DETECTION OF ANTIMICROBIAL EFFECT OF SILVER NANOWIRES EMBEDDED IN POLY LACTIC ACID (PLA) AND FILTER PAPER ON PATHOGENIC BACTERIA

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ABSTRACT

DETECTION OF ANTIMICROBIAL EFFECT OF SILVER NANOWIRES EMBEDDED IN POLY LACTIC ACID (PLA) AND FILTER PAPER ON PATHOGENIC BACTERIA

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The use of silver nanowires (Ag NWs), newly developed nanomaterials, have been demonstrated in prototypes devices such as solar cells, touch screens and light emitting diodes. Although silver nanoparticles have been used as an antimicrobial agent due to their disrupting effect on proliferation of microorganisms, testing Ag NWs in this field is the virgin side of the food packaging design. In this study antimicrobial effect of Ag NWs embedded in filter paper (FP) and polylactic acid (PLA) were investigated.

The aim of this study was to determine the antimicrobial effects of silver nanowires using Disk Diffusion method (DD), Viable Bacteria Count (VBC) and modified version of ISO Plastic-Measurement of antibacterial activity on plastic surfaces on both Gram negative and positive bacteria.

By disk diffusion method (DD), we observed 8-11 mm clear zone on Gram negative, 8-14 mm on Gram positive bacteria using Ag NWs embedded in FP.

On the other hand, no clear zone formation was observed for Ag NWs embedded in PLA and no antimicrobial growth underneath. Results obtained herein gave a clear understanding of the antimicrobial effect of Ag NWs, contact packaging material that can be used in food industry. Ag NWs are highly promising to be used in packaging applications with no direct contact with food.

Keywords: Antimicrobial, silver nanowire, filter paper, poly lactic acid.

ÖZ

POLİLAKTİK ASİT FİLMLERİNE VE FİLTRE KAĞITLARINA TUTTURULMUŞ GÜMÜŞ NANOTELLERİN ANTİMİKROBİYAL ETKİLERİNİN BELİRLENMESİ

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Yakın zamanda geliştirilmiş gümüş nanoteller; günümüzde güneş panel hücresi, kıyafet, dokunmatik ekran ve kaplamalarda kullanılmaktadır. Gümüşün antibakteriyel özelliklerinden biri olan mikroorganizmanın kendini yenileyebilme özelliğini bozmasından dolayı antimikrobiyal ajan olarak kullanılan gümüş, gıda paketlemesi alanında yeni keşiflerin yapıldığı bir materyaldir. Bu çalışmada filtrasyon kâğıdı ve polilaktik asit gümüş nanotelin özelliklerini gözlemleyeceğimiz ana paketleme ürünleri olarak kullanılmıştır.

Bu çalışmanın amacı gümüş nanotellerin Gram negatif ve pozitif bakteriler üzerindeki antimikrobiyal etkileri Disk Difüzyon, Canlı Bakteri Sayımı ve üzerinde değişimler yapılmış ISO-Plastikler-Plastik Yüzeyler Üzerinde Antibakteriyel Hareketliliğin Ölçümü metotlarını kullanarak belirlemektir.

Disk difüzyon metoduyla filtrasyon kâğıtları çevresinde Gram negatif bakterilerde 8-11 mm ve Gram pozitif bakterilerde 8-14 mm boyutlarında

inhibisyon bölgeleri gözlemlenmiştir. Fakat bu metotla yapılan polilaktik asit örneklerinin çevresinde herhangi bir temiz bölge gözlenmemiştir. Gümüş nanoteller antimikrobiyal ajan olarak polilaktik asit içerisindekine kıyasla, filtre kâğıtlarında daha etkili olmuşlardır. Bu sonuçların ışığında gümüş nanotellerin antimikrobiyal etkisine ilişkin daha net bir görüş edinilmiştir. Edinimler doğrultusunda gümüş nanotellerin gıdayla direk teması olmayan bir paketleme tasarımında kullanılabileceği düşünülmektedir. Antimikrobiyal aktiviteye sahip olduğu için gümüş nanoteller gıda sektöründe gelecek vaat eden bir ambalaj malzemesi olabilir.

Anahtar kelimeler: Antimikrobial, gümüş nanotel, filtre kağıdı, polilaktik asit.

To my family

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= number

% = percentage

(v/v) = volume by volume

(w/v) = weight by volume

≥ = equal and bigger or higher than

°C = degree celcius

µL = microliter

µm = micrometer

1D = one dimensional

1S = one surface embedded

2S = two (both) surfaces embedded

Ag^+ = Silver ion

$AgNO_3$ = Silver nitrate

Ag NP = Silver nanoparticle

Ag NW = Silver nanowire

ATP = Adenozin trifosfat

BHI = Brain heart Infusion

cfu = colony forming unit

CNT = carbon nanotubes

DAEC = Diffusely adherent *E. coli*

DD = Disk Diffusion method

EAEC = Enteroaggregative *E. coli*

EIEC = Enteroinvasive *E. coli*

EPEC = Enteropathogenic *E. coli*

et al. = *et alii* (and others)

etc. = etcetera

ETEC = Enterotoxigenic *E. coli*

EtOH = ethanol

FP = Filter paper

g = gram

Gr (-) = Gram negative

Gr (+) = Gram positive

GRAS = generally recognize as safe

LDPE = low density polyethylene

M = molarity

m/o = microorganism

mg = milligram

mg/mL = miligram per milliliter

MH = Mueller Hinton

mL = milliliter

mm = millimeter

MRSA = *Methicillin-resistant Staphylococcus aureus*

nm = nanometer

NT = Not treated after embedding process of silver nanowires

OPS = oriented polystyrene

PCA = Plate count agar

PET = polyethylene terephthalate

pH = power of hydrogen

PLA = Polylactic acid

PP = polypropylene

PVP = Poly (vinylpyrrolidone)

ROS = Reactive oxygen species

rpm = revolution per minute

sp. = species

STEC = Shiga toxin producing *E. coli*

subsp. = subspecies

UV = ultraviolet

V = volt

VBC = Viable Bacteria Count method

w/Ag NW = with silver nanowire

CHAPTER 1

INTRODUCTION

Foodborne pathogens are major threats for the humankind. The main reasons that make people to suffer from the foodborne diseases are the non-compatibility or inefficacy of current prevention techniques with the changing food conditions and dietary habits of consumers. Foods with low fat content or that are freshly consumed have become a new trend for about a decade. Decrease in the fat content and fresh foods, which are not heat treated might become the possible source for the foodborne pathogens. Therefore to maintain the safety and quality of foods, active packaging materials that collaborate with the foods are under development. Commonly used active packaging materials have been designed to track and maintain the quality of the foods. Because of the food safety issues, especially, new active packaging designs start to play bigger roles in the preservation of the food from foodborne pathogens. Using essential oils and metals as antimicrobial agents, active packaging materials have been designed as a protection layer on food against the contaminants. Silver has been used as an antimicrobial agent. Due to silver's inhibitory effect on foodborne pathogens, the use silver as in food packages has become a new trend in the active packaging field. However, the effects of silver nanowires (Ag NWs), novel one dimensional nanostructure of silver have not been investigated in food packages against pathogenic bacteria.

1.1 Foodborne Pathogens

Foodborne pathogens are one of the most important reasons to cause diseases through the contaminated foods. Due to the characteristics of the pathogens and their adaptation period to stress as well as mishandling and cross-contamination, food safety is an essential issue. In addition, trends towards the healthy foods such as low fat content or minimally processed food are some of the cases that foodborne pathogenic bacteria may easily grow and cause foodborne diseases (Danielsson-Tham, 2014a).

Foodborne diseases can be caused by a wide range of microorganisms such as bacteria, viruses and parasites. In addition, the food contamination can occur at any step from production of food to its consumption (Worl Health Organization [WHO], 2015). Although, there are different kinds of microorganisms can cause foodborne infections, pathogenic bacteria is more traceable than viruses or parasites. Common symptoms of the food infections due to pathogenic bacteria are stress in gastrointestinal system, abdominal cramps, diarrhea and vomiting. However, some foodborne pathogens can also cause life-threatening cases. Since, foodborne diseases with very mild symptoms cannot be diagnosed, the exact number of cases cannot be determined. According to World Health Organization (WHO), one of every ten people gets sick from foodborne pathogens in every year, and 420 000 people die because of it. Especially children under age 5, are at a high risk group with an annually 125 000 death occurring due to the consumption of contaminated foods (WHO, 2015). The major agents of foodborne diseases worldwide are non thyphoidal *Salmonella*, pathogenic *E. coli*, and *L. monocytogenes* (Scallan et al., 2011; WHO, 2015).There had been many outbreak cases in the history. For example, it was suspected that the Great Alexander and his soldiers died from typhoid fever, transmitted through contaminated water with *Salmonella* Typhi (Anderson, 2011).

According to the Gram Staining test, bacteria are divided through their cell membrane characteristics such as Gram negative and positive. In the Gram staining test, the Gram positive bacteria, which take the crystal violet dye up in to the cell and at the end of the test appear as purple colored. This situation occurs due to the large amount of peptidoglycan structure in the cell membrane of the Gram positive bacteria such as *Staphylococcus aureus* and *Listeria monocytogenes*. Gram negative bacteria such as *Escherichia coli* and *Salmonella* subsps., leak the crystal violet dye through the process and take the counterstain, safranin dye, up into the cell membrane. This occurs because of their thinner peptidoglycan layer. At the end of the Gram staining test, Gram negative bacteria appear pink under the microscope. The cell membrane features of the bacteria do not affect its pathogenicity. However, cell membrane characteristics may affect the bacteria's resistance against the antimicrobial agents.

1.1.1 Gram Negative Pathogenic Bacteria

Pathogenic *Escherichia coli*

Pathogenic *Escherichia coli* was first found in infant stool by Theodor Escherich in 1885. However, *Escherichia* was not identified up to 1960, and there are still some strains that are yet unknown (Bell & Kriakides, 2002). Some strains of the *E. coli* are found naturally in human and animal intestinal, however six subgroups of *E. coli* isolates are distinguished from the other isoletes, which are Shiga toxin producing *E. coli* (STEC), Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC), Diffusely adherent *E. coli* (DAEC) (Bell & Kriakides, 2002; CDC, 2014a). Infection of pathogenic *E. coli* was given in detail in Table1.1.1.

Salmonella subspecies

Salmonella is a well-known foodborne pathogen. Its first proven outbreak case occurred in 1888. A researcher called Gaertner isolated the bacteria from a cow and a man who consumed that cow meat (Wilson, 1946). Approximately 2500 different type of *Salmonella* strains are known today (Brenner, Villar, Angulo, Tauxe, & Swaminathan, 2000). Each year 1.2 million hospitalization and 450 deaths occur, due to non-thphodial *Salmonella* Only in the U.S. (CDC, 2015). Although standards are regulated by the authorities in processing plants, number of outbreaks, caused by consuming contaminated foods with *Salmonella,* has been accelerated in the last five years. As most microorganisms, *Salmonella* also adapts the geographical conditions and evolve itself according to the environment. Due to this adaptation, some *Salmonella* strains that cause outbreaks have been originated from overseas countries. *Salmonella* infection's detailed information is provided in Table1.1.1.

1.1.2 Gram Positive Pathogenic Bacteria

Listeria monocytogenes

Murray was the first researcher that isolated the *L. monocytogenes* in 1924 (Murray, Webb, & Swann, 1926). Initially *L. monocytogenes* was referred as a pathogen that causes disease in animals such as cat, dog, sheep and pig. This pathogen had not been classified as foodborne pathogenic bacteria for humankind until developing the procedures for classifying according to its pathogenicity. In 1981, *L. monocytogenes*' s early identified outbreak occurred in Canada (Bhunia, 2008; Tham & Danielsson-Tham, 2014). Especially, *L. monocytogenes* is one of the main foodborne pathogens that cause severe diseases. Immunocompromised adults, pregnant women and infants are in high

risk group that can develop severe symptoms. In addition, *Listeria monocytogenes* can survive from harsh conditions such as high salty environment or very low temperatures in the refrigerator and may contaminate. It is known that most of the outbreaks have occurred due to the mishandling or improper preparation by the consumers (Scott, 2003). Detailed information about *L. monocytogenes* infection and listeriosis is given in Table1.1.2.

Staphylococcus aureus

Staphylococcus aureus is a naturally found bacterium in nose or skin of healthy people. Roughly 30 % of the people carry this microorganism in their nose (Argudín, Mendoza, & Rodicio, 2010; CDC, 2010). Due to its enterotoxin producing capability, *S. aureus* is a noteworthy pathogenic bacterium with high food poisoning ratio around the world (Danielsson-Tham, 2014b). *S. aureus*'s enterotoxin is the main reason for foodborne diseases. *S. aureus* and its enterotoxin drew attention in Cold War due to its potential for being a biological agent (Pinchuk, Beswick, & Reyes, 2010). By inhalation or intact with open wounds both *S. aureus* and its toxin can affect significant number of people (CDC, 2010). Information about the source, incubation period and symptoms of *S. aureus* infection are given in Table1.1.2.

Table 1. 1. 1 Source, incubation period and disease symptoms of *Escherichia coli, Salmonella* subspecies as Gram negative pathogenic bacteria.

Bacteria	Source of infection	Incubation period	Symptoms	#of cell for infection
Escherichia coli	Contaminated food, not heat treated and possibly risky foods (raw milk, meat etc.) Animal skin or feces Feces of infected person	1-10 days	Diarrhea, abdominal pain, nausea, vomiting	10^6-10^8cfu
Salmonella	Contaminated food, not heat treated and possibly risky foods (raw milk, meat etc.) Animal skin or feces Feces of infected person	6-72 hours	Diarrhea, abdominal pain, nausea, vomiting	$\geq 10^5$cfu

Source: Adapted from Bell & Kriakides, 2002; Bhunia, 2008; Brenner et al., 2000; CDC, 2014, 2015b

Table 1. 1. 2 Source, incubation period and disease symptoms of *Listeria monocytogenes, Staphylococcus aureus* as Gram positive pathogenic bacteria.

Bacteria	Source of infection	Incubation period	Symptoms	#of cell for infection
Listeria monocytogenes	Commonly seen in soil Contaminated food, not heat treated and possibly risky foods (raw milk, meat etc.) Animal skin or feces	1-4 days	Fever, muscle aches, diarrhea, nausea, vomiting, abortion	10^2-10^6cfu
Staphylococcus aureus	Human and animal skins (open wounds) Handmade foods (sandwiches, bakery products, salads) Contaminated and improper treated dairy products, meat and poultry	1-6 hours	Diarrhea, nausea, vomiting, fever, appetite loss and abdominal cramps	$\geq 10^6$cfu

Source: Adapted from Bhunia, 2008; CDC, 2014b

1.2 Packaging Material

Food packaging is used to maintain the quality of the food during distribution to the consumers. Physical, chemical or biological protections are some of the functions of packaging. With these protections, extension of shelf life is aimed. Polyethylene, polystyrene, polypropylene and polyvinyl chloride are the commonly used plastic packaging materials (Bhatia, Gupta, Bhattacharya, & Choi, 2012). These packaging materials slowly degrade or decompose in the environment. Due to this degradation, hazardous materials can pollute air and water sources. To prevent plastic pollution, consumers and manufacturers have preferred environmental friendly food packaging materials. Today, roughly 3 million tons of bioplastics have been produced (European Bioplastics, n.d.). Demands in the market and regulations encourage the use of biobased plastics and it is expected that their use will increase according to European Bioplastics. By 2019, biobased production is expected to reach approximately 8 million tons (European Bioplastics, n.d.). Many companies are encouraged to produce environmental friendly packages to decrease the pollution and production cost by choosing the raw materials of food packaging from the waste food products. Among these materials, polylactic acid (PLA) is one of the most promising one (Drumright, Gruber, & Henton, 2000). PLA is one of the biodegradable and biobased packaging materials, which has been commercially produced by since it is an affordable and easy accessible raw material.

