Busting hand microbiota

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ABSTRACT

More than 100 years ago a famous scientist said: "Microbes are everywhere, microbes are powerful, it is the microbes who will have the last word" (L. Pasteur). In the past few decades there has been a rapid rise in science and many antimicrobial agents were developed. How successful were scientists in their attempt to kill as much microbes as they can? – that's a question we aimed to answer. Our results showed the effects of two different antimicrobial detergents (antibacterial gel *Balea hygiene-handgel* and antiseptic *schülke octenisept*) on hand microbiota, revealed that mostly Staphylococcus colonies live on our hands and why we shouldn't kill all the bacteria.

INTRODUCTION

An average adult human has about two square meters (2 m^2) of skin surface that varies in chemical composition and moisture content.[1.] Many microorganisms inhabit skin's outer layer – epidermis, forming a complete ecosystem called skin microbiota.[2.] According to their relationship with us, microorganisms can be divided in three groups: commensals, symbionts with a mutualistic relation and parasites.

In the case where both the host and symbiont reciprocally benefit from the relationship, it represents mutualism, whereas if the symbiont utilises the host without benefiting or harming it, it is considered as a commensal.[3.] The majority of microorganisms on our skin are commensals and can prevent colonisation of pathogenic microorganisms. They play a fundamental role in our immune system and protect our skin every day.[4.]

Parasites can cause disease if the balance in this ecosystem is not maintained (dysbiosis) or when they get somewhere they don't belong and cause infections. Skin, especially hands, can have a major role in transferring microorganisms that are not usually part of our skin microbiota. Those microbes are the most likely to cause infections and many detergents were developed in order to kill them.

According to major detergent companies brands their products kill nearly all the bacteria on our hands and prevent infections caused by skin microbiota. New studies show that antimicrobial detergents are not as effective as they're presented which could work in our favour.[5.] We expect to find both pathogenic and non-pathogenic bacteria and less bacteria after washing our hands with detergents.

The aim of our project was to test the effects of detergents on skin microbiota and try to identify some of the bacteria that are present on our hands. First of all we

cultivated microorganisms before and after using antimicrobial agents. Afterwards we used biochemical tests to characterise the colonies and universal 16S rRNA polymerase chain reaction prior to DNA sequencing for identification of the colonies.

MATERIALS AND METHODS

I. *Cultivation*

In order to grow microorganisms nutrition agar plates were prepared by mixing 400 mL of water with 2 g of peptone, 1.4 g of yeast extract, 2 g of NaCl and 6 g of agar. The mixture was boiled for 10 minutes in a pressure cooker and divided over petridish plates. Two different detergents were used; antiseptic *schülke octenisept* and antibacterial gel *Balea hygiene-handgel.* Then, handprints (before and after using detergents) were left on solidified nutrient agar and incubated at 30°C for 48 hours.

II. *Biochemical tests*

a. Gram staining

Air-dried and heat-fixed cells were dyed with crystal violet for 1 minute. They were treated then with the mordant - Gram's iodine for 1 minute in order to bind the dye to the cell wall. Afterwards they were flooded with ethanol based washing solution which interacts with the lipids of the membrane, decolorizing only gram negative bacteria. In the next step pink counterstain safranin was added. The results of the staining procedure were observed using light microscope (100X magnification).

b. Catalase activity

A few drops of H_2O_2 (hydrogen peroxide) were added on the slide with bacteria cell suspensions.

c. Coagulase test

Undiluted plasma was stirred into the cell suspension on the slide and left for 10 seconds

d. Haemolysis

One bacterial colony was inoculated on previously prepared blood agar plates.

III. *Antibiotic resistance*

Two mixtures for nutrient agar were prepared using the same composition as for the cultivation. Later, 70 μL of tetracycline or 175 μL of streptomycin was added. Lastly, The selected bacterial colonies were inoculated on these plates and incubated them.

IV. *16S PCR*

The PCR was performed using Taq polymerase, PCR buffer, dNTPs, and DNA primers targeting the 16S rRNA gene of bacteria. Each reaction consisted of 49 microliters PCR mix and 1 microliter of colonies suspended in DNase and RNase free water. The PCR was run for 35 cycles consisting of three different steps with denaturing at 95°C, annealing at 52°C and extension at 72°C. The results were visualized using gel electrophoresis.

V. *DNA sequencing*

In order to get parts of bacterial DNA sequenced, after doing the PCR those samples were sent to a company. Since we were constricted by time, our sequences wouldn't arrive on time so we used previously sequenced DNA our supervisor prepared and compared those plates (Figure 1) to ours using morphology. Afterwards we used the Bioedit programme to edit sequences and cut out parts we had no use for and also NCBI BLAST to match those sequences with bacterial species they belong to.

