface in aqueous environment (Lynch et al., 2003). *Candida* species are responsible for many infections such as vaginitis, urinary tract infections and infection of the nails and skin (Table 4). Nevertheless, more than 90% of infections are caused by *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* (Pfaller et al., 2007). Biofilms formed from *Candida* species are difficult to treat due to increased resistance to antifungal agents (Ramage et al., 2006; Klotz et al., 2007; Harriott & Noverr, 2011). Growth of biofilms is effected by some factors such as medium composition and temperature. Furthermore, the ability of *Candida* to form biofilm vary. There are strong and weak biofilm (Kumamoto & Vinces, 2005; Seneviratne et al., 2008). In other studies (Seneviratne et al., 2008; Sardi et al., 2014) showed that biofilms of *Candida* spp. were associated with increased resistance to antifungal agents.

TABLE 4: *Candida* species causing life-threatening infection to humans.

***Candida* strain**	**Clinical importance**	**References**
C. albicans	Superficial diseases (oral and vaginal trush) Endogenous fungal Endophthalmitis Sexually transmissible disease	(Molero et al., 2010) (Lingappan et al., 2012) (Ogunshe et al., 2009)
C. glabrata	Vulvo-vagina candidiasis	(Martens et al., 2004)
C. tropicalis	Nosocomial candidaemia Chronic mucocutan Eouscandidiasis Cause infecions in patient with leukemia	(Kothari & Sagar, 2009) (Weder et al., 2004) (Dixon et al., 2004) (Barasch et al., 2004)
C. krusei	Cause infections in patients with bone marrow transplant recipients	(Barasch et al., 2004)
C. parapsilosis	Candidaemia Infections	(McNeil et al., 2001)

2.7.2 Resistance of *Candida* spp. to antifungal agents

Candida spp. have ability to form biofilms due to resistance to antifungal therapy and the ability of yeast cells within the biofilms to withstand host immune defences. Several *Candida* spp. have become resistance to many antifungal drugs such as amphotericin B, fluconazole and itraconazole (Mishra et al., 2007; Pfaller et al., 2011). Al-Abeid et al. (2004) reported that *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. parapsilosis* and *C. lusitaniae* have higher resistance against fluconazole than *C. albicans*. Suggested mechanisms of antifungal drugs resistance are lower penetration of drugs to biofilm formation, presence of persister cells and overexpression of resistance genes CDR1 and CDR2 (Mukherjee & Chandra, 2004; Sardi et al., 2011).