

AN INVESTIGATION ON THE ANTIMICROBIAL ACTIVITY OF HEMP FIBER AND FABRICS AGAINIST COMMON NASOCOMIAL INFECTION AGENTS

KENEVİR LİFİ VE KUMAŞLARININ YAYGIN HASTANE ENFEKSİYON AJANLARINA KARŞI ANTİMİKROBİYAL AKTİVİTESİ ÜZERİNE BİR ARAŞTIRMA

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Geliş Tarihi/Received: 03.06.2022 Kabul Tarihi-Accepted: 24.07.2022 Available Online Date/Çevrimiçi Yayın Tarihi: 31.08.2022

Cite this article as: Yuksek A, Havuz SG, Karaduman NS, et al. An investigation on the antimicrobial activity of hemp fiber and fabrics againist common nasocomial infection agents.

J Cukurova Anesth Surg. 2022;5(2):137-144. Doi: 10.36516/jocass.1125626

Abstract

Aim: The aim of this study is to investigate the antibacterial activity of hemp fiber and fabric against common nosocomial infections in intensive care units.

Methods: Raw hemp fiber, hemp fabric in commercial production and cotton fabric with similar properties were used as test material. Antimicrobial activity against 21 common bacterial species in intensive care units was tested using the "disk diffusion method".

Results: Hemp fiber and fabric did not show antibacterial or bacteriostatic effect against any of the 21 hospital infection agents.

Conclusions: The hemp fabric, which seems suitable for use in hospitals with its robust structure, resistance to frequent washing, has not been observed to be superior to cotton fabric in terms of antibacterial properties. However, the genus, species and growing area of the cannabis plant can affect these characteristics. For this reason, more studies on the subject are required.

Keywords: Nosocomial infections, textile materials, hemp, antimicrobial activity, disc diffusion method

Öz

Amaç: Bu çalışmanın amacı yoğun bakımlarda sık karşılaşılan hastane enfeksiyonu etkenlerine karşı kenevir lifi ve kumaşının antibakteriyel etkinliğinin araştırılmasıdır. Yöntemler: Ham kenevir lifi, ticari üretimde olan kenevir kumaşı ve benzer özellikte pamuklu kumaş test materyali olarak kullanıldı. Yoğun bakımlarda sık karşılaşılan 21 bakteri türüne karşı antimikrobiyal aktivite "disk difüzyon yöntemi" kullanılarak test edildi.

Bulgular: Kenevir lifi ve kumaşı 21 hastane enfeksiyonu etkeninin hiçbirine karşı antibakteriyel ya da bakteriostatik etki göstermedi.

Sonuç: Sağlam yapısı, sık yıkanmaya karşı dayanıklığı ile hastanelerde kullanılmaya uygun görünen kenevir kumaşının antibakteriyel özellikler açısından pamuklu kumaşa göre bir üstünlüğü gözlenmemiştir. Ancak kenevir bitkisinin cinsi, türü ve yetiştirilme alanı bu özellikleri etkileyebilir. Bu sebeple konu ile ilgili daha fazla çalışma yapılması gerekmektedir.

Anahtar Kelimeler: Hastane enfeksiyonları, tekstil malzemeleri, kenevir, antimikrobiyal aktivite, disk difüzyon yöntemi

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Introduction

Hospital infections, especially those occur in intensive care units are an important threat to patients' health. These infections that are mostly avoidable lead to longer hospital time. mortality and $cost^1$. According to a report published in 2017 by the European Center for Disease Prevention and Control, 3.8 million cases of hospital infections were recorded in Europe annually². In the United States, 100,000cases of mortality occur due to hospital infections every year. Antibiotics are the most powerful weapons that we possess against infections but the increasing rate of antibiotic resistance is a serious concern³. In addition, multiple anti-biotherapies against resistant bacteria can lead to kidney disorders and immune system weaknesses⁴. Early recognition and prevention of infections is still an important step before anti-biotherapy.