1.2.1 Polylactic Acid Production and General Characteristics

PLA is produced from lactic acid monomers by fermenting the bulk starch, such as corn or sugar cane (Robertson, 2012). It is a linear aliphatic polyester with a glass transition temperature of 60^{o}C and its melting temperature is between 130-180^{o}C. PLA has high transparency and water solubility and good strength among polymers (Lunt, 1998; Robertson, 2012; Siracusa, Rocculi,

Romani, & Rosa, 2008). In some cases, PLA can give better results rather than polyethylene terephthalate (PET) or oriented polystyrene (OPS) such as holding back the aqueous vapor and resistance to oil and acids (Auras, Singh, & Singh, 2005). The first application for commercial production of PLA was done by Cargill and they developed a convenient process for its cost-effective production (Drumright et al., 2000; Lunt, 1998). PLA can be produced from petroleum components and food waste (Bhatia et al., 2012)

Once the production of PLA has achieved using the food waste, packaging using PLA started to be more affordable and environment friendly (Lunt, 1998). Because of its biodegradable and biobased nature, PLA happened to be the most feasible biobased polymer (Bhatia et al., 2012). In food industry, PLA as a packaging material was first used as a yogurt cup in 1998, and since then, it has been used as cups or bags for fresh products or bakery. According to the temperature of the drink it is also used as a coating material for the cups (Weber, Haugaard, Festersen, & Bertelsen, 2002). In addition to all these, toxicological effects of PLA were also investigated. Although PLA were subjected to aqueous, acidic and fatty food contacts apart from the migration of lactic acid the other components such as dimers or trimmers of the lactic acid were observed as a negligible and safe amounts (Conn et al., 1995). PLA has been identified as generally recognized as safe (GRAS) (Siracusa et al., 2008).

Active packaging, providing mainly biological and chemical protections, became popular because of its efficiency at increasing the shelf life and the safety of the foods. Using antioxidant and/or antimicrobial agents in the packaging materials are some of the ways to eliminate or retard the chemical or biological contamination. In particular, metals and essential oils have been used as active agents in food packaging. By increasing the efficiency of the package, producers have achieved to provide safe food. These agents, such as silver, mostly proceed to interact with the food during the shelf life.

1.3 Antimicrobial Agents

From the first antibiotic, sulfanilamide, as commercially attainable in 1935, the studies had been continued and significant number of antimicrobial agents have been used in our daily life (Heikinheimo, 2014). However, stress adaptation is one of the main characteristics of the bacteria and they gain the resistance from the start of the stress. Because of this adaptations ability to the antibiotics (or any other stress), researchers have proceed to develop other methods that would collaborate with the antibiotics. One of these methods is active packaging to stop or slow down the microbial growth in foods. The new trend showed that there has been a progress using metals such as silver as an antimicrobial agent in packaging materials. In fact the antimicrobial effect of silver had been used before the antibiotics. Persians, Greeks, Romans and other cultures used Ag in their water containers to keep their water safe (Alexander, 2009). Before knowing the influence of A it was also used by Hippocrates on the wounded patients (as cited in Alexander, 2009). Currently, researchers have been focusing on silver as an antimicrobial agent in packaging materials. Many studies have shown the significant inhibitory effect of Ag on the growth of foodborne pathogenic bacteria, both Gram positive and negative. Moreover, technical improvements to use different nanostructures such as nanowire or nanoparticle, have been improving its antimicrobial.

1.3.1 Silver

Ag is a valuable metal, used in jewelery and icons since 3000 B.C. (Lansdown, 2010). Initially, Ag was only affordable by a group of wealthy people. With time its use has been expanded to coins and plates. Ag and its derivatives are in use in a wide range of materials such as batteries, electronics, mirrors and medical devices (Reuters et al., 2016.; The Silver Institute, n.d.). At the

beginning of the 20th century, silver's antimicrobial efficiency, preventing or retaining the growth of microorganism, has been recalled and the new trend has extended the range in textile and food packaging for a decade.

Ag has been used for water sterilization in hospitals to prevent *Legionella* sp. and *Methicillin-resistant Staphylococcus aureus* (MRSA) growth (Hambidge, 2001; Rohr, Senger, Selenka, Turley, & Wilhelm, 1999). In addition to that, Ag was also used as dental amalgams for many years, because of its durability and being an inexpensive treatment (FDA, 2015). Ag usage is not limited with the devices or flourishing the houses, it is also used as a medicinal or medicine compound such as treating the ulcer wounds or as an antismoking remedy (Chambers, Dumville, & Cullum, 2007; Lansdown, 2010; Sreelakshmy, Mk, & Mridula, 2016). Moreover, Ag is also used in food packaging systems with the biodegradable and typical polymers to prevent biological contaminations.

Many researchers with the current technology have shown the effect and route of Ag as in cellular base. In case of bulk amount of Ag, only presence of free Ag^+ can affect bacteria or fungi. Moisture, temperature, pH and time are the requirements to release free silver ions from bulk form of silver. It is known that at 25°C, the ionization of Ag is under 1 mL^{-1} (Burrell, 2003). In order to increase ionization, the surface area of silver should be increased (Ovington, 2001). Thus, researchers have been working on Ag nanoparticles to set Ag release. Studies are conducted at nanoscale showed that ionization of nanoparticles is 70-100 times effective than the other forms such as wire or powder (Lansdown, 2010).

For about half of a century, researchers have been working on nano Ag such as nanoparticle phase or nanowire and silver's bactericide effect in the nanostructure form is one of the most popular research topics (Silver Institute, n.d.). These researchers have been conducted on cellular uptake of Ag in

eukaryotes. It is known that, nano forms of Ag can enter the cell wall, if it is small enough. Moreover, disfunction of cell may occur by free silver ion migration through the cell. Ag works as an antimicrobial agent that causes inhibitory effect on growth of eukaryotic cells.

1.3.2 Nanotechnology of Silver

1.3.2.1 Silver Nanoparticle

Nanoparticles have some unique features such as enhanced optical properties or electrical conductivity due to their enhanced surface to volume ratio and quantum coefficient effects (Evanoff & Chumanov, 2005). Today, over 1600 products are in the market that make use of nanotechnology (Vance et al., 2015).

The size of Ag nanoparticles (Ag NP) should be between 1nm and 100 nm. However, the shape of the Ag NPs can change through the practice, it can be round, cubic or spherical (Khodashenas & Ghorbani, 2015). With the shape and size differences, characteristics of Ag NPs can also change. Ag concentration, temperature and pH are some of the coefficients that change the size and alter the properties of the Ag NPs (Gurunathan, 2015). During synthesis Ag NPs have been widely produced nanostructures of Ag. To enhance Ag antibacterial effect the surface area to volume can be increased.

Because of its relatively efficient characteristics at nanoscale of Ag, bactericidal actions of Ag NPs have been investigated and reported on pathogenic bacteria. Ag NPs and free silver ions (Ag^+) basically cause damage on the membrane, ion balance and reproduction mechanisms of eukaryotic cells (Gopinath et al., 2010; Roh, Eom, & Choi, 2012; Schrand et al., 2008).

Uptakes of Ag NPs and Ag^+ occur in two ways from surface or internal interaction. Internal interaction can occur as aggregation, oxidation and free ion

releasing from the coating agent (McShan, Ray, & Yu, 2014; Reidy, Haase, Luch, Dawson, & Lynch, 2013; Sanford & Venkatapathy, 2010). Ag NPs can get through the cell by three ways, which are (i) diffusion, (ii) endocytosis and (iii) with the help of the membrane proteins. With these ways, Ag^{+} and Ag NPs interrupt mitochondrial functions and eukaryotic cell produces reactive oxygen species (ROS) (Asharani, Hande, & Valiyaveettil, 2009). This leads to inhibition of the cell growth at the end of the uptake (Haase et al., 2012; He, Dorantes-Aranda, & Waite, 2012; Li, Zhang, Niu, & Chen, 2013; Roh et al., 2012; van Aerle et al., 2013). Increasing the production of ROS causes oxidative stress in the cell. Many enzymes and enzyme activities get damaged, due to this increase in the oxidative stress. Consumption of glutathione and sulfhydryl groups, having a role in the protein bonds, is one of the reasons for altering these enzymes and their activity (Ahmadi & Kurdestany, 2010; Awasthi et al., 2013; Haase et al., 2012; He et al., 2012; van Aerle et al., 2013). Ag NPs and Ag^{+} mostly react with proteins because of silver's affection for sulfur (Ahamed et al., 2008; Asharani et al., 2009; Choi et al., 2009; Levard et al., 2013). Mitochondria, critical part of the cell, are vulnerable to Ag NP and Ag^{+}(Bressan et al., 2013). Damage given to the mitochondria triggers the ROS production as well as altering and inhibition of the ATP synthesis and lastly might impair the DNA (Asharani et al., 2009).

Although concerns about toxicity of Ag have been argued and examined by the researchers, silvers' inhibitory effect on microorganisms directs the producers to utilize Ag NPs in products. The migration of the Ag NPs should also be examined with the food to mimic the real life conditions (Gallocchio et al., 2016). Gallocchio and his colleagues conducted a study with chicken meatballs, which were stored in plastic bag with Ag NPs. Commonly observed refrigerator temperatures were set as the storage temperature. Without any treatment to Ag NPs, no migration was observed from the bag to the food.

However, studies show that plastic bag with Ag NPs were hold under 40^{o}C and treated with a 5 % ethanol v/v, 3 % acetic acid v/v solution, migration of Ag NPs and Ag^{+} to foods occurred (Echegoyen & Nerín, 2013). The results of Echegoyen and Nerin's study show that approximately 20 % of Ag NPs were migrated from the packages such as polyolefin, low density polyethylene (LDPE) and polypropylene (PP) (2013). These examples prove that only in proper conditions Ag NPs could migrate from the package. In addition, migration of Ag and its nano forms from packaging material are not authorized by the European Commission Regulation (EU) in direct contact with food No.10/2011. Because of that, indirect contact packaging or production contact surfaces could be the best practice to use Ag to eliminate microorganism and biofilm formation.

1.3.2.2 Silver Nanowire

Ag NWs are one dimensional (1D) nanoscale materials (Coskun, Aksoy, & Unalan, 2011) (Figure 1.3.2.2.1). Diameter and length of Ag NWs can be tuned through practices like changing synthesis temperature or injection rate of silver during synthesis (Coskun et al., 2011). Because of its commonly used synthesis protocol Ag NWs do not show any agglomeration (Andrew & Ilie, 2007). In addition to that, because of its chemical properties, silver is a metal that less prone to oxidation compared to other metals like copper. This feature provides long term stability within products for manufacturers and consumers. With all these features Ag NWs have high potential to be utilized in electronics, nano-sensors(Lin, Yao, McKnight, Zhu, & Bozkurt, 2016). On the other hand, toxicological studies on Ag NWs have been started to be reported, there are only a few studies on the food related ones.

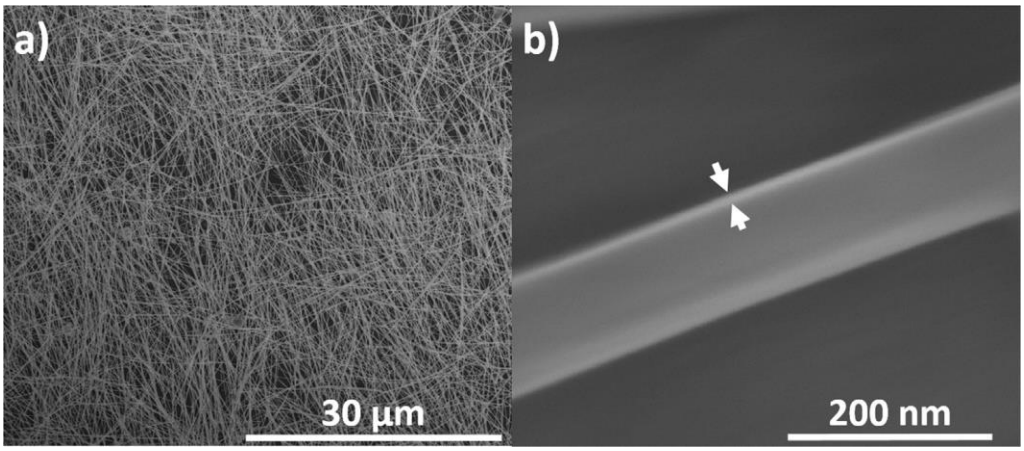

Figure1. 3. 2. 2. 1 Scanning electron microscopy (SEM) images of (a) Ag NWs and (b) an individual Ag NW (Doganay, Coskun, Kaynak, & Unalan, 2016). Arrows shows the polymer layer on the side surfaces Ag NW.

Due to Ag NW's exceptional electrical properties, most of the studies are based on their transport properties even for filtration and purification. In 2010, Schoen and his colleagues designed a filtration system. Ag NWs with carbon nanotubes (CNTs) were used together with cotton to increase the efficiency of the design. While contaminated water passes through the system, microorganism were eliminated with the help of the current flow (Schoen et al., 2010). In another example, Ag NWs and CNTs were embedded within a sponge. Due to 1D nature of the utilized nanomaterials a voltage between 5-10 volts (V) was found to be enough to disinfect the water while it flow through the system (Liu & Jiang, 2015). This process received a lot of attention since it is cheap, safe and not causing any residual materials. In addition, both studies have shown that 50 % of the bacteria can be eliminated upon the application of 5 V, whereas 100 % can be eliminated upon 10 V (Liu & Jiang, 2015; Schoen et al., 2010).

Even without an electrical field, Ag NWs were proven as a bactericidal agent. However its migration process is different from the smaller structures such as Ag NPs. Ag NWs disrupt the functions of microorganisms by releasing free

Ag^+ (Jiang & Teng, 2016; Visnapuu et al., 2013). Due to the pore size of the cell membrane, most of Ag NWs cannot get through the membrane.

In addition, due to the polyol method for the synthesis of Ag NWs poly (vinylpyrrolidone) (PVP) remains as a coating material on the side surfaces of nanowires. This PVP layer enhances the suspension of the nanowire in solutions. However, this PVP layer could also limit the release of Ag ions. To accelerate the ion release, PVP layer should be eliminated which could be practiced by a simple UV treatment. In a study about titanium and silver with titanium composites, UV treatment was used to activate the ion release (Page et al., 2007). In another study UV treatment was found to be successful in the removal of the PVP layer through oxidizing (Loraine, 2008).

From our knowledge, the mechanisms of silver nanoparticles and silver zeolites have been investigated. Although toxicology researches have been conducted on nanoparticle form of silver, the action pathways for silver nanowires are mostly assumption with respect to the Ag NPs' effect.

