Celal Bayar University Journal of Science

Microbiological Examination of Washbasin, Faucet Heads and Toilet Door Handles of the Students' Toilets at a State University

Yasemin Keleş Dinç [1], Sedat Per[2]*

[1] Yozgat City Hospital, Türkiye
[2] Department of Chemistry and Chemical Processing Technologies, Mustafa Çıkrıkçıoğlu Vocational School, Kayseri University, Türkiye
*sedatper@kayseri.edu.tr
*Orcid No: 0000-0002-6131-3321

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Abstract

This study, it was aimed to determine the presence of pathogenic bacteria in the sinks, faucets, and door handles of the toilets actively used by boys and girls in nine faculties located on Yozgat Bozok University Erdoğan Akdağ campus. 54 swab samples were taken from the sinks, faucets, and door handles of toilets, which have the largest share of the main sources of bacteria transmission, between April and June 2019. Each swab sample brought to the laboratory via a cold chain was inoculated separately on 5% sheep blood agar, EMB agar and Mac Conkey agar. In 34 (62.96%) of the swab samples taken, a total of 49 pathogenic bacteria were detected, growing singly or in multiples. Of the 49 bacteria detected, 15 were (30.6%) *Escherichia coli*, 12 were (24.5%) *Staphylococcus aureus*, nine were (18.3%) *Klebsiella spp*., six were (12.3%) *Pseudomonas* spp., five were (10.2%) *Proteus spp*. and two were (4.1%) *Enterococcus* spp.

Keywords: Contamination, Hygiene, Pathogenic bacteria, School toilet

1. Introduction

Education, which focuses on the upbringing of the individual, includes formal and informal learning that has been going on since primitive societies. While the family and the environment are influential in the upbringing of the individual in an unofficial sense; Schools have assumed this role in an official sense.[1] Schools, which have an important place in human life, are places where individuals between the ages of 6-and 24 spend most of their time. School-age individuals are more sensitive to physical, biological, and social environmental conditions than adults and are significantly affected by changes in the environment. Therefore, a healthy school environment is necessary for healthy students.[2]

Hygiene is defined as all the practices made to prevent, maintain, and improve human health factors. Hygiene covers all human activities from the moment of fertilization to death and because it has a wide range of actions, it can be divided into sub-units as an individual, public, and social hygiene. Also, hygiene always has a common purpose: protection, maintenance, and promotion of health. Personal hygiene is a branch of hygiene that deals with the factors affecting the individual's health and formulates the principles that the individual will apply to protect, maintain, and improve health.[3]

Hands play an important role in healthcare institutions, industrial settings such as the food industry, as well as in all community and home settings in the transmission of infection. However handwashing has been seen as a measure of personal hygiene for centuries, the specific link between handwashing and the spread of infectious diseases has emerged over the past 200 years.

The microbial population of the skin is divided into resident microbiota and transient microbiota. The resident microbiota is associated with the deeper layers of the skin, such as the sebaceous glands. Temporary microbiota colonizes the superficial layers of the skin and is less adherent. Also, they are more easily removed by hand washing and can be transferred by direct hand contact between human skin and the inanimate environment such as work surfaces or food.[4]

Microorganisms are the oldest living things on earth, due to their ability to adapt quickly to changing living

conditions. Thanks to these abilities, bacteria can find a way to escape from every new antibiotic developed against them.[5] Bacteria were first observed in 1676 by Antonie van Leeuwenhoek with a single-lens microscope he had designed and built. Leeuwenhoek named the creatures he observed "animalcules". The word “bacterium” was used for the first time in 1838 by Christian Gottfried Ehrenberg and later it was used in the scientific world. The word “bacterium” is originally derived is derived from the Greek word bacterion, meaning "small staff.[6]
Although there are similar studies in other countries in the literature, the fact that it was conducted in universities for the first time in our country makes the study valuable.[7-15] In this study, the presence of pathogenic bacteria in the sinks, tap heads, and door handles of the toilets are actively used by female and male students in nine faculties located on the Erdoğan Akdağ campus of Yozgat Bozok University was investigated.

