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Detection of Bacteria Types Contaminating Hotel Bedsheets and Testing their Sensitivity to Natural and Industrial Disinfectants

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Abstract

The majority of people who are travelling like to stay in hotels, or rent rooms which are considered dangerous sites for patients and vectors, and environments suitable for outbreaks and disease spreading. Bacterial contamination in hotels is a crucial concern for guests as it can cause serious infections. This study aimed to evaluate the contamination of some local hotel bedsheets with bacteria. For this, forty samples were collected (pillow covers, sheets, blankets, and mattresses) from different hotels during the summer holidays of July- August 2023. Several bacterial diagnosis tests were carried out to identify these bacterial species. Then, an antibacterial sensitivity test for ten different antibiotics and the bacteria that exhibited the highest antibiotic resistance were selected to test their sensitivity to natural plant extract and industrial disinfectant. Results showed that the highest level of bacterial contamination was registered in pillows and sheets ($22.33x10^3$cfu/g) and ($21.33x10^3$cfu/g) respectively. Also, ten bacterial species were isolated from all bedsheet types and most of them were normal flora. Moreover, Gram-positive bacteria were the most prevalent species. The antibiotic sensitivity tests showed that *Micrococcus sp*. was the most sensitive bacteria to antibiotics at 50%, while *Bacillus cereus* was more resistant to antibiotics at 80%. Also, as a result of the sensitivity of bacteria to disinfectant, crude lemon was recorded as the perfect natural disinfectant for *Staphylococcus sp.* whereas, a liquid industrial disinfectant (LID) was most effective against *Escherichia coli*, *Bacillus cereus* and *Klebsiella sp*. Therefore, it is preferable to use LID and lemon as a disinfectant and sterilizer when washing fabrics.

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1. Introduction

The average sleep rate for adults worldwide is about 8-9 hours per day, and for infants about 12-14 hours per day. Bedsheets are potential sources of biological contaminants, which can affect the health of humans especially people who have weak immunity systems. Due to its close interaction with the human body and thus it contributes to the transmission and spread of diseases [1-5]. Therefore, the bedsheet must be washed with detergents and exposed to sunlight to eliminate hazardous microorganisms [6]. Also, mattresses must be replaced to prevent microbial contamination [7].

Textiles are a reservoir for microbes as they can be transmitted and proliferate on it. Bacteria could survive from a few seconds/ hours to days or several months on fabric and surfaces, depending on fabric quality, such as cotton or polyester

and bacterial type. Hence, bedsheets serve as fomites contributing to the infection spread [8-10]. Bedsheets are one textile that is an ideal environment for the growth of bacteria, and since the accumulation of dirt and food remains and the accumulation of sweat, saliva, and skin oils, help increase the growth of microorganisms and raise the possibility of infection [11-13].
According to previous reports, the beds of undergraduate students who live in university hostels harbour harmful microorganisms, such as bacteria and fungi that might cause epidemics on campus since they found the predominance of bacteria compared to fungi in bedsheets such as *Escherichia coli, Staphylococcus aureus, Aspergillus sulphureus* and *Aspergillus niger* [6, 14, 15]. Bacteria were also found on clean and dirty hospital bedsheets and staff uniforms, as the average number of bacteria on clean bedsheets was 3cfu/25 cm^2. In comparison, on dirty bedsheets, it was 23cfu/25cm^2 [16]. In England, a *Streptococcus sp.* outbreak was due to the lack of healthcare in homes [17]. Also, outbreaks occurred because of the laundering of contaminated bedsheets [18, 19]. Bacterial colonies have been observed on textiles during laundering. Another reason for the proliferation of infectious microorganisms is the mixing of soiled bedsheets with laundered fabrics [20].

Several studies have been conducted to investigate the microbial contamination of hospital bedsheets. However, few researchers highlighted bedsheet contamination in hotels and rented rooms where many people used to stay when they travelled. Therefore, this study aimed to evaluate the bacterial contamination of bedsheets in hotels/bedrooms in Baghdad and Sulaymaniyah cities and identify the bacterial types that appeared during the investigation process.