1.4 Aim of the Study

The aim of this study is to examine the antimicrobial effect of Ag NWs on both Gram negative and positive foodborne pathogens. The efficiency of Ag NWs while they are embedded in packaging materials such as filter paper and polylactic acid was investigated. This work is the first study on the antimicrobial effect of Ag NWs on the elimination of the foodborne diseases using Ag NWs in the food packaging. This study was conducted on the most common foodborne pathogens such as *Salmonella enterica* subsp. *enterica*, *L. monocytogenes*, *E. coli* and *S. aureus*. These pathogens were particularly chosen because of their effects on human body and their sources. To observe

the efficiency of Ag NWs on foodborne pathogens, disk diffusion method (DD) and viable cell counting method by pour plate technique were used. In addition, ‘Plastics - Measurement of antibacterial activity on plastics surfaces’ method from Turkish Standard Institute was also conducted to detect effect of Ag NWs embedded in the packaging material.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

2.1.1 Bacterial Strains

Salmonella enterica subsp. *enterica serotypes;* Mbandaka, Enteritidis and Infantis were isolated by Durul et al. (Durul, Acar, Bulut, Kyere, & Soyer, 2015). These strains were isolated from different sources and taken from different locations of Turkey (Table 2.1.1). *Listeria monocytogenes* was isolated from chicken meat in our laboratory (Iqbal, Bulut, Acar, & Soyer, 2015). *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 were chosen strains for this study. As the source of the isolates, human and food were chosen. *Salmonella* strains were chosen regarding particularly to their multidrug resistance and poultry related foods. *S. aureus* and *L. monocytogenes* were chosen due to their membrane characteristics and sources.

Table2. 1. 1 Isolate table

Gram Staining	Genus	Species	Serotype	Isolate Source General	City	Country	Sample Collection
Gr(-)	*Salmonella*	*enterica*	Infantis	Chicken meat	Şanlıurfa	Turkey	2012
Gr(-)	*Salmonella*	*enterica*	Enteritidis	Human	Şanlıurfa	Turkey	2011
Gr(-)	*Salmonella*	*enterica*	Mbandaka	Egg surface	Ankara	Turkey	2012
Gr(-)	*Escherichia*	*coli*	O6	-	-	-	2011
Gr(+)	*Staphylococcus*	*aureus*	Rosenbach	-	-	-	2014
Gr(+)	*Listeria*	*monocytogenes*		Chicken meat	Ankara	Turkey	2014

2.1.2 Chemicals

Chemicals used in the study are given in Appendix D with details (i.e. supplier)

2.1.3 Preparation of Buffers and Solutions

Preparation of buffers and solutions are provided in detail in Appendix E.

2.1.4 Filter Paper

Heinz Herenz Hamburg standard filter papers (FP) were used.

2.2 Methods

2.2.1 Pre-culture of Bacterial Strains

Each strain was retrieved from the stock cultures stored at -80^{o}C (Thermo Fisher Scientific, US). Bacteria streaked on the Brain Heart Infusion (BHI) agar with the help of sterile inoculating loop. Inoculated agars were incubated at 37^{o}C for 16 to 24 hours (ET 120 Oven, Şimşek Laborteknik, Turkey).

After incubation, one of the colonies was chosen from the inoculated agar. The colony transferred to the laboratory test tubes filled with a 5 mL Mueller Hinton Broth by sterile inoculating loop. After this process, labeled test tubes were stirred about at least for 30 seconds and incubated at 37^{o}C for 2 hours, while the shaker of the incubation was set to 150 rpm. Following incubation, turbidity test was determined with 0.5 McFarland. The turbidity test was done by fixing the refraction of the black lines on white paper.

2.2.2 Silver Nanowire Synthesis

Polyol method was used for the synthesis of Ag NWs. For the synthesis, a 0.45 M solution of poly (vinylpyrrolidone) (PVP) was prepared using 10 mL of ethylene glycol and heated to 170^{o}C. After this step, 0.12 M silver nitrate ($AgNO_3$) solution in 5 mL of ethylene glycol was slowly added into the first solution while the mother solution was stirred at 1000 rpm. As the process continued multi twin Ag particles and Ag NPs are formed. Multi twin particles, as the reaction proceeds, form into Ag NWs. After adding the whole $AgNO_3$ solution, the mother solution was annealed for 30 minutes and then cooled to room temperature. To discard the excess PVP, Ag NWs were washed with acetone at a volume ratio of 1:5. Ag NWs were recovered by centrifugation and then were dispersed in ethanol. For following experiments, Ag NWs were taken and dispersed in chloroform solution. Following purification, the purity level of Ag NWs was estimated as 99.5 %. Length and diameter of the Ag NWs utilized in this work on average were 10 μm and 80 nm, respectively. Silver nanowire synthesis was conducted in NANOLAB at Metallurgical and Material Engineering Department of METU.

2.2.3 Preparation of Polylactic Acid Films with Silver Nanowires

To prepare nanocomposite films with Ag NWs, PLA were hold in 80^{o}C for half day. After this process 10 mL of chloroform and 1 g PLA powder were mixed and stirred until PLA dissolved completely within the solution. Solutions with 4 different Ag NWs contents (5, 9, 10, 14 volume %) were prepared with PLA. Final solution was poured carefully onto glass stand to avoid any bubbles with a thickness of 20 μm. As the last step, films were hold at 60^{o}C for a day. Films were then peeled off carefully for further experiments.

were taken from the test tubes by the help of the laboratory tweezer and placed into the petri dish again separated from each other until all the EtOH solution is evaporated.

UV Treatment

Samples were placed in a petri dish. They were not touching each other. The UV treatment (at 254 nm 73 W) was applied for 30 minutes to activate Ag NWs by removing PVP.

Acetic acid Treatment

1 mL of 3 % concentration acetic acid solution was divided to the test tubes. PLA with Ag NWs samples were treated for 30 minutes. In order to remove the excess acetic acid solution from the surface of the PLA at the end of the treatment, samples were placed on a handkerchief and held there until the excess acetic acid solution dried.

2.2.6 Determination of Antimicrobial Properties of Silver Nanowires Embedded in Packaging Materials

Disk Diffusion Method

After adjusting the turbidity of the MH Broth with bacteria, 100 µL of the solution was pipetted from the tube and poured on the MH agar. Suspension was swapped uniformly on the agar by a sterile cotton swab. 6 mm diameter filter papers and PLA discs with Ag NWs were cut and each sample was treated separately with either UV light or ethanol solution. For UV treatment, discs were hold in the cabinet under the UV light precisely for 30 minutes. For ethanol treatment, 1 mL ethanol (70 %) was poured into 6 test tubes and discs were placed in these ethanol solutions. Each treated sample was taken from the test tubes with sterile tweezer. Three replicate for each samples of FP with Ag

NWs and six replicates for each PLA with Ag NWs were done according to the methods.

After the incubation, the zone diameter of FP and PLA with Ag NWs controlled with antimicrobial drug discs for each isolates. The drug response was used as a positive control for disk diffusion tests. *Salmonella* serovars' antimicrobial disk results were already examined by Sinem Acar. The antimicrobial resistance profile were detected for *Salmonella* subsp.; (i) *Salmonella* Infantis; KSTAmpSfN, (ii) *Salmonella* Enteritidis; susceptible and (iii) *Salmonella* Mbandaka; susceptible for 18 antimicrobial drugs (Acar, 2015). The antimicrobial resistance was done according to the antimicrobial disc standard diameters given by Clinical & Laboratory Standard Institute (CLSI) (CLSI, 2002, 2011). Detailed information about antimicrobial resistance of the strains and are provided in Table F.1 (APPENDIX F).

Viable Bacteria Count with Pour Plate Culture Technique

For testing the effect of the Ag NWs in liquid media, Ag NWs within water were used. In first test tube, *S. aureus* were inoculated in BHI broth with the sterile loop and incubated at 37^{o}C for 2 hours. After incubation, 500 µL of UV treated Ag NWs were added to the inoculated BHI broth and incubated at 37^{o}C for 16 hours. In the second test tube, *S. aureus* and 100 µL of UV treated Ag NWs were added into the BHI broth and incubated at 37^{o}C for 16 hours. In the third test tube, *S. aureus* were inoculated in BHI broth and incubated at 37^{o}C for 2 hours. After incubation, 100 µL of not treated Ag NWs were added to the inoculated BHI broth and incubated at 37^{o}C for 16 hours. As positive control *S. aureus* were inoculated to the BHI broth without Ag NWs and incubated at 37^{o}C for 16 hours. Using saline solution 10 fold dilutions were done for the cell suspensions by taking 1 mL from previous dilution in phosphate-buffered

physiological saline solution. From each dilution, 1 mL was taken into the petri dish and plate count agar were poured and uniformly dispersed. The same procedure was repeated for *E. coli*. Afterwards, all the samples were incubated at 37^{o}C for 1 day.

Turkish Standards 'Plastics-Measurement of antibacterial activity plastic surfaces' ISO 22196

Test specimens were cut into 4 cm x 4 cm squares and inoculated 0.1 mL MH Broth was pipetted on the surface of the PLA with Ag NWs. On to the test surface a piece of polyethylene (PE) film was placed as a cover of the test inoculum and incubated at 37^{0}C for 1 day. After incubation, 10 fold serial dilution of the broth in phosphate-buffered physiological saline performed. 1 mL of each dilution that were recovered from the test specimen (PLA with Ag NWs) were pipetted into the sterile petri dish and plate count agar were poured and gently dispersed and incubation step was repeated at 37^{o}C for 40 to 48 hours (Plast, 2014).

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Antimicrobial Effect of Silver Nanowire Solution on Bacteria in the Liquid Media

Viable Cell Count

Ag NWs in water were used to detect the antimicrobial effect of Ag NWs in the liquid environment. Different concentrations of Ag NW solution were used to compare the antimicrobial effect of Ag NWs. For this experiment, the effects of Ag NWs effects on bacterial growth were investigated at different phases of the bacterial growth such as exponential and stationary phases. *E. coli* and *S. aureus* were chosen as representatives for Gram negative and positive pathogenic bacteria respectively.

First, *S. aureus* was inoculated in BHI broth with the sterile loop and incubated at 37^{o}C for 2 hours. After incubation, 500 µL of UV treated Ag NWs was added to the inoculated BHI broth and incubated at 37^{o}C for 16 hours (Figure 3.1.1.a). In this procedure, the effect of Ag NWs on cell growth in exponential phase was observed. A 4 log reduction was observed. At fourth dilution, cell number in the phosphate-buffered physiological saline solution was 45 x 10^4 for this sample.

Then *S. aureus* and 100 µL of UV treated Ag NWs were added into the BHI broth and incubated at 37^{o}C for 16 hours (Figure 3.1.1.b). After this procedure,

a 4 log reduction was observed again. The process gave an idea of the Ag NWs effect on lag phase of the growth of *S. aureus.* At fourth dilution, number of cells in the phosphate-buffered physiological saline solution was 40 x 10^4 for this sample.

Finally, *S. aureus* and 100 µl of Ag NWs were added into the BHI broth and incubated at 37^{o}C for 16 hours (Figure 3.1.1.c). This time a 5 log reduction was observed using Ag NWs without any treatment. At fifth dilution, number of cells in the phosphate-buffered physiological saline solution was 58 x 10^5 for this sample.

As a positive control, *S. aureus* was inoculated to the BHI broth without Ag NWs and incubated at 37^{o}C for 16 hours (Figure 3.1.1.d).

The same procedure was conducted for *E. coli*. Afterwards, all the samples were incubated at 37^{0}C for 1 day. However there was no significant reduction in the growth of the *E. coli* at any step of the viable cell count experiment (FigureA.1).

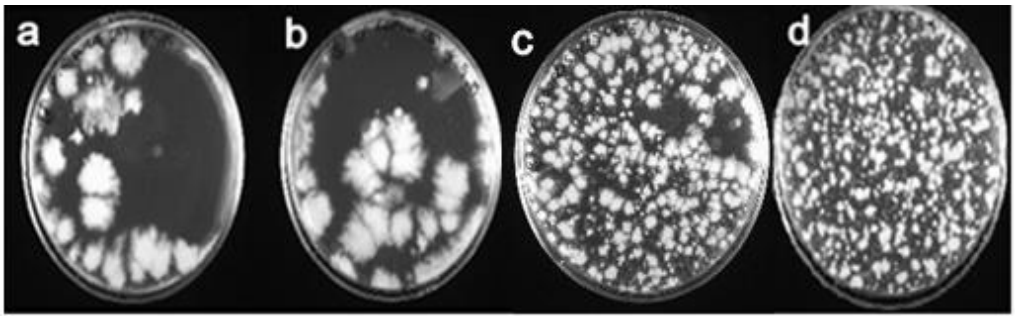

Figure 3. 1. 1 Antimicrobial effect of Ag NWs with water on *S. aureus* in liquid medium. Given photos were taken at fourth dilution: (a) Cell suspension of *S. aureus* with Ag NW solution treated with UV (500 μL); (b) Incubated at the same time with Ag NW solution treated with UV (100 μL); (c) Incubated at the same time with Ag NW solution (100 μL); (d) Incubated *S. Aureus, S. aurues*'s cell number in the dilution was higher than 300 x 10^4 cfu / mL

It is clear (Figure 3.1.1.b) UV treated Ag NWs were more effective than (Figure 3.1.1.c) non-UV treated counterparts. Therefore, it can be said that Ag NWs were retained the cell growth at lag phase of growth of the *S. aureus*. Although higher concentration of Ag NWs was used for (Figure 3.1.1.a) exponential growth phase experiment, UV treated Ag NWs were not very effective at exponential phase of growth of the *S. aureus*. This situation may occur due to cellular uptake of Ag NWs into the *S. aureus* cells at exponential phase of the growth.

3.2 Antimicrobial Effect of Polylactic Acid with Silver Nanowires

Disk Diffusion Method

Disk diffusion method is commonly used to detect the antimicrobial effect of antimicrobial agents like drugs on bacteria.

In this study, Ag NWs were used as antimicrobial agents in PLA (Figure 3.2.1). However, Ag NWs that are used in food packaging films cannot be compared with antimicrobial drugs by disk diffusion method. Since, there is no standardization of foodborne pathogen resistance for metals. In this study, the results of the disk diffusion method cannot give the resistance of the foodborne pathogens to Ag NWs. This method can only demonstrate the effect of Ag NWs on food borne pathogens.

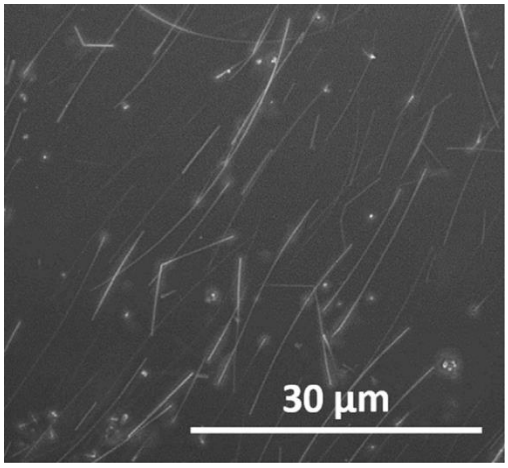

Figure 3. 2.1 Top-view SEM image of 1.74 volume % Ag NW/PLA

For disk diffusion method; petri dishes were divided into six parts to observe the clear zone differences distinctly. PLA films and PLA films with Ag NWs were cut into 6 mm diameter discs. In addition, one sample was separated from each one of the PLA films with Ag NWs and was not subjected to any treatment. Not treated samples were used as a negative control for each experiment. Discs were placed onto the inoculated MH agars with *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *S.* Infantis , *S.* Mbandaka, *S.* Enteritidis and *L. monocytogenes* respectively and incubated 37^{o}C for 1 day. At the end of incubation, it was found that the PLA films without Ag NWs did not inhibit the growth of the pathogens. However, without any treatment, PLA films with Ag

NWs inhibit the growth of the pathogens underneath the PLA films with Ag NWs.