2. Materials and Methods

The study examined swab samples taken from the toilet door handles, faucet heads, and washbasin of the toilets belonging to 9 different faculties during the education period (in the Figure that will cover the months of April, May, June) on the campus. A total of 54 swab samples were taken by taking three samples from each of the male and female student toilets, which are actively used in the faculties. The samples taken were transported to the laboratory at the cold chain for cultivation (considering the season in which the samples were collected, they were transported in a bag containing ice wrapped in gauze without contacting the battery in order to protect the characteristics of the medium and the presence of bacteria).

2.1. Cultivation of Specimens

Each swab sample was cultivated separately on 5% sheep blood agar, EMB agar, and MacConkey agar. The media were incubated at 37°C for 36-48 hours. At the end of the incubation, non-growth media were noted. To colonies with multiple growths on 5% sheep blood agar were applied Gram staining. After that gram-negative colonies were passaged on EMB and MacConkey agar. In addition, were passaged again to obtain pure colonies from the mixed growing colonies on EMB and MacConkey agar. The media taken into the passage were again incubated at 37°C for 36-48 hours.

2.2. Macroscopic Examination

Grown on EMB, Macconkey agar, and 5% sheep blood agar and macroscopic appearance of bacteria with characteristic morphology were used.

2.3. Gram Staining

Colonies spread on the slide were dried and fixed, and crystal violet dye solution was dropped on it and waited for one minute. After the preparations were washed with distilled water, Lugol was dripped and waited for one minute. It was washed with distilled water again, 95% ethanol was dropped and waited for 10-15 seconds, then washed with distilled water and covered with aqueous fuchsin and waited for 30 seconds. After washing with distilled water, air-dried preparations were examined under a light microscope with oil immersion.

2.4. Catalase Test

Catalase activities of all bacterial colonies that fell pure after the passage process was performed with hydrogen peroxide (H_2O_2). Colonies that formed gas by releasing O_2 when the colony was mixed with the catalase reagent dripped on the slide were evaluated as catalase positive.

2.5. Coagulase Test

The pure colony grown in the medium was suspended in the tube containing 0.5 ml of blood plasma. Tubes with plasma were incubated at 37°C. The tubes were evaluated for the presence of fibrin at the 4th hours, 8th hours, and 24th hours. Tubes with full clotting in the 4th hour were evaluated as positive results. Tubes without complete fibrin formation were allowed to incubate again. At the end of the incubation, the coagulase test of tubes with coagulation plasma was evaluated as positive, and the homogeneous liquid plasmas coagulase test was evaluated as negative.

2.6. Oxidase Test

For the oxidase test, the colony was rubbed into blotting paper and oxidase solution was dripped onto it. After 30-60 seconds, the formation of violet-purple color on the colony applied area was evaluated as oxidase-positive.

2.7. PYR (Pyrrolidonyl Arylamidase) Test

One drop of distilled water was dropped on the sticks that came out of the PYR test kit, and it was waited for 30 seconds for the stick to absorb the water evenly, and then the colony was applied to the place where the distilled water was dripped. The PYR solution included in the kit was dripped onto the colony. After 40-50 seconds, the formation of a pink-fuchsia color on the colony applied area was considered as a positive result.

2.8. TSI Agar (Triple Sugar Iron Agar) Test

Bacteria determined to be gram-negative as a result of gram staining were inoculated into a TSI medium and incubated at 37°C for 16-18 hours. Blackening in the

medium was evaluated as H_2S positive. The gas formation seen with the disintegration of the medium was evaluated. In addition, the bottom of the medium turning from red to yellow was evaluated as the presence of bacteria that use glucose. The presence of bacteria that use lactose when the slanted part turned from red to yellow was evaluated as positive and the absence of color change was evaluated as negative.

2.9. Urea Test

Bacteria determined to be gram-negative as a result of gram staining were inoculated into a urea medium and incubated at 37°C for 16-18 hours. The fuchsia-pink color formed in the medium at the end of the incubation was evaluated as the presence of bacteria that use urea.

2.10. Citrate Test

Bacteria determined to be gram-negative as a result of gram staining were inoculated into a citrate medium and incubated at 37°C for 16-18 hours. The color change from green to blue that occurred in the medium at the end of incubation was evaluated as the presence of bacteria that use citrate.