hospital environment plays The an important role on the transmission of bacteria. Textile materials, which are widely used in hospitals, are important routes for bacterial transmission and carriage. Bed sheets, pillowcases, clothing of patients and health workers, protective equipment, surgical gowns, napkins, cloths, and other textile materials used in hospitals play an important role on the transmission and spread of infections. Especially health workers can carry infection between patients in this way. A study showed that 4.6 % of health workers are MRSA carriers⁵. Another study found that nurses that had contact with the belongings of MRSA patients have MRSA on their gloves and can carry them to the other patients ⁶. The Covid-19 pandemic showed us that the virus can be spread by textiles as well as by aerosols and the practice of changing clothes after being in patients' rooms increased as an adaptation to pandemic 7 conditions In previous studies. Acinetobacter baumannii, MRSA, VRE, Coagulase-negative staphylococci, Corynebacterium spp., Micrococcus spp., Bacillus

spp., Non-fermentative gram-negative bacilli, Enterococcus spp., Saprophytic gram-negative bacilli and RNA viruses such as rotavirus have been detected in textile products used on the surgical field⁸. In the hospital environment, blood and tissue remains, food residues and other organic materials create perfect conditions for bacteria growth and transmission if the right textile materials are not used. It was shown that most bacteria can live up to a month on cotton fabrics that are commonly used in hospitals ⁹. Antibacterial finishing of textile materials can suppress bacterial growth and transmission ¹⁰⁻¹². There are various methods to achieve antimicrobial activity on textile materials. Metals and salts. quaternary metal ammonium compounds, triclosan, dyes and other materials are commonly used for this purpose. Depending on the type of fibers and textile materials, different application methods of these compounds can be used such as a) coating the fabrics with these substances b) absorption of these materials into the textile structure and fixation by chemical bonding, c) use of antibacterial metal fibers and yarns and d) use of inherently antimicrobial natural fibers such as chitin and chitosan¹³. However, most of the finishing materials can only last a few weeks or months on fabrics due to repeated washing of textile materials ¹⁴. This fact should also be taken into consideration when dealing with the hospital textiles that are subject to intensive washing.

The use of natural fiber materials against pathogenic bacteria has a long tradition. Literature shows some natural fibers antimicrobial possess effectiveness including hemp fiber ¹⁴⁻¹⁷. The use of hemp fiber and textiles dates back to ancient times. However, during the industrial revolution, the improvements in cotton processing and later the invention of synthetic fibers bring about a loss of interest in natural bast fibers such as flax and hemp. The cultivation of industrial hemp was forbidden in many countries due to the psychoactive THC content in some hemp

varieties. However, in recent years, there is an increasing interest in industrial hemp due to its environmentally friendly nature and various other advantages. One of these advantages of hemp plant is its antimicrobial effectiveness shown in various studies^{18,19}. Hemp plant contains various chemical ingredients most of which has been shown to be antibacterial such as cannabinoids, nitrogen compounds, amino acids, proteins, glycoproteins, enzymes, sugars, hydrocarbons, alcohols, aldehydes, ketones, acids, esters, lactones, steroids, terpenes, flavonglycosides, vitamins, and pigments ^{20,21}. Especially a group of cannabinoids extracted from hemp plant showed excellent antibacterial properties ^{18,} ^{19, 22}. Cannabinoids are found in every part of hemp plant but they are especially abundant in leaf and flowers. Stalk, seed and roots contain much lower amounts of cannabinoids ^{23,24}.

Hemp fibers are more nature-friendly and stronger when compared to cotton fibers and they are mostly referred to as antibacterial and antiallergenic by nature. Hemp fabrics are promising candidates as a potential replacement to the cotton fibers in hospital textiles due to their durability and antibacterial effectiveness. Inprasit et al., ²⁵ showed that Neem extract-applied hemp very effective against fabrics are 26 al.. staphylococcus. Nissen et investigated the microbial inhibition properties of extracts from three different hemp varieties. They showed that essential oils extracted from hemp especially those of Futura can effectively inhibit microbial growth. Radu and Sirghie ²⁷ tested hemp fabrics treated with Ag nanoparticles and detected bacteriostatic activity against S. aureus and E. coli. To date, most of the studies dealt with the antimicrobial properties of hemp fabrics treated with antimicrobial agents. There are only a limited number of studies investigating the antimicrobial effectiveness of raw hemp fiber^{23,25,27,28}.

In this study, the antibacterial effectiveness of raw hemp fibers and two types of hemp fabrics against common hospital infections were investigated and compared with that of cotton which is commonly used in hospital textiles.