In order to release silver ions from nanostructures, one of the four conditions should be met such as temperature, pH, moisture and time. In Fernández's study, polarity of the environment was changed using ethanol solution, acetic acid and water to increase the free silver ion release from PLA with silver zeolites (Fernández, Soriano, Hernández-Muñoz, & Gavara, 2010). For this study Fernández et al., investigated the antimicrobial effect of silver zeolites on *E. coli* and *S. aureus*. 95 % and 5 % ethanol solutions, 3 % acetic acid solution and distilled water treatments were done to investigate the effect of silver zeolites on *E. coli* and *S. aureus*. The results showed that the use of acetic acid and ethanol solution changed the release rate of silver ions (Fernández et al., 2010). In our work; a 3 % acetic acid solution was found to dissolve PLA films with 9 % Ag NWs (v/v). Therefore, acetic acid treatment was withdrawn. Following a 5 % ethanol solution treatment, no clear zone was observed. Also, after serial treatments of 70 % and 95 % ethanol solutions, we did not observe any clear zone. However, no microbial growth was observed underneath the PLA with 9 % Ag NWs (v/v). These results may be due to low silver ion release capacity of Ag NWs embedded in PLA films (FigureA.1 &FigureA.2). Moreover, additional polymer coating other than PVP may hold the release of the free ions from the Ag NWs in PLA films. Therefore, 70 % ethanol solution treatment did not affect the silver ion release properties in Ag NWs in PLA films.

Page et al. (2007) used titania and silver titania composite films on glass as an antimicrobial layer (Page et al., 2007). UV radiation was used to activate titania and silver- titania composites. In another study, oxidation of PVP was done by Loraine (Loraine, 2008). In Page's study, coating film release capacity was

significantly increased by UV treatment (365 nm). Therefore, UV treatment (254 nm and 73 W) was conducted on PLA with Ag NWs for 30 minutes. After this treatment, PLA with Ag NWs were placed on inoculated MH agar and incubated 37^{o}C for 1 day. At the end of the procedure, no clear zones were observed around the PLA with Ag NWs (Figure 3.2.2-Figure 3.2.4). However, there was no cell growth underneath the PLA films with Ag NWs for all foodborne pathogens used in this study. This may due to insufficient power of the light or non-uniform placing of Ag NWs on PLA film surfaces. More powerful UV light bulb could be more sufficient to increase the free ions amount on the surface of the PLA with Ag NWs. These results showed that Ag NWs inhibit growth of the pathogens as a bactericidal; however its effectiveness was retained (FigureA.3-FigureA.7).

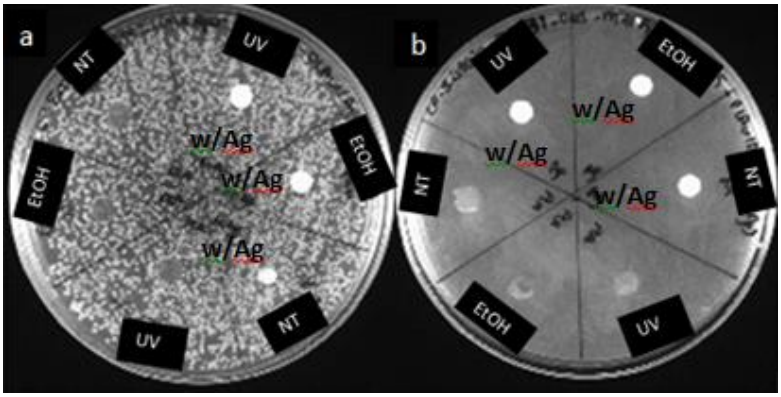

Figure 3. 2. 2 Photos of antimicrobial effects of Ag NWs embedded in PLA on (a) *E. coli* ATCC 25922 & (b) *Listeria monocytogenes*. 10 % Ag NWs /PLA were used in these examples. These figures were taken under trans-illuminator UV light. The PLA with Ag NWs were shown as "w/Ag".

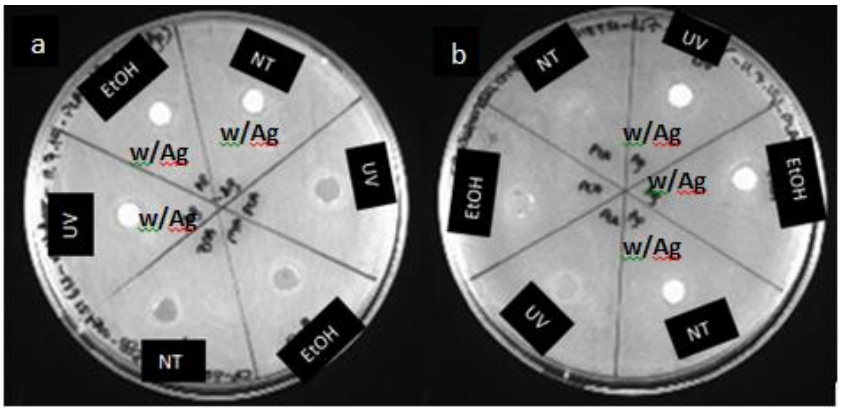

Figure 3. 2. 3 Photos of antimicrobial effects of Ag NWs embedded in PLA on (a) *Salmonella* Mbandaka & (b) *Salmonella* Enteritidis. 10 % Ag NWs /PLA were used in these examples. These figures were taken under trans-illuminator UV light. The PLA with Ag NWs were shown as "w/Ag".

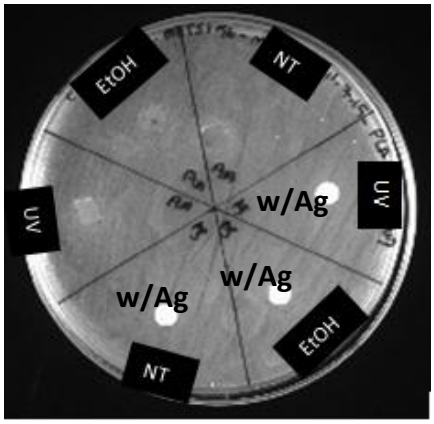

Figure 3. 2. 4 Photo of antimicrobial effects of Ag NWs embedded in PLA on *Salmonella* Infantis**.** 10 % Ag NWs /PLA were used in these examples**.** These figures were taken under trans-illuminator UV light. The PLA with Ag NWs were shown as "w/Ag".

PLA with Ag NWs were also tested using the *Turkish Standards 'Plastics-Measurement of antibacterial activity plastic surfaces' ISO 22196* on *E. coli* ATCC 25922. Inoculum of *E. coli* ATCC 25922 in BHI broth was adjusted

with 0.5 McFarland (approximately $1.5*10^8$ cfu / mL). After 10 fold dilution, 1 mL of phosphate-buffered physiological saline solution with recovered inoculum of pathogenic bacteria was pipetted on petri dishes and plate count agar (PCA) was poured. After incubation of PCA with *E. coli* ATCC 25922 at 37^oC for 24 hours, the results showed that, the recovery part of the experiment was unsuccessful. This was because the inoculum on the PLA with Ag NWs dried during the incubation process and cover film and PLA got clanged to each other. As a cover, acetate films were used. Clinging of PLA and acetate films may occur due to the humidity of the incubator or the acetate film might not be suitable for this process. Therefore, cover film was changed with polyethylene. Switching to polyethylene as a cover film, no antimicrobial effects of Ag NWs in PLA films were observed on *E. coli* ATCC 25922. This might be due to insufficient amount of Ag NWs in PLA films or insufficient conditions to release free silver ions from Ag NWs in PLA films.

3.3 Antimicrobial Effect of Filter Paper with Silver Nanowires

Disk Diffusion Method

In this work, Ag NWs were used as antimicrobial agent on FPs (Figure 3.3.1). To detect the antimicrobial efficiency of Ag NWs disk diffusion test were used. Ag NWs embedded filter papers cannot be compared with the results of the antimicrobial drug resistance test due to absence of standardization for Ag NWs in literature.

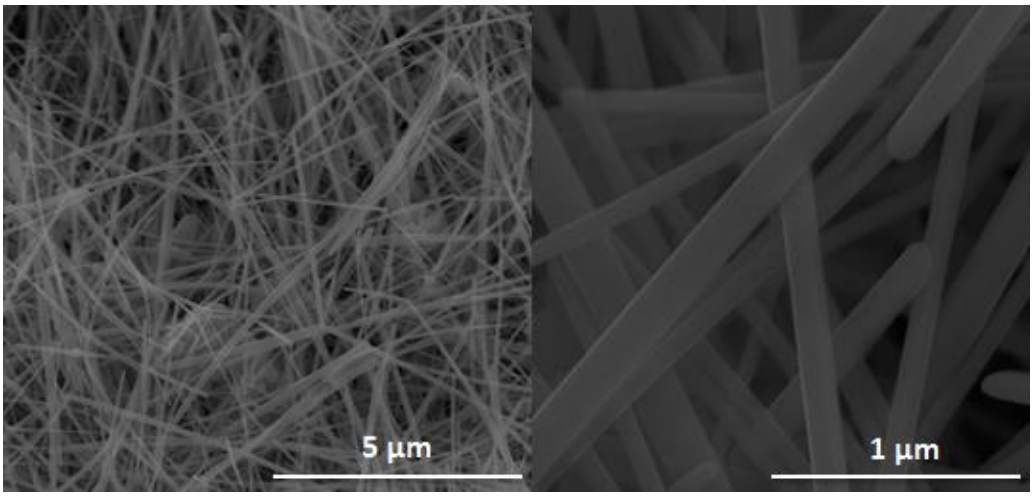

Figure 3. 3. 1 SEM image of 0.750 mg Ag NWs on FP

Different concentrations of Ag NWs (i.e.: 0.0625 mg Ag NWs/ mL EtOH solution, 0.125 mg Ag NWs/ mL EtOH solution, 0.250 mg Ag NWs/ mL EtOH solution, 0.500 mg Ag NWs/ mL EtOH solution, 0.750 mg Ag NWs/ mL EtOH solution, 1.000 mg Ag NWs/ mL EtOH solution) were embedded into filter papers in two ways. Ag NWs were embedded either onto only one surface or both surfaces of the filter papers. Ag NWs were embedded onto filter papers through vacuuming 4 mL solution of Ag NWs in ethanol. For embedding Ag NWs on both surfaces of the filter paper, 2 mL of silver solution was filtered onto first side and the second 2 mL Ag NWs solution was filtered onto second side of the filter paper. Petri dishes were divided into six parts to clearly observe the clear zone diameter differences. After incubation process, for the FP without Ag NWs there were no clear zones as expected. FP with Ag NWs had antimicrobial effect on pathogenic bacteria and clear zone diameters were changed by the amount of Ag NWs in FP. To increase the ion releasing capacity of Ag NWs in FP, 70 % EtOH solution treatment was used. By changing the pH of the environment with 70 % EtOH solution, releasing rate of

the free silver ions was increased. Following this treatment, larger clear zone diameters were observed. Filter papers with Ag NWs were exposed to UV light to remove the PVP layer on Ag NWs. All concentrations of Ag NWs embedded in FP and treated versions were tested against Gram (+) and Gram (-).

Gram Positive Pathogenic Bacteria

For *Listeria monocytogenes*, filter papers, one surface with Ag NWs inhibit the growth of *L. monocytogenes* and clear zones were observed around the FP with Ag NWs discs (Figure 3.3.1 & Figure 3.3.2). According to these results, there were significant differences in the clear zone diameters between 0.0625 mg and 1.000 mg of Ag NWs on *L. monocytogenes.* At 0.250 mg and above Ag NW concentrations, there was dramatical inhibition on the cell growth (Figure 3.3.2). FPs without Ag NWs did not inhibit the cell growth.

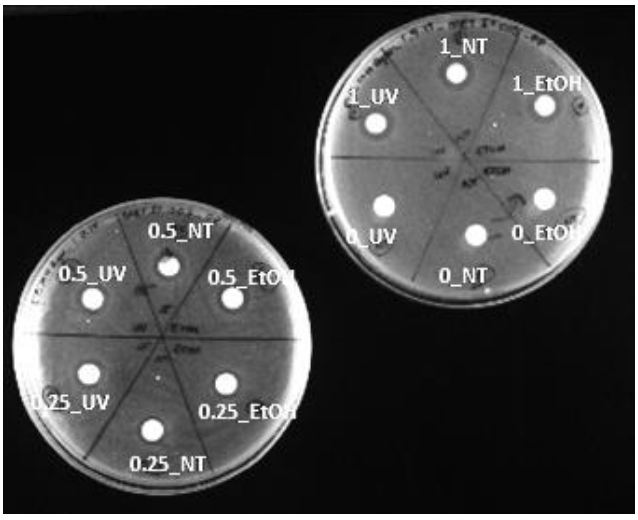

Figure 3. 3. 2 Photos of antimicrobial effects of Ag NWs at different concentrations embedded in FP on *L. monocytogenes.* 4 mL of 0.25, 0.5, 1 mg Ag NWs/ EtOH solution were used in these examples. Samples were shown as "amount of Ag NWs in Filter paper (FP)_treatment type" (e.g.: 1_UV)

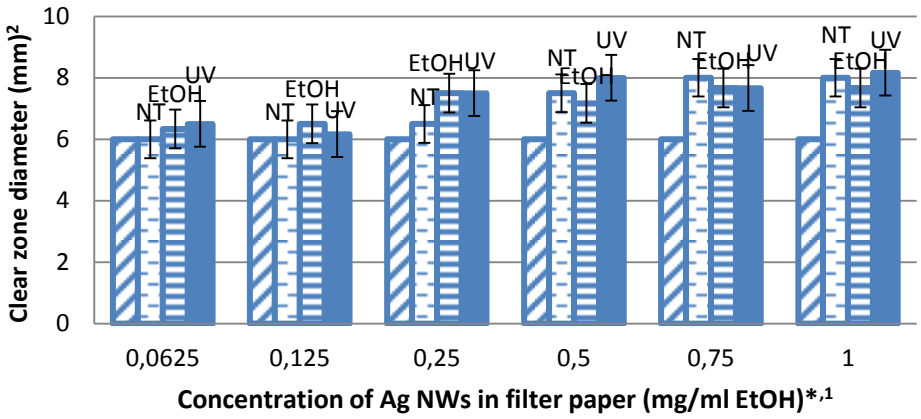

Figure 3. 3. 3 Antimicrobial effects of Ag NWs embedded in FP on *L. monocytogenes*. (*) 4 mL of each concentration were embedded only in one surface of the FP silver. (1) Each filter paper was treated with these treatments and every filter paper was treated with the stress separately. For 70 % EtOH solution treatment, to prevent any transition of silver ions each filter paper was hold in a different EtOH solution and evaporation was done separately. (2) The columns in the group of one concentration show that clear zone diameter at that concentration of Ag NWs in filter paper. First columns showed diameters of FPs with no Ag NWs. Second columns, FPs with Ag NWs that were not treated with 70 % EtOH solution and UV light (NT). Third columns in the groups show clear zone diameter at that concentrations FP with Ag NWs treated with 70 % EtOH solution. The last columns show clear zone diameters at that concentration of Ag NWs in FP that were treated with UV light.

For *Staphylococcus aureus*, Ag NWs embedded on one surface of the filter papers inhibit the growth of *S. aureus* and clear zones were observed around the FP with Ag NWs discs (Figure 3.3.4 & Figure 3.3.5). According to the results, there were significant differences in the clear zone diameters between 0.0625 and 1.000 mg on *S. aureus.* At 0.250 mg and above concentrations of Ag NWs was dramatically inhibit the cell growth. FPs without Ag NWs were used as control samples.