2.11. Motility Test

The colony which was taken with the help of a loop into the movement medium was cultured perpendicularly and incubated at 37°C for 16-18 hours. The spread of growth towards the periphery of the culture area after incubation was evaluated as positive motility of the cultured bacteria.

2.12. Indole (Tryptophan) Test

Bacteria determined to be gram-negative as a result of gram staining were inoculated into liquid media and incubated at 37°C for 16-18 hours. After incubation, 3-4 drops of Kovac's reagent were dropped into a liquid indole medium. After 4-5 seconds, the red ring formed on the liquid media was evaluated as the indole test was positive.

3. Results and Discussion

No growth was observed in 20 of the 54 swab samples examined. In the remaining 34 swab samples, a total of 49 bacteria were detected, 21 of which were single and 13 were multiple growths. A total of 15 (30.6%) *E. coli* bacteria were detected, 8 (53.33%) single and 7 (46.67%) multiples. A total of 12 (24.5%) *S. aureus* bacteria were detected, 6 (50%) single and 6 (50%) multiples. A total of 9 (18.3%) *Klebsiella spp*. bacteria were detected, 2 (22.22%) single and 7 (77.78%) multiples. A total of 6 (12.3%) *Pseudomonas spp*. bacteria were detected, 2 (33.33%) single and 4 (66.67%) multiples. A total of 5 (10.2%) *Proteus spp*. bacteria were detected, 1 (20%) single and 4 (80%) multiples. Finally, 2 (100%) single (4.1%) *Enterococcus spp*. bacteria were detected (Figure 1-5).

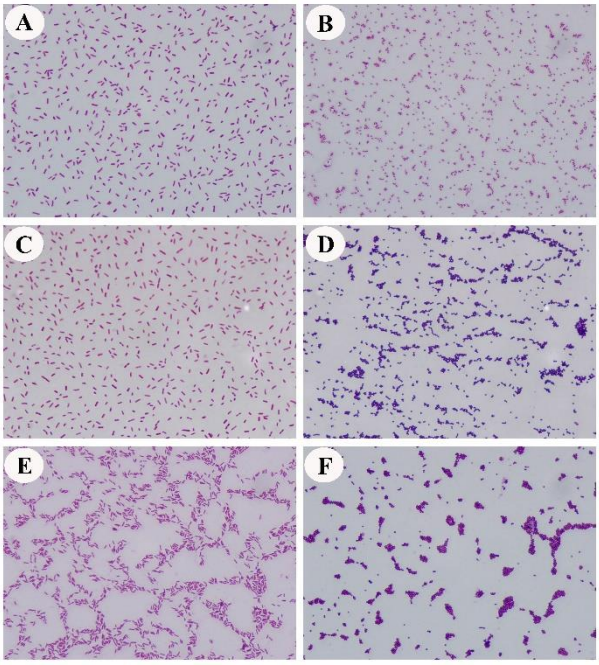

Figure 1. Microscope images **A)** *Escherichia coli*, **B)** *Proteus spp.*, **C)** *Klebsiella spp.*, **D)** *Enterecoccus spp.*, **E)** *Pseudomonas spp.*, **F)** *Staphylococcus aureus.*

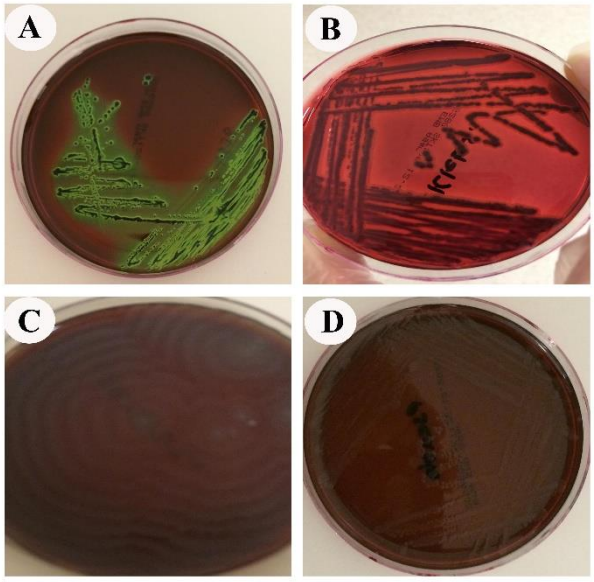

Figure 2. Images on Eosin Methylene Blue agar **A)** *Escherichia coli* **B)** *Klebsiella spp.*, **C)** *Proteus spp.*, **D)** *Pseudomonas spp.*.