2. Materials and Methods

2.1 Sample Collection

Forty samples of bacteria were randomly collected from bedsheets from different local hotels in Baghdad and Sulaymaniyah cities between 11th July and 23rd August 2023. This was achieved by rubbing on the surface of the bedsheets (pillow covers, sheets, blankets, and mattresses) with swab sticks immersed in normal saline as previously reported by [21]. Each sample was inoculated separately into sterile tubes containing nutrient broth medium at 37 °C for 24 h directly. Tubes were transferred to the microbial laboratory in Al Ramadi Teaching Hospital for Maternity and Children to conduct bacterial diagnosis tests and identify isolated bacterial strains.

2.2 Culture Media

MacConkey agar (Biomark labs/India), Blood agar (Biomark labs/India), and Mannitol salt agar (Biomark labs/India) were used to detect *Staphylococcus sp.*, *Streptococcus sp.*, and gram-negative bacteria. Eosin Methylene Blue Agar (ENBA) medium (Biomark labs/India) was also used to detect *Escherichia sp.*, and Nutrient agar (Biomark labs/India), was used to count the colony's numbers. Then, all cultures were incubated at 37 °C for 24 h. All media were prepared according to the manufacturer-recommended procedure.

2.3 Bacterial Identification

Bacteria species were identified based on standard protocol starting with the morphology of colonies, gram stain, and the description of colonies. Then, several biochemical tests were conducted for the bacterial colony (catalase, oxidase, IMVIC, urease, and nitrate) test, as previously described [22]. Bergey's Manual was used to identify types of bacteria as already reported [23].

2.4 Antibacterial

Disc diffusion technique was employed to determine the sensitivity of bacteria against ten types of bacterial antibiotics, Augumentin (AMC) 30mg, Ampicillin(AMP) 10mg, Chloramphenicol (C) 25mg, Cephalexin (CN) 10mg, Doxycycline (DO) 30mg, Erythromycin (ERY) 10mg, Rifampicin (RD) 25mg, Streptomycin (STR) 10mg, Bactrim (SXT) 25mg, and Tetracycline (TET) 30mg. Muller Hinton agar (MHA) (Biomark labs/India) was used as the culture medium for bacteria isolated in this study [24].

2.5 Natural and Industrial Disinfectant Test

A liquid industrial disinfectant (LID) of 2.10% and phenol of 5% were used, according to the dilution rate recommended by the manufacturer (Soap and Chemicals Industrial & Trading Co. /Dubai, U.A.E.). Crude lemon and cloves were used as a natural disinfectant. 20µl of each disinfectant was inoculated onto holes (6 diameters and 5 depth) made in MHA of each resistant bacteria [25]. Then all cultures were incubated at 37°C overnight. After that, the inhibition zone diameters(mm) were measured.

2.6 Statistical Analysis

The Excel system was used to explain the presence and spread of bacterial isolates. ANOVA was also used to analyze all data statistically under a probability level of 0.05 to make comparisons.

3. Results and Discussion

The results of evaluating the bacterial content of the study samples showed that there were significant differences in bacterial count between the different types of bedsheets at a probability level of less than (0.05), the highest significant levels of bacterial content in pillow covers and sheets were ($22.33x10^3$cfu/g) and ($21.33x10^3$cfu/g) respectively. Blankets and mattresses had the lowest significant level ($19.33x10^3$cfu/g) and ($17.33x10^3$cfu/g) respectively. As shown in Table (1).

Table (1): Statistical Analysis of Bacterial Contamination

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	3	44.250	14.750	8.045	0.00845
Error	8	14.667	1.833		
Total	11	58.917			

Treatment	Character	
	Mean ±SD	S.E.
Pillow covers	22.333	0.882
Sheets	21.333	1.202
Blanket	17.333	0.333
Mattress	19.333	0.333
LSD 5%	2.589	
SE(m)	0.782	
SE(d)	1.106	
C.V.	6.742	

DF= Degree of Freedom, C.V. Coefficient of Variation
S.E. Standard Errors, SE(m)= Standard Errors means, SE(d)= Standard Errors division

3.1 Bacterial Contamination Based on Gram-Stain

The results showed that the rate of bedsheet contamination with gram-positive bacteria (66%) is much higher than that of contamination with gram-negative bacteria (34%) Figure (2). *Escherichia coli* (G^-) and *Staphylococcus aureus* (G^+) recorded the highest percentage of bacterial content on bedsheet types. As shown in Figure (1).