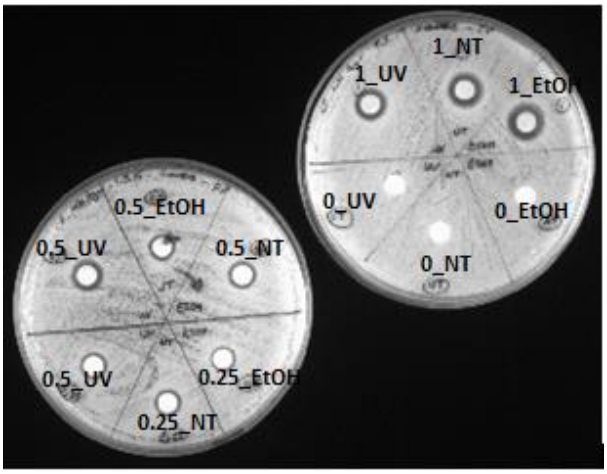

Figure 3. 3. 4 Photos of antimicrobial effects of Ag NWs embedded in FP on *S. aureus*. 4 mL of 0.25, 0.5, 1 mg Ag NWs/ EtOH solution were used in these samples. Samples were shown as "amount of Ag NWs in Filterpaper (FP)_treatment type" (e.g.: 1_UV)

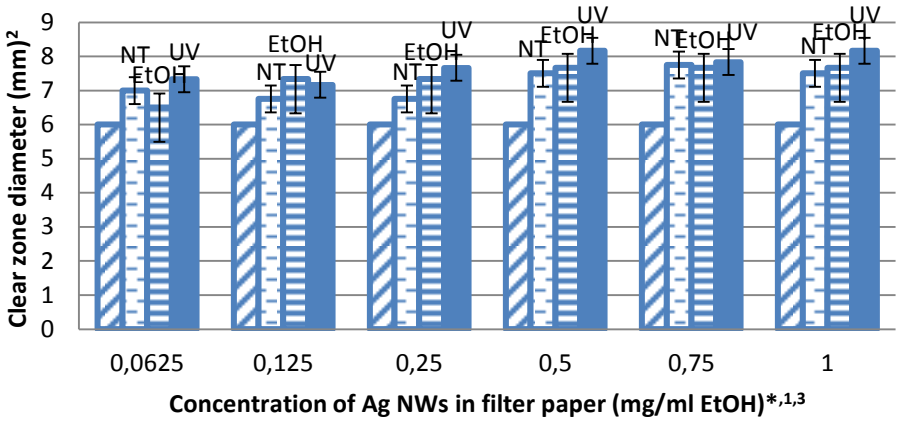

Figure 3. 3. 5 Antimicrobial effects of Ag NWs embedded in FP on *S. aureus.* (*)4 mL of each concentration were embedded only in one surface of the FP. (1) Each filter paper was treated with these treatments and every filter paper was treated with the stress separately. For 70 % EtOH solution treatment, to prevent any transition of silver ions each filter paper was hold in a different EtOH solution and evaporation was done separately.(2) The columns in the group of one concentration show that clear zone diameter at that concentration of Ag NWs in filter paper. At first columns showed diameters of FPs with no Ag NWs. Second columns, FPs with Ag NWs were not treated with 70 % EtOH solution and UV light (NT). Third columns in the groups show clear zone diameter at that concentrations FP with Ag NWs treated with 70 % EtOH solution. The last columns show clear zone diameters at that concentration of Ag NWs in FP that were treated with UV light.

S. aureus and *L. monocytogenes* are Gram positive bacteria. However, their reactions to the different concentrations of Ag NWs were not shown any uniform results. Ag NWs were found to be more effective on *L. monocytogenes* than *S. aureus*. As a Gram positive pathogen, *Staphylococcus aureus* did not show any discriminative response to the treatment stress as *L. monocytogenes.* Increase in the clear zone diameter for *Listeria monocytogenes* may be explained by the motif of the cell membrane, especially, binding structure or matrix of peptidoglycan. In addition to that, the amount of protein in *L. monocytogenes* cell membrane might be higher than that of *S. aureus'*s. As mentioned before, silver ions tend to bind with sulfur and sulfur based proteins.

Due to that, silver ions might bind with the sulfur based proteins on *L. monocytogenes* cell membrane that takes place in the cell proliferation. The opposite situation might happen, proteins that interact with the Ag NWs or silver free ions may not take a role at the cell proliferation of *S. aureus*. Moreover, the surface of the *L. monocytogenes* has more negative charge rather than *S. aureus* (Dickson, Koohmaraie, & Hruska, 1989). Because of that silver free ions may prefer to bind the proteins on the *L. monocytogenes* cell membrane than that of *S. aureus's*. However, the antimicrobial effect of Ag NWs was not substantially affected from the cell membrane characteristics such as G (+) and G (-).

Gram Negative Pathogenic Bacteria

FPs with Ag NWs were affective on *Escherichia coli* and the clear zone diameters did change with respectively to the concentration. Increase in Ag NWs concentration directly affected the zone diameter and the clear zone diameter was changed between 6 to 9 mm depending to the amount of the Ag NWs (Figure 3.3.6).

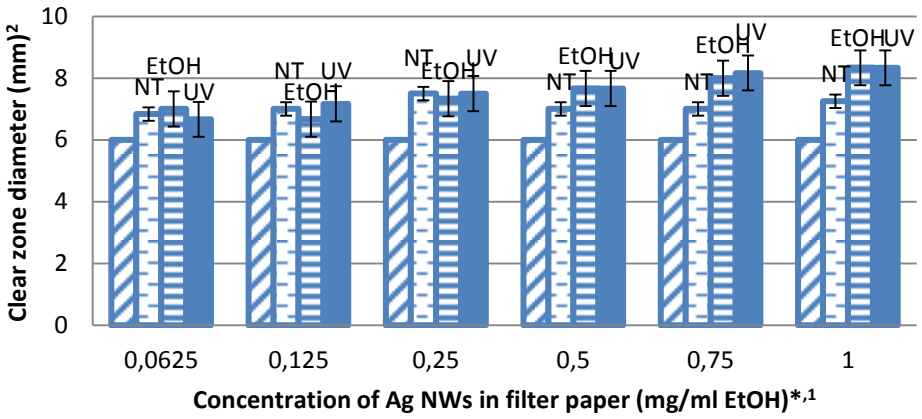

Figure 3. 3. 6 Antimicrobial effects of Ag NWs embedded in FP on *E. coli*(*)4 mL of each concentration were embedded only in one surface of the FP. (1) Each filter paper was treated with these treatments and every filter paper was treated with the stress separately. For 70 % EtOH solution treatment, to prevent any transition of silver ions each filter paper was hold in a different EtOH solution and evaporation was done separately.(2) The columns in the group of one concentration show that clear zone diameter at that concentration of Ag NWs in filter paper. At first columns showed diameters of FPs with no Ag NWs. Second columns, FPs with Ag NWs were not treated with 70 % EtOH solution and UV light (NT). Third columns in the groups show clear zone diameter at that concentrations FP with Ag NWs treated with 70 % EtOH solution. The last columns show clear zone diameters at that concentration of Ag NWs in FP that were treated with UV light.

Among the *Salmonella* isolates, *S.* Infantis did not show any varied response to the stress alterations. Treatment types did not affect the releasing rate of the free Ag ions (Figure 3.3.7). The effects of Ag NWs on *S.* Infantis did not differentiate according to the amount of Ag NWs in filter papers. This might explained by *S.* Infantis's adaptation characteristics to the treatment stress.

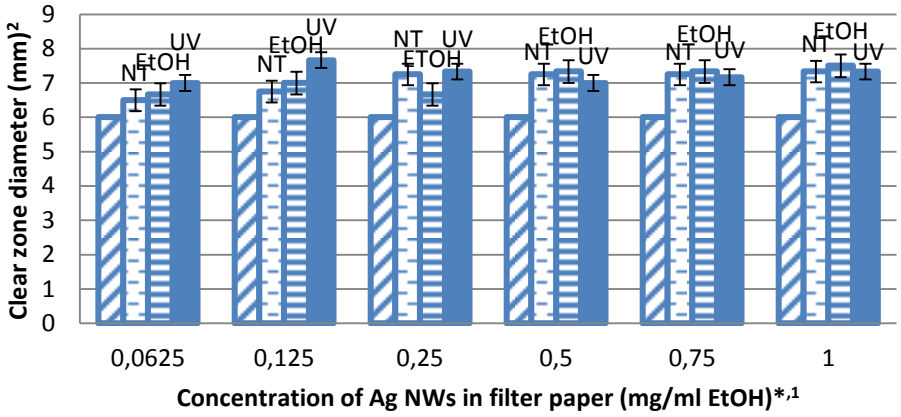

Figure 3. 3. 7 Antimicrobial effects of Ag NWs embedded in FP on *S.* Infantis. (*)4 mL of each concentration were embedded only in one surface of the FP. (1) Each filter paper was treated with these treatments and every filter paper was treated with the stress separately. For 70 % EtOH solution treatment, to prevent any transition of silver ions each filter paper was hold in a different EtOH solution and evaporation was done separately.(2) The columns in the group of one concentration show that clear zone diameter at that concentration of Ag NWs in filter paper. At first columns showed diameters of FPs with no Ag NWs. Second columns, FPs with Ag NWs were not treated with 70 % EtOH solution and UV light (NT). Third columns in the groups show clear zone diameter at that concentrations FP with Ag NWs treated with 70 % EtOH solution. The last columns show clear zone diameters at that concentration of Ag NWs in FP that were treated with UV light.

The result of the *S.* Mbandaka also collaborated with the treatment type and amount of Ag NWs in FP. UV treatment was effective on the Ag NWs and with this treatment, clear zone diameters were increased (Figure 3.3.8). Clear zone diameters were changed between 6 to 9 mm also. Effect of 70 % EtOH solution was also evident.

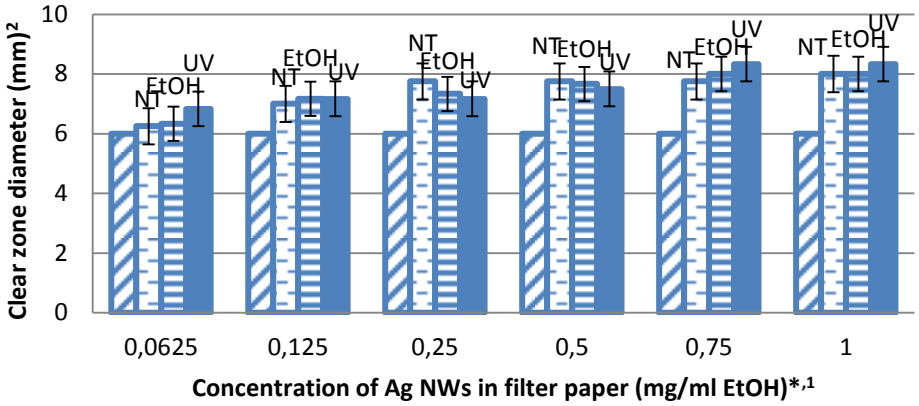

Figure 3. 3. 8 Antimicrobial effects of Ag NWs embedded in FP on *S.* Mbandaka. (*)4 mL of each concentration were embedded only in one surface of the FP. (1) Each filter paper was treated with these treatments and every filter paper was treated with the stress separately. For 70 % EtOH solution treatment, to prevent any transition of silver ions each filter paper was hold in a different EtOH solution and evaporation was done separately.(2) The columns in the group of one concentration show that clear zone diameter at that concentration of Ag NWs in filter paper. At first columns showed diameters of FPs with no Ag NWs. Second columns, FPs with Ag NWs were not treated with 70 % EtOH solution and UV light (NT). Third columns in the groups show clear zone diameter at that concentrations FP with Ag NWs treated with 70 % EtOH solution. The last columns show clear zone diameters at that concentration of Ag NWs in FP that were treated with UV light.

Moreover in Figure 3.3.8, the peak in the clear zone diameter occurred due to the excessive oxidation of Ag NWs in UV treated filter paper with 0.75 mg Ag NW/EtOH solution. Because of this oxidation, the inhibition effect was increased on *S.* Enteritidis just for one disc. At the edge of the disc, Ag NWs might be cut into half and UV treatment might increase the oxidation level of these Ag NWs.

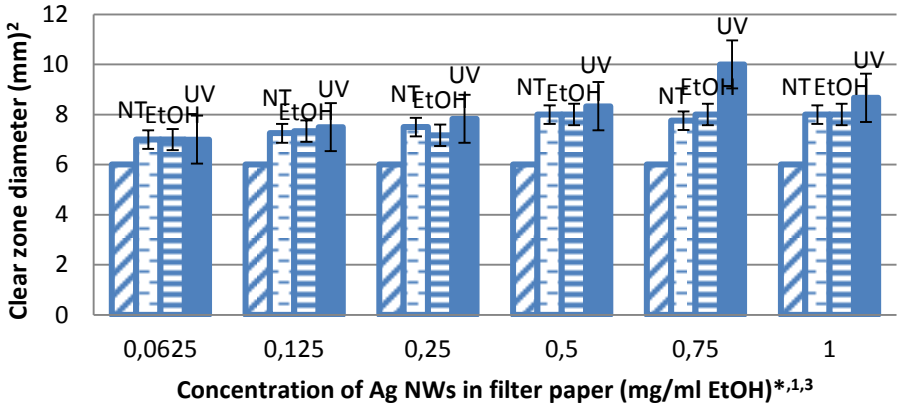

Figure 3. 3. 9 Antimicrobial effects of Ag NWs embedded in FP on *S.* Enteritidis. (*)4 mL of each concentration were embedded only in one surface of the FP. (1) Each filter paper was treated with these treatments and every filter paper was treated with the stress separately. For 70 % EtOH solution treatment, to prevent any transition of silver ions each filter paper was hold in a different EtOH solution and evaporation was done separately.(2) The columns in the group of one concentration show that clear zone diameter at that concentration of Ag NWs in filter paper. At first columns showed diameters of FPs with no Ag NWs. Second columns, FPs with Ag NWs were not treated with 70 % EtOH solution and UV light (NT). Third columns in the groups show clear zone diameter at that concentrations FP with Ag NWs treated with 70 % EtOH solution. The last columns show clear zone diameters at that concentration of Ag NWs in FP that were treated with UV light.

According to the results of both Gram positive and negative pathogenic bacteria used in this study, FP with Ag NWs inhibit the cell growth. The clear zone diameters were found to depend on their Ag NW concentrations. In addition to that, FPs with Ag NWs showed results that are highly efficient following UV treatment as opposed to non-treated samples. Therefore, it can be said that porous bases like filter papers are more appropriate for the removal of the PVP using UV light. Among *Salmonella* serovars, used in our study (Enteritidis, Mbandaka, Infantis) Infantis was the most susceptible one. This may be explained by membrane proteins and stress adaptation. Comparing the disc

diffusion results of FP with Ag NWs between Gram negative and positive bacteria, there were no significant differences in the clear zone diameters.

Surface Effect of Filter Papers with Silver Nanowires on Pathogenic Bacteria

To detect the effect of the embedding process on the surfaces of the filter papers, Ag NWs were embedded on one surface (1S) and two surfaces (2S). FP discs were treated with the exact same amount of the stress and time. The planting process of Ag NWs kept same and the overall concentrations of Ag NWs were 4 mL of the Ag NWs/EtOH solution.

Gram positive pathogenic bacteria

L. monocytogenes colonies were affected even from the lower concentration of Ag NWs. 0.25 mg and higher concentrations of Ag NWs in filter papers were more effective on *L. monocytogenes.* Embedding process of Ag NWs did not change the antimicrobial effect of Ag NWs in filter papers on *L. monocytogenes*.