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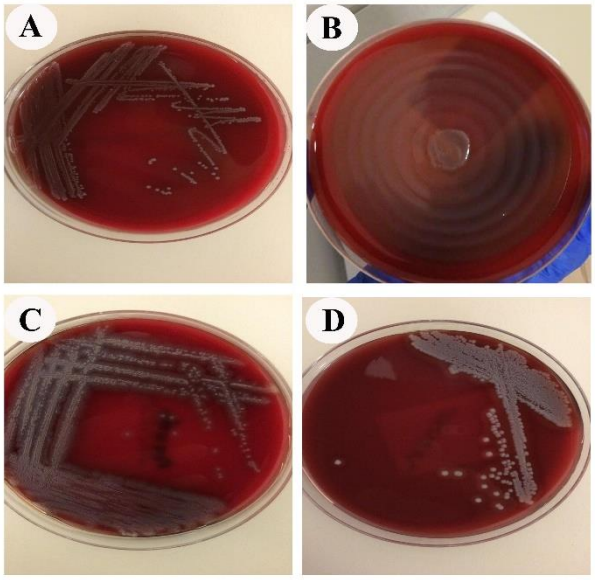

Figure 3. Images on blood agar **A)** *Enterecoccus spp.*, **B)** *Proteus spp.*, **C)** *Pseudomonas spp.*, **D)** *Stafilococcus aureus*.

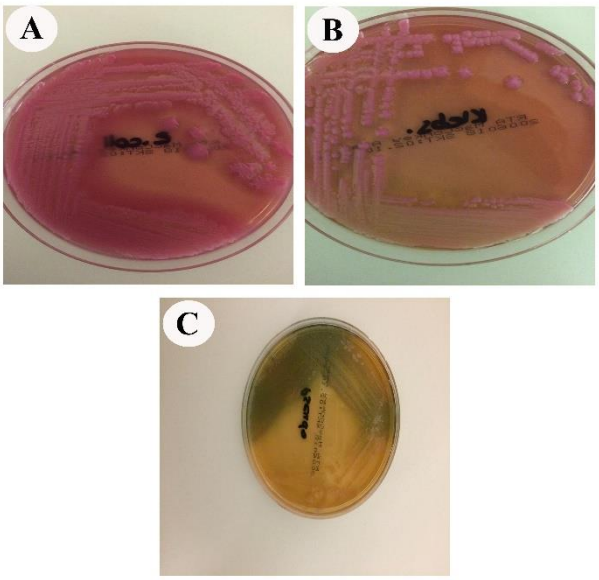

Figure 4. Images on Mac Conkey agar **A)** *Escherichia coli*, **B)** *Klebsiella spp.*, **C)** *Pseudomonas spp*..

Staphylococcus aureus was reported to be the most common organism in a study conducted by taking swab samples from 200 door handles in two universities (Khartoum University, Sudan University of Science and Technology, and Al Neelain University) in the state of Khartoum in Sudan.[15]

Alonge et al. (2019) in their study, in which they conducted a microbiological scan of the door handles of 12 toilets in the Abuja campus of Baze University of Nigeria, stated that there was general contamination by seven bacterial species. (*Staphylococcus aureus* 2.9%; *Salmonella typhimurium* 21.4%; *Escherichia coli* 14.3%; *Pseudomonas aeruginosa* 9.5%; *Proteus mirabilis* 4.8%; *Klebsiella oxytoca* 4.8%; *Klebsiella pneumoniae* 2.3%).[16] In the study in which the water flowing from the sinks in some schools in Erzurum (Turkey) city center and the swab samples taken from faucet heads of the toilets were examined microbiologically, bacteria were detected in 136 (90.7%) of the 150. The isolated bacteria were *Escherichia coli* in 54 samples (36%), and *Staphylococcus aureus* in 52 (34.6%) of the swab samples. Total coliform bacteria and fecal coliform bacteria were not found in any of the water samples examined.[17]