Materials and Methods

• Tested textile materials

Two types of hemp fabrics such as bleached hemp woven fabric (BHW), bleached hemp woven fabric with sizing (BHW-S) as well as raw hemp fibers (RHF) and cotton woven fabrics (CW) were used. Table 1 lists the characteristics of fibers and fabrics used in this study. Textile materials go through a series of processes from raw fibers to fabrics such as mercerization, bleaching, other preparatory processes, spinning, sizing, weaving, dyeing and finishing. In this study we used raw hemp fibers obtained from retting process without any further chemical treatment to especially keep lignin on the fibers which is known for its antibacterial properties. Besides raw hemp fibers, bleached and bleached/sized hemp fabrics were also used to see if there is any change in antimicrobial properties of raw fibers after these treatments. Commercial cotton fabrics were used as reference materials. Water-retted raw hemp fibers were purchased from a local hemp farmer in Kastamonu, Turkey. Plain-weave bleached hemp woven fabrics (BHW) were produced using a laboratory-type weaving machine located in our Materials Laboratory at Hemp Research Institute, Yozgat, Turkey. Bleached hemp woven fabrics with sizing (BHW-S) were purchased from Maeko Tessuti S.r.l., Milano, Italy. The characteristics of fabrics and raw hemp fibers were shown in Figure 1.



• Selection of nosocomial infection agents

Within the scope of this study, 21 microorganisms that were previously isolated and identified in the laboratory and stored at a temperature of -20 °C were tested Staphylococcus (3 methicillin-resistant aureus (MRSA), 3 methicillin-susceptible S. aureus (MSSA), 2 methicillin-resistant S. epidermidis (MRSE), 2 extended-spectrum beta-lactamase (ESBL) positive Escherichia coli, 2 ESBL positive Klebsiella pneumoniae. 4 vancomycin-resistant Enterococcus fecalis (VRE), 2 Carbapenem-resistant Pseudomonas aeruginosa, 2 Carbapenem-resistant Acinetobacter baumannii and 1 Colistin-resistant Acinetobacter baumannii]. Identification of microorganisms and antibiotic susceptibility tests performed according were to the manufacturer's recommendations on the Vitek 2 Compact (Biomerieux, France) device. Colistin susceptibility for A. baunmanii was tested using the liquid microdilution method in accordance with the recommendations of the European Committee on Antimicrobial Susceptibility Testing (Eucast). Standard strains (E. faecalis WDCM 00009, E. coli ATCC 25922, P. aeruginosa ATCC 27853, S. aureus ATCC 25923, S. aureus ATCC 4330) were used for quality control.

• Antimicrobial activity testing

The fabrics were cut into 5 mm2 pieces to determine their antibacterial activity, and then sterilized by autoclaving. Hemp plant fiber was used after being cut into 1cm long pieces and sterilized (Figure 2). The presence of the antibacterial effect of fabrics and hemp fiber against selected microorganisms was tested with the "Disc method" diffusion according to the standards of "Clinical and Laboratory Standard Institute (CLSI - M02-A10). Suspensions of 0.5 McFarland (108 microorganisms/ml) were prepared for the microorganisms to be tested. Inoculum

taken from the suspensions with sterile swab was spread over the surface of Mueller-Hinton Agar (MHA) plates. 5 mm2 pieces of fabric and 1 cm hemp fibers were placed on agar plates inoculated. Media were evaluated for the presence of an inhibition zone after incubating at $35 \pm 1^{\circ}$ C for 18-24 hours.

Results

For this study, common intensive care infectious agents were tested. Hemp fibers, which are claimed to have antibacterial activity in some studies, were compared with other fabrics and fibers. Figure 2 and Table 2 show the antibacterial activity testing of fibers and fabrics, and various pathogens responsible for nosocomial infections and quality control strains, which were used for study. It was determined that hemp fiber (RHF), hemp fabrics (BHW and BHW-S) and cotton fabric (CW) do not have antibacterial activity against 21 bacteria, the most common cause of nosocomial infections including 3 MRSA, 3 MSSA, 2 MRSE, 2 ESBL-producing E. coli, 2 ESBL-producing K. pneumoniae, 4 VRE, 2 Carbapenem-resistant P. aeruginosa, 2 Carbapenem-resistant A. baumannii and 1 Colistin-resistant A. baumannii. Also, antimicrobial activity was not observed against quality control strains including E. faecalis WDCM00009, E. coli ATCC 25922, P. aeruginosa ATCC 27853, S. aureus ATCC 25923 and S. aureus ATCC 4330. The results obtained showed that hemp fibers and natural fabrics obtained from hemp fibers do not have antibacterial activity and do not have an advantage over cotton fabric in this respect.