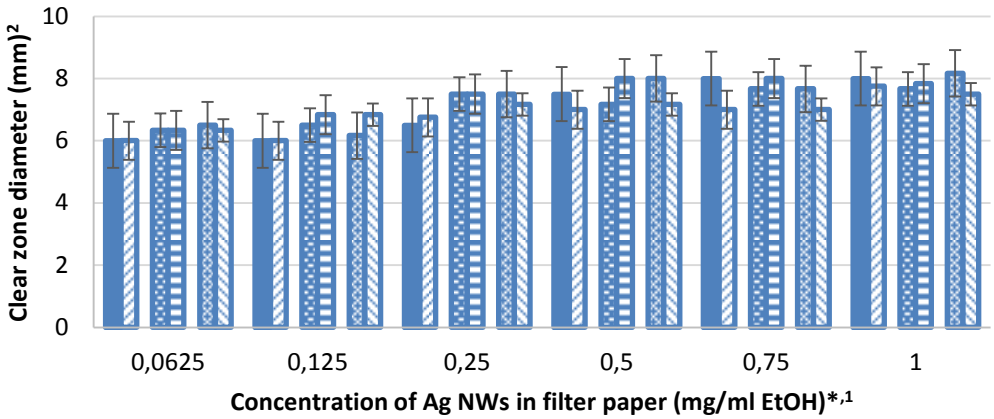

Figure 3. 3. 10 Surface effect of antimicrobial capacity of Ag NWs embedded in FP on *L. monocytogenes*. (*) 4 mL of each concentration were embedded in surface/surfaces of the FP. (1) Each FP was treated with these treatments and every FP treated with the stress separately. For 70 % EtOH solution treatment, to prevent any transition of Ag^{+} each FP was hold in a different EtOH solution and evaporation was done separately. (2)The columns in the group of each concentration show that clear zone diameter at that concentration of Ag NWs on FP. The first columns in the first pairs at that concentrations show the clear zone diameter at that concentration of Ag NWs in FP which embedded only 1 surface of the FP. The second columns in the first pairs at that concentrations show the clear zone diameter at that concentration of Ag NWs in FP that embedded both surfaces of the FP. At first pairs of columns FP with Ag NWs were not treated with 70 % EtOH solution and UV light. Second pairs of columns in the groups show the clear zone diameter at that concentrations FP with Ag NWs treated with 70 % EtOH solution. The third columns show the clear zone diameters at that concentration of Ag NWs in FP which treated with UV light.

For *S. aureus*, treatments were effective on activating and increasing the ion release capacity of Ag NWs in FP for both embedding processes of the Ag NWs into the FP. Between the concentrations 0.0625 and 0.25 mg of Ag NWs in filter papers, antimicrobial effects of Ag NWs were observed at similar zone diameters. Although after 0.5 mg of Ag NWs, the bactericidal effects of filter

paper with Ag NWs was increase on *S. aureus*, clear zone diameters between 0.5 and 1 mg of Ag NWs in filter papers were same.

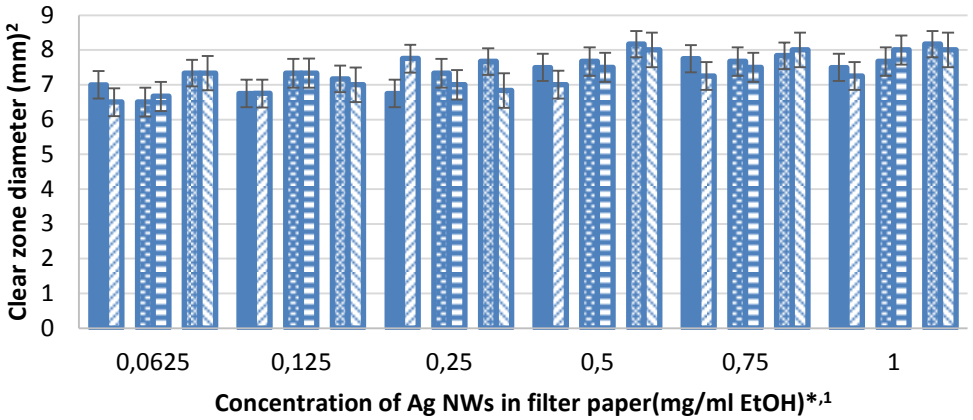

Figure 3. 3. 11 Surface effect of antimicrobial effect of Ag NWs embedded in FP on *S. aureus.* (*) 4 mL of each concentration were embedded in surface/surfaces of the FP. (1) Each FP was treated with these treatments and every FP treated with the stress separately. For 70 % EtOH solution treatment, to prevent any transition of Ag^{+} each FP was hold in a different EtOH solution and evaporation was done separately. (2)The columns in the group of each concentration show that clear zone diameter at that concentration of Ag NWs on FP. The first columns in the first pairs at that concentrations show the clear zone diameter at that concentration of Ag NWs in FP which embedded only 1 surface of the FP. The second columns in the first pairs at that concentrations show the clear zone diameter at that concentration of Ag NWs in FP that embedded both surfaces of the FP. At first pairs of columns FP with Ag NWs were not treated with 70 % EtOH solution and UV light. Second pairs of columns in the groups show the clear zone diameter at that concentrations FP with Ag NWs treated with 70 % EtOH solution. The third columns show the clear zone diameters at that concentration of Ag NWs in FP which treated with UV light.

Gram negative pathogenic bacteria

Effects of Ag NWs on *E. coli* were changed by altering the concentrations in FPs not by the embedding process into the FP. Moreover, filter paper with 1 mg

Ag NWs which Ag NWs were planted on both surfaces of filter paper and treated with UV light showed better inhibition of growth of the *E. coli*.

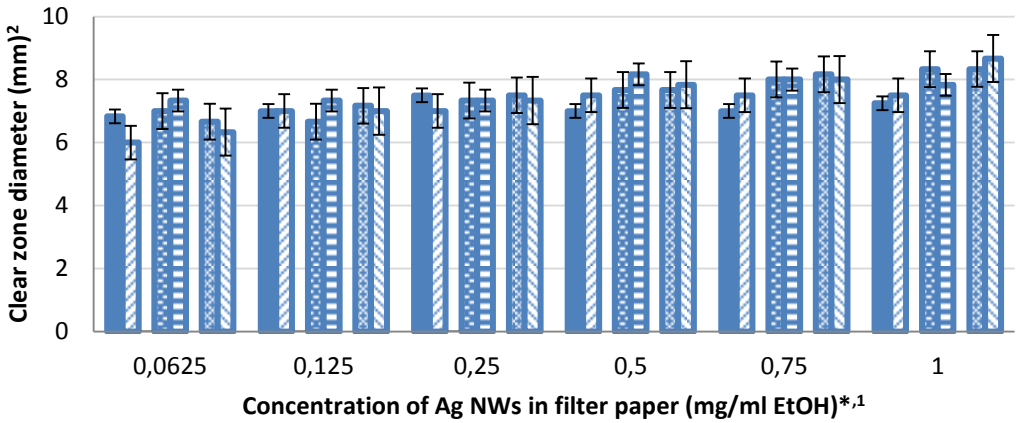

Figure 3. 3. 12 Surface effect of antimicrobial effect of Ag NWs embedded in FP on *E. coli*. (*) 4 mL of each concentration were embedded in surface/surfaces of the FP. (1) Each FP was treated with these treatments and every FP treated with the stress separately. For 70 % EtOH solution treatment, to prevent any transition of Ag^{+} each FP was hold in a different EtOH solution and evaporation was done separately. (2)The columns in the group of each concentration show that clear zone diameter at that concentration of Ag NWs on FP. The first columns in the first pairs at that concentrations show the clear zone diameter at that concentration of Ag NWs in FP which embedded only 1 surface of the FP. The second columns in the first pairs at that concentrations show the clear zone diameter at that concentration of Ag NWs in FP that embedded both surfaces of the FP. At first pairs of columns FP with Ag NWs were not treated with 70 % EtOH solution and UV light. Second pairs of columns in the groups show the clear zone diameter at that concentrations FP with Ag NWs treated with 70 % EtOH solution. The third columns show the clear zone diameters at that concentration of Ag NWs in FP which treated with UV light.

Effect of Ag NWs on *S.* Infantis also showed that, there was no noticeable effects on the bacteria of the differences in embedding the Ag NWs on surface of the FPs. In addition to that, increase in Ag NWs concentrations was not effective on *S.* Infantis as *E. coli* or other *Salmonella* serovars like Mbandaka

(Figure 3.3.14). According to the Figure 3.3.13, filter paper with the 1 mg Ag NWs concentration that was embedded on both surfaces of filter paper and treated with UV light gave the best result for *S.* Infantis same as *E .coli*.

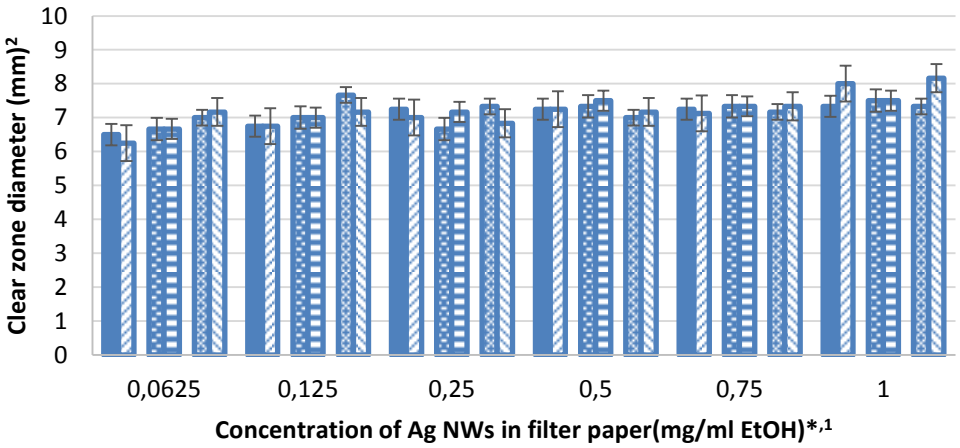

Figure 3. 3. 13 Surface effect of antimicrobial effect of Ag NWs embedded in FP on *S.* Infantis(*) 4 mL of each concentration were embedded in surface/surfaces of the FP. (1) Each FP was treated with these treatments and every FP treated with the stress separately. For 70 % EtOH solution treatment, to prevent any transition of Ag^{+} each FP was hold in a different EtOH solution and evaporation was done separately. (2)The columns in the group of each concentration show that clear zone diameter at that concentration of Ag NWs on FP. The first columns in the first pairs at that concentrations show the clear zone diameter at that concentration of Ag NWs in FP which embedded only 1 surface of the FP. The second columns in the first pairs at that concentrations show the clear zone diameter at that concentration of Ag NWs in FP that embedded both surfaces of the FP. At first pairs of columns FP with Ag NWs were not treated with 70 % EtOH solution and UV light. Second pairs of columns in the groups show the clear zone diameter at that concentrations FP with Ag NWs treated with 70 % EtOH solution. The third columns show the clear zone diameters at that concentration of Ag NWs in FP which treated with UV light.

For *S.* Mbandaka, filter paper with 1 mg Ag NWs concentration that were planted on both surfaces of FP and treated with UV light gave the best result as shown in Figure 3.3.14.

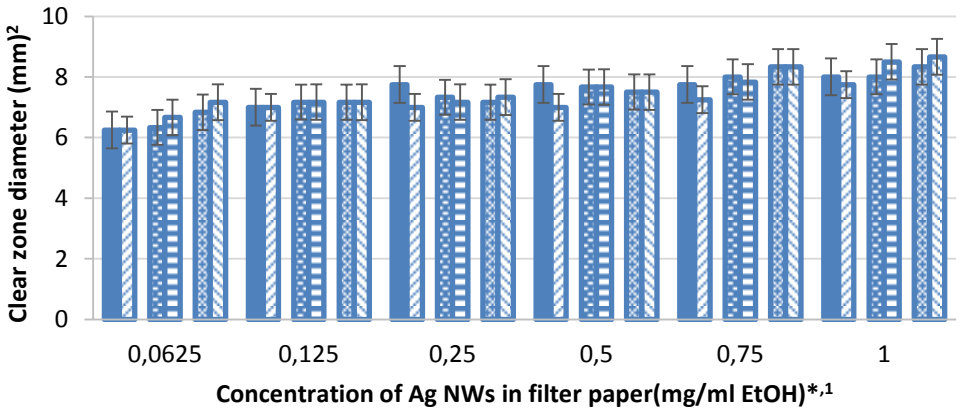

Figure 3. 3. 14 Surface effect of antimicrobial effect of Ag NWs embedded in FP on *S.* Mbandaka. (*) 4 mL of each concentration were embedded in surface/surfaces of the FP. (1) Each FP was treated with these treatments and every FP treated with the stress separately. For 70 % EtOH solution treatment, to prevent any transition of Ag^{+} each FP was hold in a different EtOH solution and evaporation was done separately. (2)The columns in the group of each concentration show that clear zone diameter at that concentration of Ag NWs on FP. The first columns in the first pairs at that concentrations show the clear zone diameter at that concentration of Ag NWs in FP which embedded only 1 surface of the FP. The second columns in the first pairs at that concentrations show the clear zone diameter at that concentration of Ag NWs in FP that embedded both surfaces of the FP. At first pairs of columns FP with Ag NWs were not treated with 70 % EtOH solution and UV light. Second pairs of columns in the groups show the clear zone diameter at that concentrations FP with Ag NWs treated with 70 % EtOH solution. The third columns show the clear zone diameters at that concentration of Ag NWs in FP which treated with UV light.

For *S.* Enteritidis, UV treated Ag NWs on both surfaces of the FP gave larger clear zone diameter as shown in Figure 3.3.15. Clear zone diameters were found to be mostly in the same range. However, one of the samples showed an unexpected peak as shown in Figure 3.3.15. This situation might have occurred

due to changes in the characteristics of Ag NWs. The main reason is the environmental conditions. This peak might have also occurred due to excess oxidation of Ag NWs. Excess oxidation might have been developed during the production period or discontinuous PVP layer on the Ag NWs.

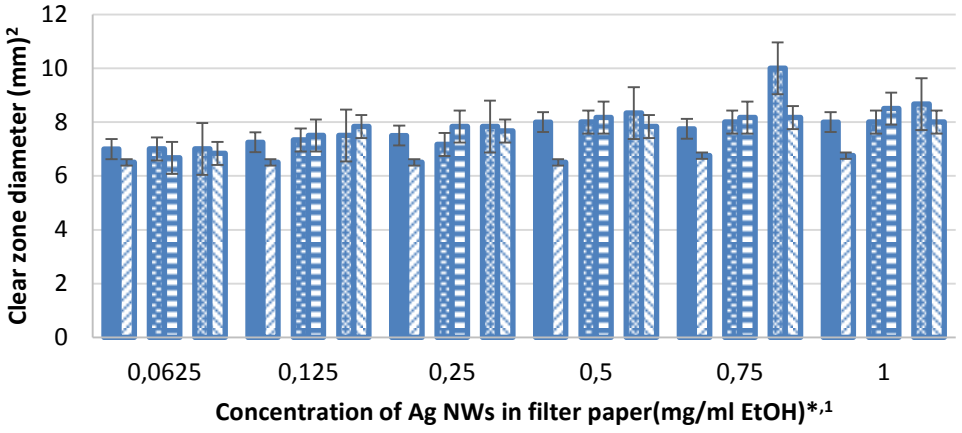

Figure 3. 3. 15 Surface effect of antimicrobial effect of Ag NWs embedded in FP on *S.* Enteritidis. (*) 4 mL of each concentration were embedded in surface/surfaces of the FP. (1) Each FP was treated with these treatments and every FP treated with the stress separately. For 70 % EtOH solution treatment, to prevent any transition of Ag^{+} each FP was hold in a different EtOH solution and evaporation was done separately. (2)The columns in the group of each concentration show that clear zone diameter at that concentration of Ag NWs on FP. The first columns in the first pairs at that concentrations show the clear zone diameter at that concentration of Ag NWs in FP which embedded only 1 surface of the FP. The second columns in the first pairs at that concentrations show the clear zone diameter at that concentration of Ag NWs in FP that embedded both surfaces of the FP. At first pairs of columns FP with Ag NWs were not treated with 70 % EtOH solution and UV light. Second pairs of columns in the groups show the clear zone diameter at that concentrations FP with Ag NWs treated with 70 % EtOH solution. The third columns show the clear zone diameters at that concentration of Ag NWs in FP which treated with UV light.