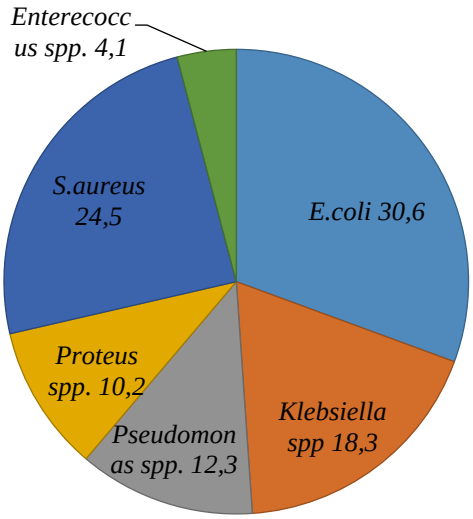

Figure 5. The growth results of pathogenic bacteria on washbasin, faucet heads and toilet door handles.

Approximately 10 surfaces were sampled from each of the 12 public toilets and 19 bacterial phyla were identified using high-throughput barcoded pyrosequencing of 16 S rRNA genes. Most sequences belonged to four phyla: *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria*.[18]

It was determined that, except for one of the toilets used by the students in the faculties (Faculty of Engineering and Architecture, men's toilet), foaming hand soap was found in the rest. It was determined that the availability of toilet paper varies according to faculties (Table 1). A total of 26 (53.1%) of the toilets used by female students and 23 (46.9%) of the toilets used by male students were found to have bacterial growth.

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Table 1. Availability of soap and toilet paper in faculty toilets.

Faculty names	Gender	Soap	Toilet paper
Faculty of Medicine	F	+	+
	M	+	-
Faculty of Engineering and Architecture	F	+	+
	M	-	-
Faculty of Health Sciences	F	+	-
	M	+	+
Faculty of Theology	F	+	-
	M	+	+
Faculty of Agriculture	F	+	+
	M	+	-
Faculty of Education	F	+	+
	M	+	+
Faculty of Economics and Administrative Sciences	F	+	-
	M	+	-
Faculty of Communication	F	+	-
	M	+	-
Faculty of Art and Sciences	F	+	-
	M	+	+

Percentage distribution of bacteria detected from toilets used by female students; *E. coli* 38.5%, *S. aureus* 19.2%, *Klebsiella spp*. 15.4%, *Pseudomonas spp*. 11.5%, *Proteus spp*. 11.5%, *Enterococcus spp*. 3.9% (Figure 6).

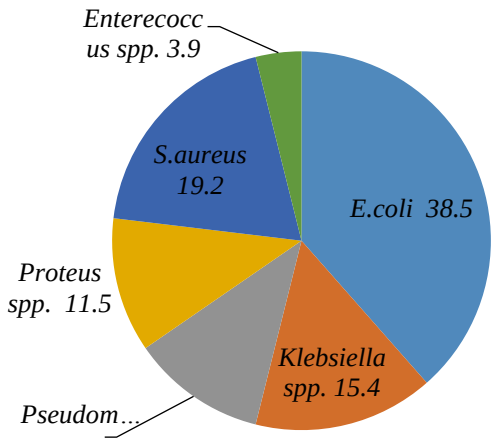

Figure 6. The growth results of pathogenic bacteria on washbasin, faucet heads and toilet door handles used by female students.

Percentage distribution of bacteria detected from toilets used by male students; 30.4% *S. aureus*, 21.7% *E. coli*, 21.7% *Klebsiella spp*., 13.1% *Pseudomonas spp*., 8.8% *Proteus spp*., 4.3% *Enterococcus spp*. (Figure 7).

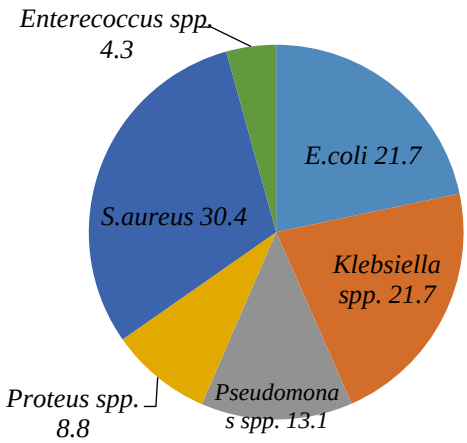

Figure 7. The growth results of pathogenic bacteria on washbasin, faucet heads and toilet door handles used by male students

Table 2. According to faculties, the distribution of pathogenic bacteria growth on the washbasin, faucet heads, and toilet door handles..