Sample code	Sample description	Fabric type	Yarn count (Nm)	Filling density (yarns/cm)	Warp density (yarns/cm)	Fabric areal weight (g/m ²)
RHF	Raw Hemp Fiber	_	_	_	-	_
BHW	Bleached Hemp Woven Fabric	Plain- weave (1/1)	26	40	10	160
BHW-S	Bleached Hemp Woven Fabric with sizing	Plain- weave (1/1)	24	20	22	170
CW	Cotton Woven Fabric	Plain- weave (1/1)	26	20	22	170

 Table 1. Characteristics of fibers and fabrics used in the study.

Raw hemp fibers (RHF); bleached hemp woven fabric (BHW); bleached hemp woven fabric with sizing (BHW-S); cotton woven fabric (CW).

Table 2. The common nosocomial infection pathogens selected for the study and the antimicrobial activity testing results of hemp and cotton fabric.

Bacteria;	Fibers and fabrics used			
Patient isolates and Quality control strains (n = 26)	RHF	BHW	BHW-S	CW
Enterococcus faecalis WDCM00009	-	-	-	-
Escherichia coli ATCC 25922	-	-	-	-
Pseudomonas aeruginosa ATCC 27853	-	-	-	-
Staphylococcus aureus ATCC 4330 (methicillin- resistant)				
Staphylococcus aureus ATCC 25923	-	-	-	-
Methicillin-resistant Staphylococcus epidermidis (patient isolate-1)	-	-	-	-
Methicillin-resistant Staphylococcus epidermidis (patient isolate-2)	-	-	-	-
Methicillin-resistant Staphylococcus aureus (patient isolate-1)	-	-	-	-
Methicillin-resistant Staphylococcus aureus (patient isolate-2)	-	-	-	-
Methicillin-resistant Staphylococcus aureus (patient isolate-3)	-	-	-	-
Methicillin- susceptible Staphylococcus aureus (patient isolate-1)		-	-	-
Methicillin-susceptible Staphylococcus aureus (patient isolate-2)	-	-	-	-
Methicillin-susceptible Staphylococcus aureus (patient isolate-3)	-	-	-	-
ESBL-producing Escherichia coli (patient isolate-1)	-	-	-	-
ESBL-producing Escherichia coli (patient isolate-2)	-	-	-	-
ESBL-producing Klebsiella pneumoniae (patient isolate-1)	-	-	-	-
ESBL-producing Klebsiella pneumoniae (patient isolate-2)	-	-	-	-
Vancomycin-resistant Enterococcus fecalis (patient isolate-1)	-	-	-	-
Vancomycin-resistant Enterococcus fecalis (patient isolate-2)	-	-	-	-
Vancomycin-resistant Enterococcus fecalis (patient isolate-3)	-	-	-	-
Vancomycin-resistant Enterococcus fecalis (patient isolate-4)	-	-	-	-
Carbapenem-resistant Pseudomonas aeruginosa (patient isolate-1)	-	-	-	-
Carbapenem-resistant Pseudomonas aeruginosa (patient isolate-2)	-	-	-	-
Carbapenem-resistant Acinetobacter baumannii (patient isolate-1)	-	-	-	-
Carbapenem-resistant Acinetobacter baumannii (patient isolate-2)	-	-	-	-
Colistin- resistant Acinetobacter baumannii (patient isolate-1)	-	-	-	-

Raw hemp fibers (RHF); bleached hemp woven fabric (BHW); bleached hemp woven fabric with sizing (BHW-S); cotton woven fabric (CW).

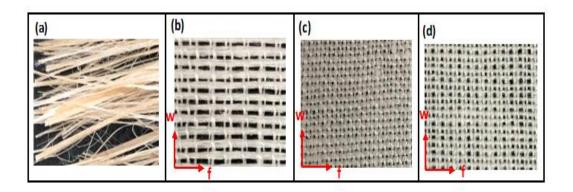


Figure 1. Fibers and fabrics used in the study (w: warp direction, f: filling direction). (a) Raw hemp fibers (RHF); (b) bleached hemp woven fabric (BHW); (c) bleached hemp woven fabric with sizing (BHW-S); (d) cotton woven fabric (CW).