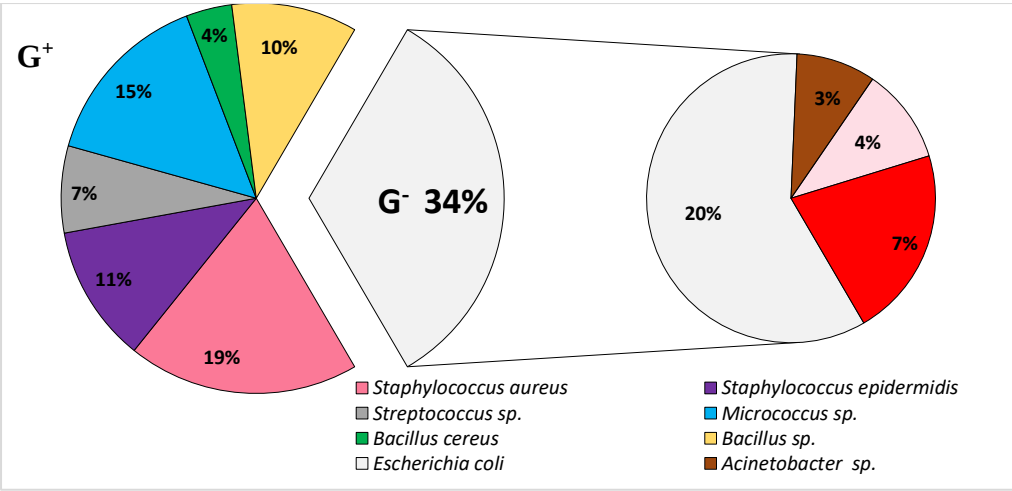

Figure 1. Bacterial Contamination Based on Gram-Stain

The results showed that hotel bedsheets contain different bacteria species. This may be due to the lack of effective detergents used when washing lines, or worse clean-up service. These results agree with previous studies [6, 16] that have reported many bacterial species in a bedsheet environment and 57% were found on human skin. Another study was conducted about microbial contamination of mattresses, which found 42 microbes were isolated from mattresses before utilization:

Staphylococcus epidermidis 38%, *Staphylococcus aureus* 14%, and *Pseudomonas aeruginosa* 12%; and 40 microbes isolated from mattresses after utilization: *Staphylococcus epidermidis* 25%, *Acinetobacter sp*. 13%, *Enterobacter sp*. 10%, and *Pseudomonas sp.* 10% [7]. Environmental conditions and fabric type affect the load of bacteria on bedsheets [26]. Therefore, the adhesion, growth, and transmission of bacteria to fabrics depend on these main factors, as each type of bacteria has surface properties that distinguish it from other types [27].

3.2 Bacterial species in bedsheets

Ten bacterial species were obtained from hotel bedsheets. *Staphylococcus aureus* and *Escherichia coli* recorded the highest percentage of bacterial content on all bedsheet types, while in mattresses, *Bacillus sp*. had the highest percentage of bacterial contamination at 18.2%. On the other hand, *Acinetobacter sp.* was found on the pillowcase and blanket at 10.5% and 1.3% respectively but was not found on the mattress and sheets.