Faculty names	Gender	W	FH	TDH	W	FH	TDH	W	FH	TDH	W	FH	TDH	W	FH	TDH	W	FH	TDH
Faculty of Medicine	F				+												+		
	M					+				+							+		
Faculty of Engineering and Architecture	F							+											+
	M					+	+		+			+				+			
Faculty of Health Sciences	F		+	+								+	+						
	M		+					+						+					
Faculty of Theology	F		+	+	+			+			+								
	M	+																	+
Faculty of Agriculture	F	+																	
	M	+				+									+				
Faculty of Education	F						+								+				
	M												+		+				
Faculty of Economics and Administrative Sciences	F		+												+				
	M		+			+										+			
Faculty of Communication	F	+		+	+														
	M	+													+				
Faculty of Art and Sciences	F	+	+					+						+		+			
	M																		
		E. coli			***Klebsiella spp.***			***Pseudomonas spp.***			***Proteus spp.***			***S. aureus***			***Enterococcus spp.***		

***W: Washbasin FH: Faucet heads TDH: Toilet door handles**

The material with the highest growth was the faucet heads with 21 (42.9%) bacterial growth. In total, 21 (42.9%) of 49 bacteria were found at the faucet heads, 15 (30.6%) in the washbasins, and 13 (26.5%) on toilet door handles (Figure 8-10). Scott et al., (1892) were examined the kitchens, toilets, and bathrooms of more than 200 homes to determine the occurrence and contamination levels of potential pathogens and were found high numbers to be mostly in wet areas associated with bathrooms, bathtubs, washbasin, washing machines, and diaper pails.[19]

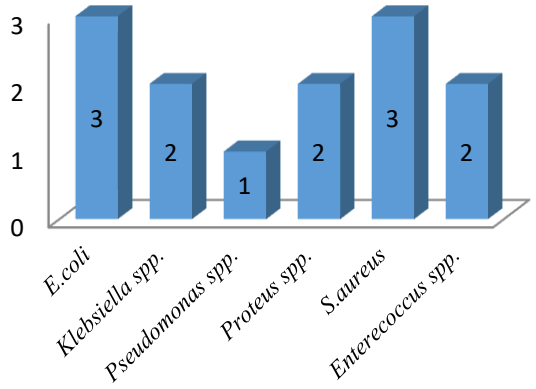

Figure 8. The growth results of pathogenic bacteria on toilet door handles

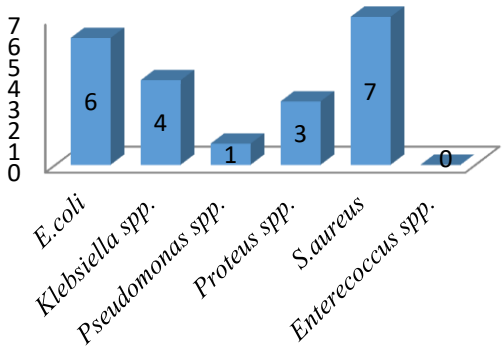

Figure 9. The growth results of pathogenic bacteria on faucet heads

In a study in Kathmandu (Nepal), one hundred and ninety swab samples were collected from five household toilets and total coliform and *Escherichia coli* concentrations were measured using membrane filtration methods. The faucet, spray arm, or bucket used for anal cleaning, where toilet stones have the highest median bacterial concentrations (mean total coliform = 214 / cm^2 and mean *E. coli* = 56 / cm2), have the highest median bacterial contamination in household (mean total coliform = 56 / cm^2) = 1/cm^2 and mean *E. coli* = 0.4/cm^2).[20]