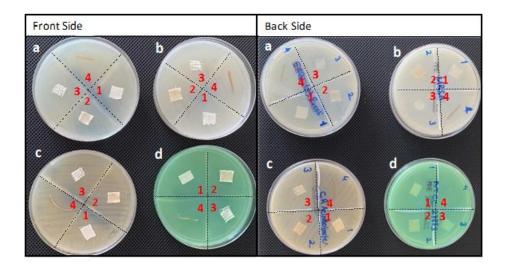


Figure 2. Antibacterial activity of hemp fiber and fabrics and cotton fabric by disc diffusion method against common nosocomial infection agents. (a) ESBL- producing *Escherichia coli*, (b) MRSA, (c) Carbapenem- resistant *Acinetobacter baumannii* (d) *Pseudomonas aeruginosa* ATCC 27853) 1: BHW-S, 2: CW 3: BHW, 4: RHF; MRSA: Methicillin-resistant *Staphylococcus aureus*; ESBL: Extended-spectrum beta-lactamases.

Discussion

The fact that the beds and covers used by the patients in the intensive care units have bactericidal properties or prevent the development of bacteria can make an important contribution to the prevention of hospital infections. Chairs, fabrics and curtains frequently used by patients and their relatives in waiting rooms or wards should be washed frequently. The durability of fabrics that are changed so frequently is

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valuable in terms of costs. Hemp fabric, on the other hand, is a promising product in both respects with its durability and potential antibacterial activity²². Our study is designed to test this added value. We aimed to test the factors that are seen in intensive care units and constitute an important problem in terms of mortality and morbidity in hospital infections, which are the biggest cost expenses. However, this antibacterial property could not be seen in the hemp fabric in our tests. This situation can be explained by multiple reasons. First, it may have activity against an agent other than the 21 bacterial species we use. Another possible reason is that the raw material from which our hemp fabric is produced can have many sub-types.

The hemp plant is referred to as cannabis sativa (*C. sativa*) in the literature. Today, a total of 545 different compounds were isolated from *C. sativa*. Out of these, more than 100 compounds are phytocannabinoids that are specific to hemp varieties and show cannabinoid activity 29,30 .

Phytocannabinoids are produced in leafs, flowers, bracteates and trichomes ³¹. Resins secreted form trichomes protects the plant from its natural enemies and acts as a defense mechanism. It also protects the plant from moisture and heat. Cannabinoids are found in every part of a hemp plant but they are mostly present in leafs and flowers. Stalk, seeds and roots contain much lower amounts of cannabinoids ²³. Therefore it is reasonable to think that the antimicrobial effect is higher in leafs and flowers and lower in stalk and fibers. This can be the reason that an antimicrobial effect is not found in this study in any of the hemp fibers and fabrics against the tested bacteria which are common hospital bacteria found in intensive care units. Another reason for the lack of antimicrobial activity may be that we used fibers from a male plant. It is wellknown that the male plants usually have fewer amounts of cannabinoids when compared to female ones³². A similar study conducted on flax fibers which are similar to hemp in terms of structure and the

amount of chemical ingredients showed that there is not an apparent bacteriostatic effect of flax fiber against gram positive and gram-negative bacteria ³³. It was concluded that further study is needed to determine the effectiveness of hemp fiber and fabric against different types of pathogens.

Conclusion

Since hemp fibers are natural and durable products, they attract attention with their potential in hospital textiles. Although previous studies suggested antimicrobial activity of hemp extracts, in the current work, there is not a clear indication of the antimicrobial activity of raw hemp fiber and fabrics against tested infection agents. These findings can be interpreted as the end of a myth. It was concluded that further study is needed to determine comparatively the antimicrobial activity of hemp fiber produced from a female plant containing higher amounts of cannabinoids against nosocomial infection agents by both disc diffusion method and microdilution method.

Author contributions

All authors contributed to the study conception and design. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

Authors declared no financial support.

Ethical approval

Ethical approval is not required

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