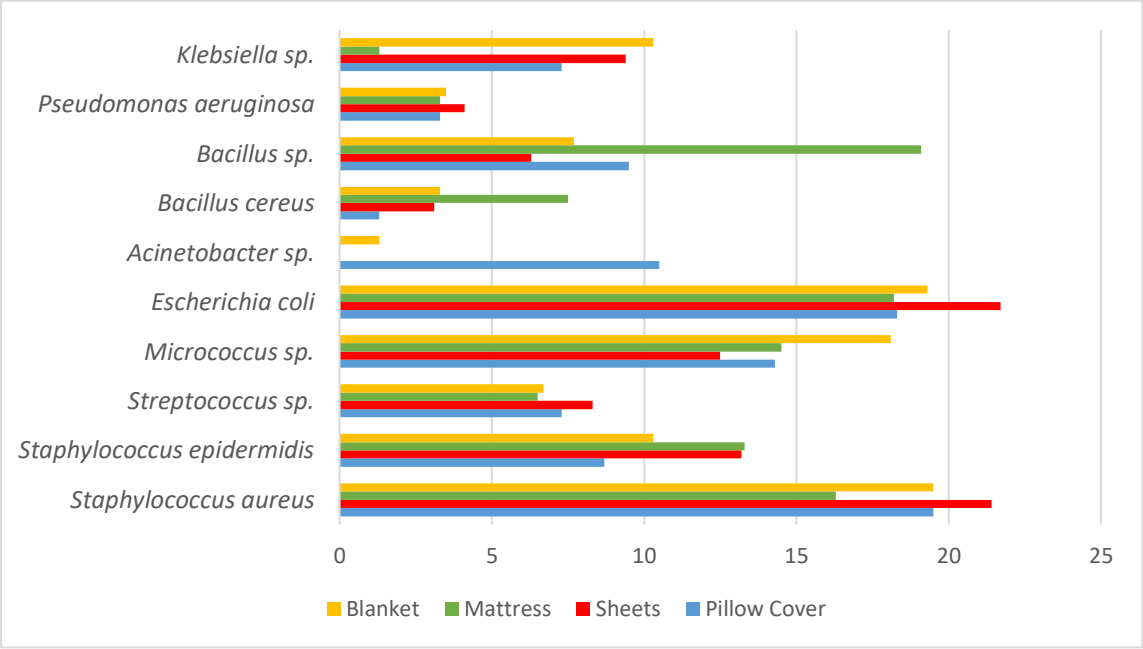

Figure 2. Bacterial Species in Bedsheet

Sixty-six per cent were found with gram-positive bacteria. *Staphylococcus aureus*, *Micrococcus sp*., and *Escherichia coli* were the dominant bacteria. These results are coherent with the literature data [15, 18, 19]. the higher percentage of Gram-positive bacteria in textiles could be due to the presence of bed bugs that carry many *Staphylococci sp.*, *Micrococcus sp*., and *Kocuria kristinae* that may cause highly contagious skin diseases in communities [28-30].

The presence of several bacterial species may be due to the mixing of clean with dirty bedsheets during laundering in the washing machine, which is considered the other main source of infections [31]. In addition, the bacterial load on laundry is affected by many key factors including fabric type, usage, storage conditions, and type and quality of detergent [32].

3.3 Sensitivity of bacteria to antibiotics

The result showed that *Micrococcus sp*. was the most sensitive bacteria against antibiotics among other bacteria isolated from bedsheets at 50%. At the same time, *Bacillus cereus* was more resistant bacteria to antibiotics by about 80%.

AMC and RD were recorded as the wide-spectrum antibiotics for bedsheet bacteria at 70%, followed by TET at 50%. While the narrow-spectrum antibiotics were AMP and DO at 20%. Table (2).

Table (2): The Effect of Antibiotics on Bacteria

Bacteria isolated	Antibiotics										%
	AMC	AMP	C	CN	DO	ERY	RD	STR	SXT	TET	
Staphylococcus aureus	+	-	+	-	-	-	+	-	+	-	30
Staphylococcus epidermidis	+	-	-	-	-	-	+	-	+	-	30
Streptococcus sp.	+	+	-	+	-	-	+	-	-	-	40
Micrococcus sp.	+	-	+		+	-	+	-	-	+	50
Escherichia coli	+	-	+	+	-	-	-	-	-	-	30
Acinetobacter sp.	+	+	-	+	-	-	-	-	-	+	40
Bacillus cereus	-	-	-	-	-	-	+	-	-	+	20
Bacillus sp.	-	-	+	-	+	-	+	-	-	-	30
Pseudomonas aeruginosa	-	-	-	+	-	-	+	-	+	+	40
Klebsiella sp.	+	-	-	-	-	-	-	-	+	+	30
35	7	2	4	4	2	0	7	0	4	5	
%	70	20	40	40	20	0	70	0	40	50	

+ Sensitive to antibiotics - Resistance to antibiotics

Different species of bacteria showed varying levels of resistance towards antibiotics. Several factors make bacteria resistant to antibiotics. One reason is the cell membrane permeability to antibiotics and enzymatic degradation of the antibiotics. Another factor is the specific mechanism of action of the antibiotic. Bacteria may be more resistant to antibiotics that inhibit protein and nucleic acid synthesis than those that affect the cell wall and cytoplasmic membrane synthesis. Also, the genetic makeup of bacteria and the presence of specific genes responsible for resistance. Furthermore, target molecule alteration [33-36].

3.4 Bacteria sensitivity to industrial and natural disinfectants

Drastically, LID and crude lemon produced good antibacterial activity with inhibition zones range (of 10.67-14. mm) and (17.67- 9.33 mm) respectively whether to G^+/ G^- bacteria. Crude lemon was the most powerful natural disinfectant for *Staphylococcus sp.* while, LID was the superlative industrial disinfectant for *Escherichia coli*, *Bacillus cereus* and *Klebsiella sp*.