Ag NWs have been synthesized with the PVP layer and nanowires and dispersed to packaging materials non-homogeneously. Therefore, UV treatment

might not affect the all Ag NWs in the FP. Although the diameter of PVP layer was accepted as 2 nm, in the process, thickness of the PVP could not be controlled for all Ag NWs. Therefore, increase in the diameter of the PVP layer might retain the release rate of free Ag ions and use of the UV treatment might not be very effective for higher diameters of PVP layer. PVP layers can also change according to batch to batch as mentioned. More importantly, the exact numbers of silver nanowires are not countable for experimental samples (6 mm diameter discs). Because of that, in some cases although treatments of the samples were effective, the clear zone diameter between treated and not treated ones might have not shown any difference.

In addition, the processing area of Ag NWs was not monitored so, moisture and temperature changes were not controlled. Because of that, fluctuation in the room conditions can affect the characteristics of the Ag NWs. All of these situations might cause alteration of effectiveness of the samples.

Some of the cases Ag NWs were not ionized enough to form clear zones after the treatments. The samples were directly used one day after embedding into the filter paper. At some parts of the mother FP, ethanol solution did not vaporize right after the embedding process of Ag NWs. In order to vaporize the ethanol, samples were placed in a cabinet to achieve vaporization in a dark place. However after evaporation, Ag NWs did not show any antimicrobial effect on pathogenic bacteria for these samples. This may be explained by effect of light during the evaporation of ethanol solution.

Overall, clear zone formations were explained by the ion release mechanism of silver. Embedding Ag NWs onto either two surfaces or one surface of the filter papers did not significantly change the clear zone diameter both on Gram negative and positive bacteria.

CHAPTER 4

CONCLUSIONS AND FUTURE RECOMMENDATIONS

Ag nanoparticles' antimicrobial effects on pathogenic bacteria have been demonstrated in literature. This study is the first time reporting antimicrobial effect of Ag NWs on pathogenic bacteria in food packaging from our knowledge. Studies on the antimicrobial effect of Ag NPs in nanocomposite films reported that Ag^+ could move freely in the packaging films and affect the pathogenic bacteria. In this study, two different bases were used to embed Ag NWs namely, filter paper and PLA. PLA with Ag NWs did not show any inhibition effect around the PLA films. However, PLA with Ag NWs inhibit the growth of the pathogenic bacteria with the contact, but not any further.

Changing the base from PLA to filter paper provided enough places to free silver ions for their movement. Therefore, filter papers with Ag NWs inhibit the microorganisms more efficiently than PLA with Ag NWs by releasing the free silver ions from Ag NWs. In addition, different treatment procedures resulted in formation of different clear zone diameters. Treating with 70% EtOH increased the ion release rate and UV treatment increased the exposure of Ag NWs in filter papers by removing the PVP layer on the side surfaces of NWs. Regardless of the cell membrane structure of pathogenic bacteria, Ag NWs were effective at each concentration in FP. As Gram negative *E. coli* and *Salmonella* Infantis, *S.* Enteritidis and *S.* Mbandaka were used and all of these Gram negative bacteria were inhibited at the beginning by UV treated Ag NW on FP. It was observed that, FP with Ag NWs was more effective on

Salmonella subsps. than *E. coli*. For *Salmonella* subsps., increasing Ag NW concentration in FP was not effective on *S.* Infantis according to DD method. Among *Salmonella* serotypes studied here, *S.* Mbandaka gave better results for antimicrobial effect of FP with Ag NWs. The clear zone diameters of *S.* Mbandaka increased respectively to the concentration of Ag NWs. For Gram positive foodborne pathogen, *Listeria monocytogenes* and *Staphylococcus aureus* were chosen. Silver nanowires were also effective on Gram positive pathogens such and *S. aureus* and *L. monocytogenes.* The results of the *L. monocytogenes* stand out amongst the other foodborne pathogens that used in this study because of its stress adaptation characteristics.

In conclusion, Ag NWs on filter papers inhibit growth of the both Gram positive and negative bacteria. UV treated filter papers suppress the cell growth and give larger clear zone diameters.

As a future work, the antimicrobial effect of Ag NWs embedded in FPs that are treated with 70 % ethanol solution can be examined.

It can be recommended that, FP with Ag NWs may be used as a food packaging material for foods or surface material in food storage and production area. Egg cartons might be one of the possible packaging designs for demonstrating the antimicrobial effect of Ag NWs. Since the health effects of Ag are still under debate, packages with Ag NWs may be provided that the package is not in direct contact with the food (i.e. egg cartons). In addition to that, UV treatment of the packaging material with Ag NWs may increase the efficiency of antimicrobial capability of Ag NWs. More studies should be conducted to design the food packaging material and storage/production area of foods that provide UV treatment like in refrigerators in future. Moreover, instability of antimicrobial effect of Ag NWs should be resolved for future studies.

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APPENDIX A

DETAILS OF ANTIMICROBIAL EFFECT OF SILVER NANOWIRES IN LIQUID MEDIUM

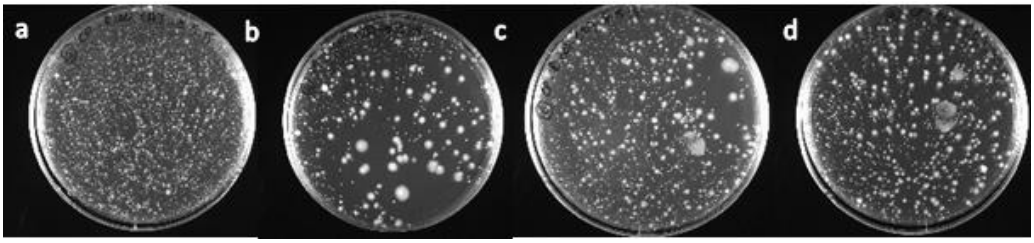

Figure A. 1 Antimicrobial effect of silver nanowire with water solution on *E. coli* in liquid medium. Given pictures were taken at fourth dilution. (a) Cell suspension of *E.coli* with Ag NW solution treated with UV (500 microliter) (b) Incubated at the same time with Ag NW solution treated with UV (100 microliter) (c) Incubated at the same time with Ag NW solution (100 microliter) (d) Incubated *E. coli.* (d) *E. Coli's* cell nnumber in the dilution was higher than 300 x 10^4

APPENDIX B

DETAILS OF ANTIMICROBIAL EFFECT OF POLYLACTIC ACID WITH SILVER NANOWIRES

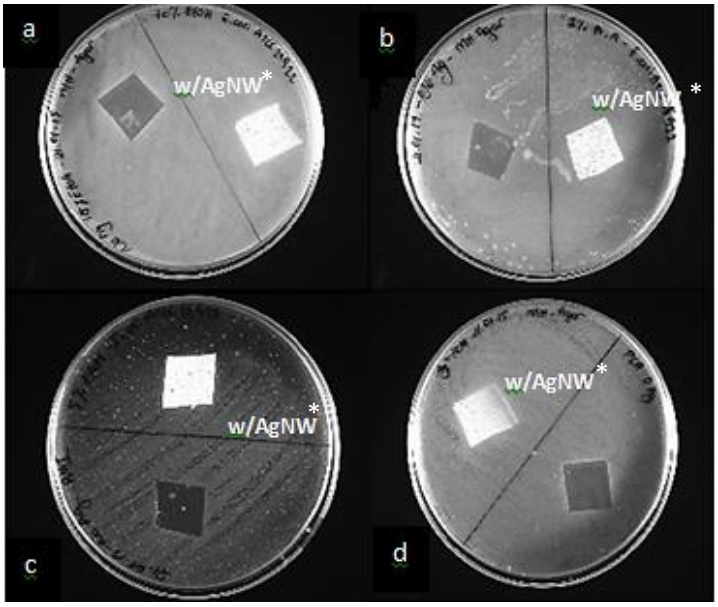

Figure B. 1 Antimicrobial effects of PLA with 9 % Ag NWs on *E. coli*. (*)PLA with Ag NWs treated with (a) 70 % ethanol solution, (b) 3 % acetic acid solution, (c) 5 % ethanol solution and (d) not treated

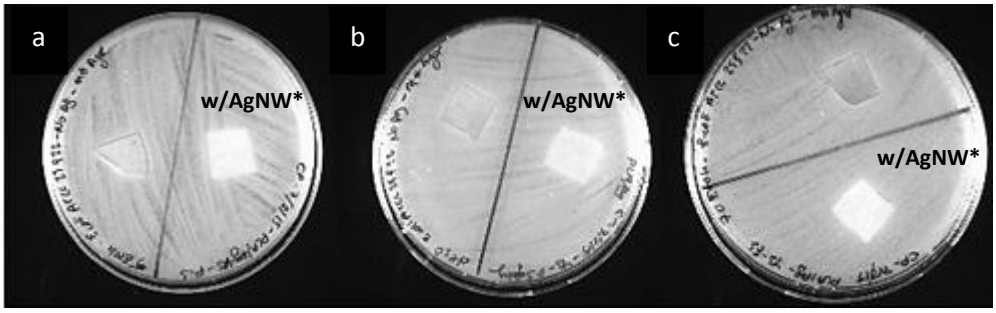

Figure B. 2 Antimicrobial effects of PLA with 9 % Ag NWs on *E. coli*. (*)PLA with Ag NWs treated with (a) 70 % ethanol solution, (b) 3 % acetic acid solution, (c) 5 % ethanol solution and (d) not treated

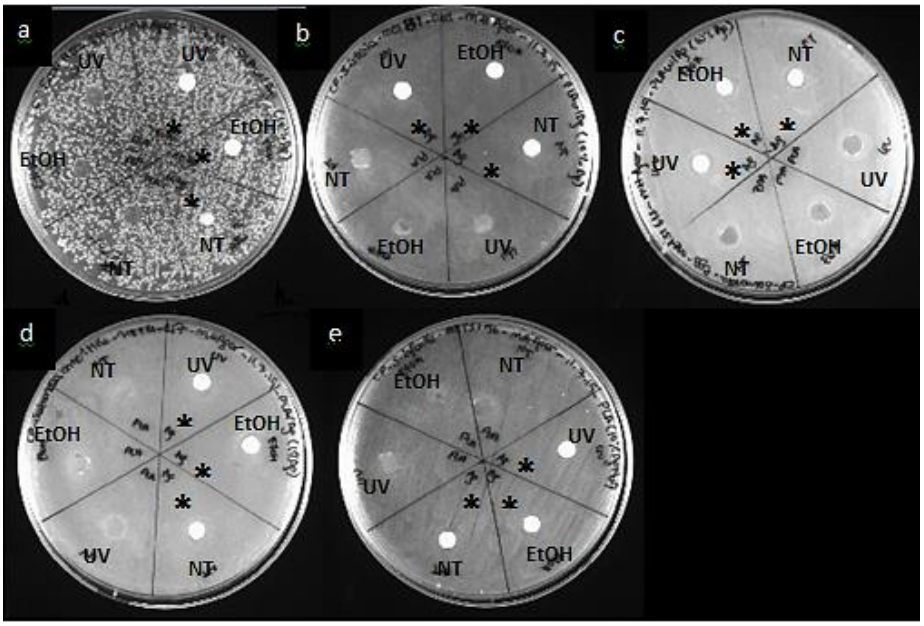

Figure B. 3 Antimicrobial effects of PLA with 10 % Ag NWs on (a) *E. coli* ATCC 25922, (b) *L. monocytogenes*, (c) *S.* Mbandaka, (d) *S.* Enteritidis and *(e) S.* Infantis. (*)PLA with Ag NWs treated with UV light, 70 % ethanol solution and not treated. PLAw/AgNWs were shown with"*".

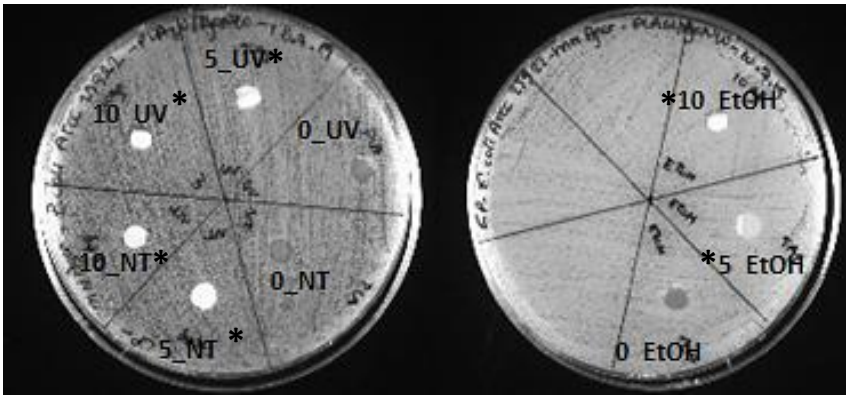

Figure B. 4 Antimicrobial effects of PLA with 10 % Ag NWs and 5 % Ag NWs on *E. coli* ATCC 25922. (*) PLA with Ag NWs treated with UV light, 70 % ethanol solution and not treated. Samples were shown as concentration percentages of Ag NWs in PLA_treatment type (e.g: 1_UV)

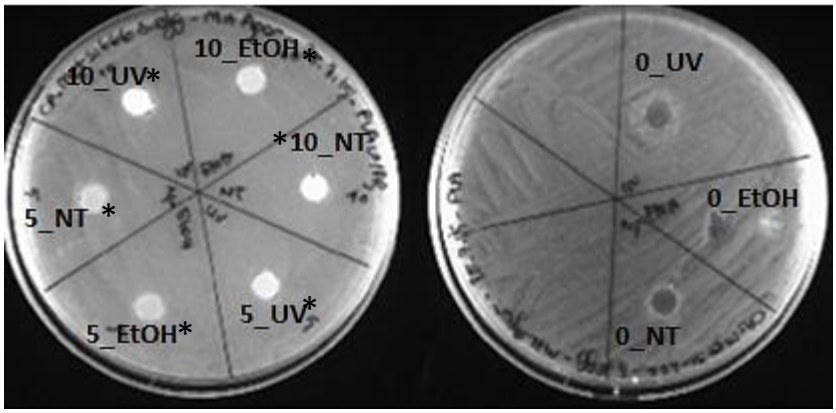

Figure B. 5 Antimicrobial effects of PLA with 10 % Ag NWs and 5 % Ag NWs solution on *S.* Mbandaka. (*)PLA with Ag NWs treated with UV light, 70 % ethanol solution and not treated. Samples were shown as concentration percentages of Ag NWs in PLA_treatment type (e.g: 5_UV)