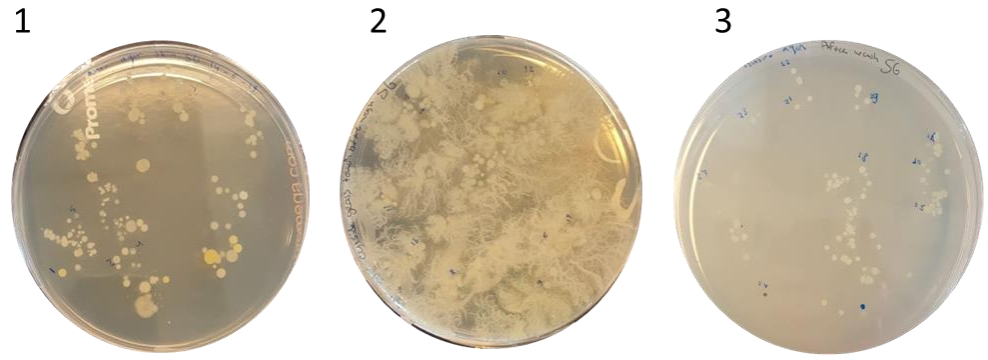

Figure 1 Previously prepared plates. 1) Before washing hands, 2) After going outside in the grass and 3) After washing hands with soap.

RESULTS & DISCUSSION

I. *Cultivation*

The final results of the cultivation fit to our hypothesise; there were less bacteria on our hands after washing our hands. Different colonies were visible on *before* and *after* plates. On the *after* plate of the antibacterial gel (Figure 2) none of the fungi

were present, while on the *after* plate of the antiseptic (Figure 3) the number of fungi was only reduced.

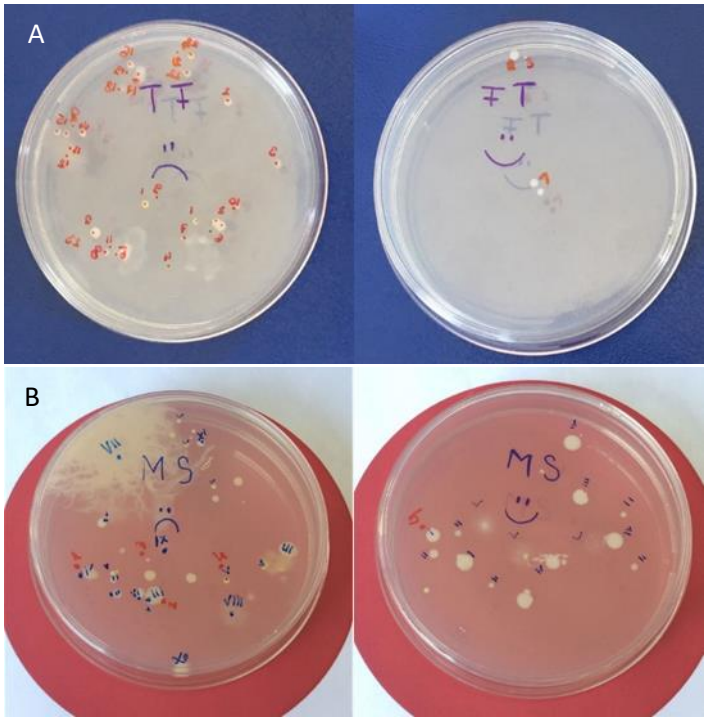

Figure 2 A: Cultivation of microorganisms from the hands before and after washing with the antibacterial gel *Balea hygiene-handgel. B:* Cultivation of microorganisms from the hands before and after washing with the antiseptic *schülke octenisept.*

Our results after washing hands with antiseptic don't fit to what's pointed out in its commercial videos. It should have a broad-spectrum antimicrobial activity effective against gram-positive and gram-negative bacteria, fungi and viruses.[6.]

Comparing to another similar article, results clearly differentiate. In the article[7.] the Octenidine dihydrochloride is declared as a very effective agent against Pseudomonas aeruginosa-contaminated, full-skin thickness burn wounds in rats. Since we rather focused on regular skin microbiota and not on the ones present in contaminated burn wounds it's not very likely to find P. aeruginosa. But it's important to mention that it's said in the article that Octenidine dihydrochloride exhibits a broad spectrum of antimicrobial efficacy against gram-positive and gram-negative bacteria and fungi. Which means that we might had a contamination after using the antiseptic what explains the numerous colonies after washing our hands.

II. *Biochemical tests*

Table 1 shows that two out of ten bacteria were gram positive, five were catalase positive, only one was coagulase positive, four of them were rods. For colonies TF1, TF3, TF4 and TF17 we didn't do any further biochemical tests after gram staining.

	GRAM	CATALASE	COAGULASE	SHAPE
MS1	-	+	-	rods
MS2	+	+	+	rods
MS3	-	+	-	rods
MS5	-	-	-	cocci
SG1	-	+	-	rods
SG2	+	+	-	cocci
TF1	-	/	/	cocci
TF3	-	/	/	cocci
TF4	-	/	/	cocci
TF17	-	/	/	cocci

Table 1 The results of gram staining, catalyse test, coagulase test and shape of the bacteria

Figures 3 and 4 show the results of gram staining of the gram negative bacteria MS1 and gram positive bacteria MS2. Surprisingly, the results of gram staining colony KS3 (Figure 5) were both purple and pink stain. It is possible that the amount of bacteria on the slide wasn't evenly spread on the surface or that we might had mixed colonies on one glass. Another option is that these bacteria have the acid-fast cell wall.