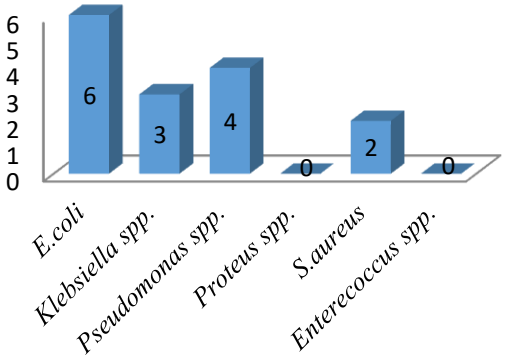

Figure 10. The growth results of pathogenic bacteria on washbasin

Here, *E. coli*, which was 10 out of a total of 26 bacteria grown in the girls' toilet, and *S. aureus*, which was 7 (30.4%) of the 23 bacteria that grew in the boys' toilet, were determined as the dominant bacteria. In our study, *E. coli* 15 (30.6%) was the most encountered bacteria. In addition, the bacteria detected from the targeted places of the faculties are given in Table 2. Microbial contamination research was carried out on 7,482 samples collected in public toilets in Tehran, the capital of Iran. The data were obtained by analyzing the inner and outer door handles of 804 toilets, 1062 faucet heads, 826 sink faucets, 1.062 toilet hoses, 804 flush handles, 643 soap dispenser bases, 643 liquid soaps, 99 bar soap, 169 toilet paper, 50 hand dryers. It was determined that 89.25% (6678 samples) of the samples were contaminated and 10.75% (804 samples) were not contaminated. In the study, it was reported that Escherichia coli with 28.48% and Pseudomonas species with 0.39% were the most common bacteria, respectively.[21]

Samples were taken from frequently touched surfaces in Mecca, Mina, Arafat, and Medina to detect the presence of respiratory bacteria and viruses during Hajj (in 2016 and 2018). 70/142 (49.3%) environmental samples collected were determined to be positive for at least one respiratory pathogen. Among the positive samples, the most frequently tested positive bacteria was *Klebsiella pneumoniae* (57.1%), followed by Streptococcus pneumoniae (12.9%), and *Staphylococcus aureus* (10.0%), and *Haemophilus influenzae* (7.1%). The surfaces with the highest positive sample rate were kitchen tables (100%), water fountain faucet heads (73.3%), and water cooler cover edge (84.6%).[22]

Samples were taken from a total of 99 toilet door handles and 45 faucet heads, which are frequently used by health personnel, patients, and visitors in different units of the Fırat University Faculty of Medicine hospital (Elazığ-Turkey). Growth was observed in 55 (55.5%) of 99 toilet

door handles and 31 (68.8%) of 45 faucet heads. Multiple growths were detected in 87% of positive cultures.[16]

4. Conclusion

The risk of infection is increasing the shared use of areas such as toilets in schools. With the study, results were obtained regarding the bacterial presence in the toilets with swab samples from washbasin, faucet heads, and toilet door handles of the toilets actively used by female and male students in 9 faculties located on the Erdoğan Akdağ campus of Yozgat Bozok University.

The swab samples were taken to the laboratory in accordance with the cold chain rules and pathogenic bacteria researches were carried out. In 34 (62.96%) of the swab samples taken, a total of 49 pathogenic bacteria were detected, growing singly or in multiples. Of the 49 bacteria detected, 15 were (30.6%) *Escherichia coli*, 12 were (24.5%) *Staphylococcus aureus*, nine were (18.3%) *Klebsiella spp*., six were (12.3%) *Pseudomonas spp.*, five were (10.2%) *Proteus spp*. and two were (4.1%) *Enterococcus spp*..

Extremely important results in terms of hygiene have been revealed in the research. Hand hygiene is one of the most effective ways to prevent the transmission of microorganisms that cause infection in the school environment. Improving the cleaning services of shared toilets, supply of cleaning materials, correct and hygienic use and compliance with hand washing rules will greatly reduce bacterial contamination and infection. In addition, monitoring, monitoring, and protection of the cleaning processes in the nine faculties of Yozgat Bozok University Erdoğan Akdağ campus, training on personal hygiene, and ensuring the continuity of these training are extremely important in preventing the transmission and spread of infectious diseases.

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Author's Contributions

Yasemin KELEŞ DİNÇ: Performed the experiments' progress, result interpretation and helped in manuscript preparation.

Sedat PER: Prepared experimental method, supervised the experiment's progress, result in interpretation and helped in manuscript preparation

Ethics

There are no ethical issues after the publication of this manuscript.

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