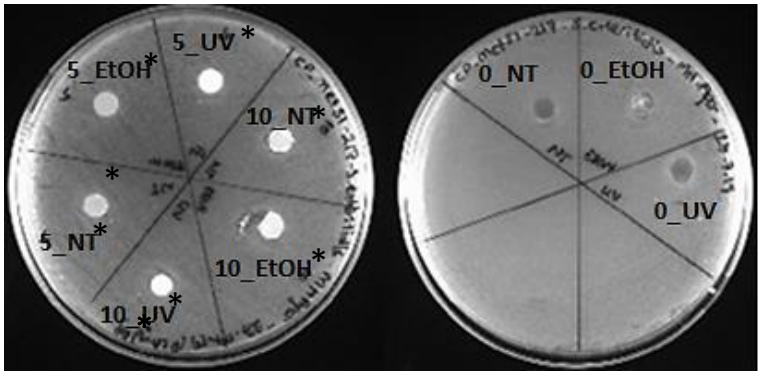

Figure B. 6 Antimicrobial effects of PLA with 10 % Ag NWs and 5 % Ag NWs on *S.* Enteritidis. (*) PLA with Ag NWs treated with UV light, 70 % ethanol solution and not treated. Samples were shown as concentration percentages of Ag NWs in PLA_treatment type (e.g: 10_UV)

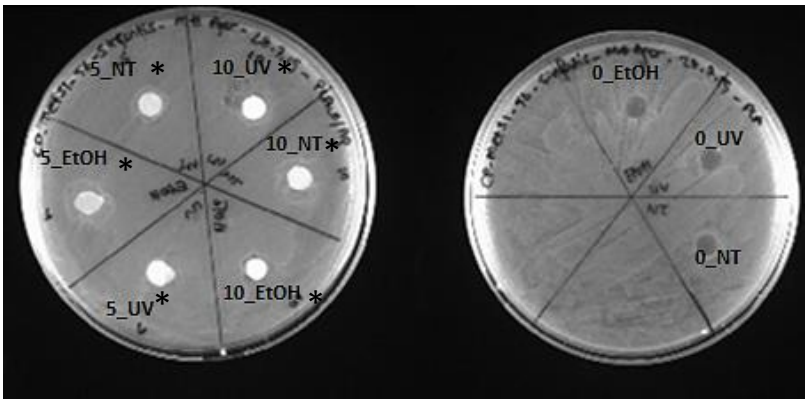

Figure B. 7 Antimicrobial effects of PLA with 10 % Ag NWs and 5 % Ag NWs on *S.* Infantis. (*) PLA with Ag NWs treated with UV light, 70 % ethanol solution and not treated. Samples were shown as concentration percentages of Ag NWs in PLA_treatment type (e.g: 1_UV)

APPENDIX C

DETAILS OF ANTIMICROBIAL EFFECT OF FILTER PAPER WITH SILVER NANOWIRES

Table C. 1 Antimicrobial effect of Ag NWs embedded in FP on foodborne pathogenic bacteria used in this study

Bacteria	Concentration of AgNWs in FP (mgAgNWs/mL EtOH)	Clear zone diameter of 1S UV (mm)	Clear zone diameter of 1S EtOH (mm)	Clear zone diameter of 1S NT (mm)	Clear zone diameter of 2S UV (mm)	Clear zone diameter of 2S EtOH (mm)	Clear zone diameter of 2S NT (mm)
E. coli	0,0625	6,5	8	7,5	6,5	7,5	6
E. coli	0,0625	6,5	6,5	6,5	6	8	6,5
E. coli	0,0625	7	6,5	6,5	6,5	6,5	6
E. coli	0,125	7,5	6,5	8	7	8	7
E. coli	0,125	7,5	6,5	6	6,5	7,5	6,5
E. coli	0,125	6,5	7	6,5	7,5	6,5	6
E. coli	0,25	7	8	8	7,5	8,5	7
E. coli	0,25	8	7	6,5	7,5	8	7
E. coli	0,25	7,5	7	8	7	7	6,5
E. coli	0,5	7	8	7	8	8,5	7,5
E. coli	0,5	8	7	7	8	8	7
E. coli	0,5	8	8	7,5	7,5	8	7
E. coli	0,75	7,5	8	6,5	8	8	7,5
E. coli	0,75	9	7,5	7,5	8	8	7,5
E. coli	0,75	8	8,5	7,5	8	8	7,5
E. coli	1	9	8	7	9	7,5	7
E. coli	1	8	9	7,5	9	8	8
E. coli	1	8	8	8	8	8	8
***S.* Infantis**	0,0625	6	6	6,5	8	7	6
***S.* Infantis**	0,0625	8,5	6	6,5	6,5	6,5	6,5
***S.* Infantis**	0,0625	6,5	8	6	7	6,5	6,5
***S.* Infantis**	0,125	8	6,5	6,5	7,5	8	7
***S.* Infantis**	0,125	6,5	6,5	7	6,5	6,5	6,5
***S.* Infantis**	0,125	6,5	8	7	7,5	6,5	6,5
***S.* Infantis**	0,25	8	6,5	6,5	7	7,5	7

Table C. 2 Antimicrobial effect of Ag NWs embedded in FP on foodborne pathogenic bacteria used in this study (continued)

Bacteria	Concentration of AgNWs in FP (mgAgNWs/mL EtOH)	Clear zone diameter of 1S UV (mm)	Clear zone diameter of 1S EtOH (mm)	Clear zone diameter of 1S NT (mm)	Clear zone diameter of 2S UV (mm)	Clear zone diameter of 2S EtOH (mm)	Clear zone diameter of 2S NT (mm)
***S*. Infantis**	0,25	7	6,5	8	7	7	7
***S*. Infantis**	0,25	6,5	7	7	6,5	7	7
***S*. Infantis**	0,5	7,5	7	6,5	7,5	7	7,5
***S*. Infantis**	0,5	8	7	8	7	8	7
***S*. Infantis**	0,5	7	8	7,5	7	7,5	7
***S*. Infantis**	0,75	7	7,5	7	7	7,5	7
***S*. Infantis**	0,75	7	7,5	7,5	7,5	7	7
***S*. Infantis**	0,75	8	7	7,5	7,5	7,5	7,5
***S*. Infantis**	1	8	8	6,5	8	8	8
***S*. Infantis**	1	7	6,5	8	8,5	6,5	8
***S*. Infantis**	1	8	8	7,5	8	8	8
***S*. Mbandaka**	0,0625	6	6	6,5	8	7	6
***S*. Mbandaka**	0,0625	8	7	6	7	7	6,5
***S*. Mbandaka**	0,0625	6,5	6	6	6,5	6	6
***S*. Mbandaka**	0,125	8	7	8	6,5	7	7,5
***S*. Mbandaka**	0,125	6,5	8	6	7	8	6,5
***S*. Mbandaka**	0,125	7	6,5	6,5	8	6,5	6,5
***S*. Mbandaka**	0,25	6,5	7,5	8,5	8	7	7
***S*. Mbandaka**	0,25	8	8	7	7,5	8	7
***S*. Mbandaka**	0,25	7	6,5	7	6,5	6,5	7
***S*. Mbandaka**	0,5	8	7,5	8,5	7	7,5	7
***S*. Mbandaka**	0,5	7	8,5	7	7,5	8,5	7
***S*. Mbandaka**	0,5	7,5	7	8	8	7	7,5
***S*. Mbandaka**	0,75	7,5	8	8	10	7,5	7
***S*. Mbandaka**	0,75	7,5	9	7,5	7,5	9	7,5
***S*. Mbandaka**	0,75	10	7	8	7,5	7	7,5
***S*. Mbandaka**	1	9	8	8	8	8,5	7,5
***S*. Mbandaka**	1	8	9	8	9	9	8
***S*. Mbandaka**	1	8	7	8	9	8	7,5
***S*. Enteritidis**	0,0625	8,5	8	6,5	7	6,5	7
***S*. Enteritidis**	0,0625	6	6	7,5	6,5	6,5	6
***S*. Enteritidis**	0,0625	6,5	7	6	7	7	6,5
***S*. Enteritidis**	0,125	8,5	8	6,5	8	7	7
***S*. Enteritidis**	0,125	8	6,5	8	8	8	6
***S*. Enteritidis**	0,125	6	7,5	6,5	7,5	7,5	6,5
***S*. Enteritidis**	0,25	8,5	8,5	7	7,5	7,5	7
***S*. Enteritidis**	0,25	8,5	6,5	8	8	8,5	7
***S*. Enteritidis**	0,25	6,5	6,5	7	7,5	7,5	6
***S*. Enteritidis**	0,5	9	9	8	8	7,5	7
***S*. Enteritidis**	0,5	9	7	8	8	9	6
***S*. Enteritidis**	0,5	7	8	7,5	7,5	8	7

Table C. 3 Antimicrobial effect of Ag NWs embedded in FP on foodborne pathogenic bacteria used in this study (continued)

Bacteria	Concentration Of AgNWs in FP (mgAgNWs/mL EtOH)	Clear zone diameter of 1S UV (mm)	Clear zone diameter of 1S EtOH (mm)	Clear zone diameter of 1S NT (mm)	Clear zone diameter of 2S UV (mm)	Clear zone diameter of 2S EtOH (mm)	Clear zone diameter of 2S NT (mm)
***S*. Enteritidis**	0,75	8	9	7,5	8	8	7,5
***S*. Enteritidis**	0,75	9	7	8	8	8,5	6
***S*. Enteritidis**	0,75	13	8	8	8,5	8	7,5
***S*. Enteritidis**	1	9,5	8	8	8	8	7,5
***S*. Enteritidis**	1	8	8	8	8	9	6
***S*. Enteritidis**	1	8,5	8	8,5	8	8,5	7,5
S. aureus	0,0625	8	6	7	7,5	8	7
S. aureus	0,0625	6,5	6,5	7	8	6	6
S. aureus	0,0625	7,5	7	6	6,5	6	6
S. aureus	0,125	6,5	6	6,5	7,5	9	7
S. aureus	0,125	8	7	7	7	6,5	6,5
S. aureus	0,125	7	9	7	6,5	6,5	6
S. aureus	0,25	7,5	6,5	6,5	7	8	9
S. aureus	0,25	8,5	7,5	7	7	6,5	6,5
S. aureus	0,25	7	8	7	6,5	6,5	6,5
S. aureus	0,5	7,5	7	7	8	8	7
S. aureus	0,5	9	8	8	7,5	7,5	7
S. aureus	0,5	8	8	7,5	7,5	7	7,5
S. aureus	0,75	7,5	7	7,5	8	7	7,5
S. aureus	0,75	8	8	8	8	8	7
S. aureus	0,75	8	8	8	8	7,5	7,5
S. aureus	1	8,5	8	7	8	8	7
S. aureus	1	8	8	8	8	8	7,5
S. aureus	1	8	7	8	8	8	8
L. monocytogenes	0,0625	7	6	6	7	6	6
L. monocytogenes	0,0625	6	6,5	6	6	6,5	6
L. monocytogenes	0,0625	6,5	6,5	6,5	6	6,5	6,5
L. monocytogenes	0,125	6	7	6	7,5	7	6
L. monocytogenes	0,125	6	6,5	6,5	7	6,5	6
L. monocytogenes	0,125	6,5	6	7	6	7	6,5
L. monocytogenes	0,25	7,5	7	6,5	7,5	8,5	7
L. monocytogenes	0,25	8	6,5	7	7	7	6,5
L. monocytogenes	0,25	7	7	7,5	7	7	7
L. monocytogenes	0,5	8,5	7,5	7,5	7	10	7

Table C. 4 Antimicrobial effect of Ag NWs embedded in FP on foodborne pathogenic bacteria used in this study (continued)

Bacteria	Concentration Of AgNWs in FP (mgAgNWs/mL EtOH)	Clear zone diameter of 1S UV (mm)	Clear zone diameter of 1S EtOH (mm)	Clear zone diameter of 1S NT (mm)	Clear zone diameter of 2S UV (mm)	Clear zone diameter of 2S EtOH (mm)	Clear zone diameter of 2S NT (mm)
L. monocytogenes	0,5	8	7	7	7	7	7
L. monocytogenes	0,5	7,5	7	7,5	7,5	7	7
L. monocytogenes	0,75	7	7,5	8	6,5	9,5	7
L. monocytogenes	0,75	7,5	7,5	8	7	7,5	7
L. monocytogenes	0,75	8,5	8	8	7,5	7	7,5
L. monocytogenes	6	8,5	7,5	8	7	8,5	7,5
L. monocytogenes	6	8	7,5	8	7,5	7,5	8
L. monocytogenes	6	8	8	8,5	8	7,5	8

APPENDIX D

CHEMICALS

Table D. 1 List of chemicals

Chemical	Supplier
Brain Heart Infusion (BHI) Broth	Oxoid Ltd. (UK)
Agar Bacteriological (Agar No.1)	Oxoid Ltd. (UK)
Ethylenediaminetetraacetic acid (EDTA) Stock Solution [0.5M]	Bioshop (Canada)
Ethanol, absolute	Merck (Germany)
Polylactic acid (PLA) granules [1.25g/cm^3]	Nature Plast (PLI 003)

APPENDIX E

PREPARATION OF BUFFERS AND SOLUTIONS

1. McFarland Solution

The 0.5 McFarland's solution was prepared with;

1.0 % Barium Chloride (mL)	0.05
1.0 % Sulfuric acid (mL)	9.95
Transmittance, %*	74.3
Absorbance*	0.08 to 0.1

*at wavelength of 600nm

Prepared solution was measured with spectrophotometry (Shimadzu 1700 Spectrophotometer, Japan) and the density of cell suspension approximately is $1.5*10^8$ cfu / mL.

2. Ethanol Solution

Ethanol was supplied from MERK with the purity of 96 %. Adding distilled water 70 % ethanol solution was prepared.

3. Physiological Saline Solution

To prepare saline solution 1000 mL distilled water were mixed with 8 gram sodium chloride. After dissolving the salt the solution was autoclaved at 121^{o}C 15 minutes.

4. Phosphate Buffer Solution

250 mL distilled water were mixed with 8 gram potassium dihydrogen phosphate and mixed until the chemical agent was dissolved. To adjust the pH of the solution (6.8 to 7.2) at 25^{o}C, sodium hydroxide was slowly added.

5. Phosphate Buffered Physiological Saline Solution

Prepared phosphate buffered solution were diluted by physiological saline solution to 250 fold volume and autoclave the mixture 121^{o}C for 15 minutes, the remaining mix were hold in cold room (4^{o}C).

APPENDIX F

ANTIMICROBIAL RESISTANCE PROFILE OF THE PATHOGENIC STRAINS

Table F. 1 Antimicrobial resistance profile of pathogenic strains used in this study

Genus	Species	Serotype	Antimicrobial Resistance Profile
Salmonella	*Enterica*	Infantis	KSTAmpKfSfSxtCN
Salmonella	*Enterica*	Enteritidis	susceptible
Salmonella	*Enterica*	Mbandaka	susceptible
Escherichia	*coli*	O6	
Staphylococcus	*aureus*	Rosenbach	
Listeria	*monocytogenes*		-

*Antimicrobial resistance profile (AR Profile):Cro: Ceftriaxone, Eft: Ceftiofur, Sf: Sulfisoxazole, Sxt: Trimethoprim-sulfamethoxazole, C: Chloramphenicol, Imp: Imipenem, Ak: Amikacin, Cn: Gentamicin, K: Kanamycin, S: Streptomycin, Cip: Ciprofloxacin, N: Nalidixic acid, Amp: Ampicillin, Amc: Amoxicillin-clavulanic acid, T: Tetracycline, Fox: Cefoxitin, Kf: Cephalothin, Etp: Ertapenem