The acid-fast cell wall consists of a thick, outer lipid-rich layer composed primarily of the fatty acid "mycolic acid". This lipid layer lies on top of a layer of peptidoglycan and the sugar arabinogalactan. The thick outer mycolic acid layer renders acid-fast bacteria resistant to gram stain. When stained with alternative dyes, the cell wall is resistant to decolorization with acid alcohol.[8.] That's why for acid-fast bacteria regular gram staining procedure doesn't work so scientists have to use a special staining method.[9.]

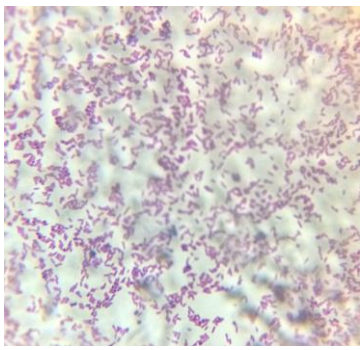

Figure 3 Gram staining MS1

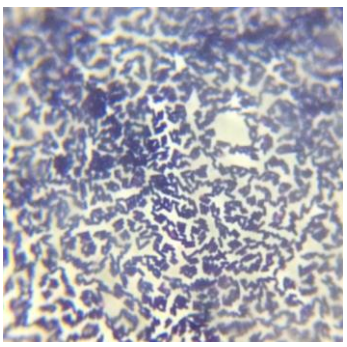

Figure 4 Gram staining MS2

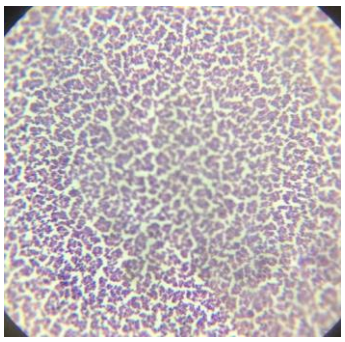

Figure 5 Gram staining KS3

Unfortunately, all previously prepared agar plates were contaminated except for one on which we tested haemolysis for colony SS1 (Figure 5) and it appears to be γ-haemolytic.

Figure 5 Results of haemolysis SS1

III. *Antibiotic resistance*

Table 2 shows that none of the four colonies were resistant to the antibiotics we used.

Staphylococcus aureus is one of the extremely resistant pathogens which could be found on our hands. Half of all S. aureus infections in the US are resistant to penicillin, methicillin, tetracycline and erythromycin.[10.] According to our data none of these bacteria was Staphylococcus aureus but DNA sequencing was needed for exact identification of the bacterial colonies.

Considering that the flame we used to anticipate contamination ran out, contamination shouldn't be excluded. That could be a reason why all the tests came out negative.

	TETRACYCLIN	STREPTOMYCIN
MS1	-	-
MS2	-	-
MS5	-	-
SG2	-	-

Table 2 The results of antibiotic resistance test using tetracycline and streptomycin

IV. *16S PCR*

The first lane of the agarose gel contains a molecular marker of known sizes, to which the amplicons after PCR were compared (Figure 6). The size of the amplicons was found to be in between 1400 and 1500 base pairs, which was

expected. The size calculated before was 1465 bp. This figure also shows that not all PCR reactions resulted in an amplicon with the same light intensity, or even no amplicon at all. This could be due to several reasons such as, the PCR program, the bacteria themselves (not enough or cell wall did not break) or that the DNA of the cells picked on the nutrient plate could not be detected by these primers.

Figure 6 Results of electrophoresis

V. *DNA sequencing*

According to morphology, it is very likely that we would have found Staphylococcus epidermis, *Moraxella osloensis* and *Micrococcus luteus* or *yunnanensis* on our plates before washing our hands, based on morphology similarity to the plates of our supervisor. Therefore, this cannot be stated with certainty. After using antibacterial gel we would have most likely found Staphylococcus species, which are part of the commensal bacteria on our hands.

Table 3 Bacteria species found on previously prepared plates

BEFORE
1. Micrococcus yunnanensis/aloeverae
2. Staphylococcus epidermis
3. Moraxella osloensis
4. Staphylococcus hominis
8. Paenibacilus pasadenensis/humicus/glycoanilyticus
13. Bacillus mycoldes/cereus/weihenstephanensis
15. Pseudomonas koreensis/fluorescens
18. Bacilus megaterium/aryabhattai/ huizhiuensis
19. Paenibacilus terrigena/gleabe/gsoil/susongensis
AFTER
20. Lysinibacillus spahaericus/xylanilyticus
22. Staphylococcus warneri/pasteuri
23. Staphylococcus epidermis
27. Staphylococcus warneri/pasteuri
28. Staphylococcus warneri/pasteuri
29. Staphylococcus hominis
30. Staphylococcus epidermis

CONCLUSION

Our results showed the effects of two different antimicrobial detergents (antibacterial gel *Balea hygiene-handgel* and antiseptic *schülke octenisept*) on hand microbiota, revealed that mostly Staphylococcus colonies live on our hands and why we shouldn't kill all the bacteria.

References

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