Table (2) Industrial and Natural Disinfectant

	LID	Phenol	Lemon	Cloves
Staphylococcus aureus	10.67	4.67	14.67	7.33
Staphylococcus epidermidis	14.33	9.00	16.00	6.00
Escherichia coli	17.67	7.67	9.67	8.67
Bacillus cereus	14.00	4.67	9.33	9.67
Klebsiella sp.	21.33	13.67	17.67	6.33
LSD 5%	2.048	1.879	2.349	2.048
P- value	0.000**	0.000**	0.000**	0.0123**

Statistical analysis showed significant differences between the effect of plant extracts on bacterial isolates with variability related to the bacteria species at level ($P \leq 0.05$). The reason for the difference in bacterial species sensitivity against these natural disinfectants might be due to the composition of the bacteria wall and the active compounds of the plant extracts on the other hand [37]. These results matched with [38] when they investigated lemon activity against microbes, they found it possesses antibacterial activity to *Staphylococcus aureus*, *Klebsiella sp.*, *Escherichia coli* and *Pseudomonas aeruginosa*.

The superiority of lemon over clove extract in inhibiting bacteria can be attributed to the pH and the active substances included in the extract, which affect the permeability of the cell membrane of bacteria [39]. Most of the bacteria species isolated were mostly opportunistic pathogens for people, such as staphylococci or micrococci [16].

4. Conclusion

The study showed that several bacteria were found in all kinds of bedsheets in the investigated hotels .Therefore, changing and washing bedsheets after each use is necessary because they are a risk source of disease transmission among travelers. Choosing the best types of detergent and disinfectant is also necessary to reduce the presence of bacteria. It is preferable to use LID when washing disinfectant fabrics, in addition to using lemon as a natural disinfectant.

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الكشف عن أنواع البكتيريا الملوثة لأغطية الأسرة الفندقية واختبار حساسيتها للمطهرات الطبيعية والصناعية

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المستخلص:

يفضل غالبية الأشخاص الذين يسافرون الإقامة في الفنادق، أو أستئجار الغرف التي تعتبر بؤرة خطرة للمرضى وحاملي الأمراض، وبيئة مناسبة لتفشي المرض وانتشاره. يعد التلوث البكتيري في الفنادق مصدر قلق بالغ للضيوف لأنه يمكن أن يسبب التهابات خطيرة. هدفت هذه الدراسة إلى تقييم تلوث بعض أغطية أسرة الفنادق المحلية بالبكتيريا. ولهذا تم جمع أربعين عينة (أغطية وسائد، ملاءات، بطانيات، ومراتب) من فنادق مختلفة خلال العطلة الصيفية لشهر تموز – آب 2023. وتم اجراء العديد من الاختبارات التشخيصية البكتيرية للتعرف على هذه الأنواع البكتيرية. بعد ذلك، تم اختيار اختبار الحساسية المضادة للبكتريا لعشر مضادات حيوية مختلفة والبكتيريا التي أظهرت اعلى مقاومة للمضادات الحيوية لاختبار حساسيتها للمستخلصات النباتية الطبيعية والمطهرات الصناعية.
أظهرت النتائج أن أعلى مستوى للتلوث الجرثومي كان في الوسائد والملاءات ($22.33x10^3$cfu/g) و ($21.33x10^3$cfu/g) على التوالي. كما تم عزل عشرة أنواع بكتيرية من جميع أنواع الملاءات وكان معظمها من البكتريا الطبيعية. علاوة على ذلك، كانت البكتيريا إيجابية الجرام هي أكثر الأنواع انتشارا.
أظهرت اختبارات الحساسية للمضادات الحيوية أن *Micrococcus sp.* كانت البكتيريا الأكثر حساسية للمضادات الحيوية بنسبة 50%، في حين كانت *Bacillus cereus* أكثر مقاومة للمضادات الحيوية بنسبة 80%. وأيضاً نتيجة لحساسية البكتيريا للمطهرات فقد تم تسجيل الليمون الخام كمعقم طبيعي مثالي لبكتيريا *Staphylococcus sp.* في حين كان المطهر الصناعي السائل (LID) الأكثر فعالية تجاه لبكتيريا *Escherichia coli* و *Bacillus cereus* و *Klebsiella sp.*