ANALYSIS OF CUTANEOUS MICROBIOTA OF PIGLETS WITH HEREDITARY MELANOMA*

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Malignant melanoma may be a life-threatening disease caused by various conditions. Cutaneous bacteria could play a role in melanoma development or regression. The present work aimed to analyze the bacterial species present on the epidermis of piglets with hereditary melanoma. Bacteria isolated by swabs directly from melanomas and healthy epidermis were analysed using MALDI-TOF mass spectrometry. From the total of 290 isolates, 92 were identified, while the extraction by ethanol turned out to be more efficient compared to identification by direct transfer. *Staphylococcus sciuri, Staphylococcus cohnii,* and *Lactococcus lactis* were significantly more frequent on healthy skin, whereas *Staphylococcus chromogenes*, *Staphylococcus hyicus,* and *Enterococcus faecalis* have thrived significantly better on melanoma. Overall, the results indicate that the microbiota of melanoma is different from that of healthy epidermis, so piglet skin bacteria inspections are recommended.

bacteria, skin, cancer, pig, MeLiM

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INTRODUCTION

Malignant melanoma is the most aggressive form of skin cancer. Due to its metastatic potential it causes up to 90% of deaths from skin cancer (Cervinkova et al., 2017)spontaneous regression of the metastatic form of tumour is a rare phenomenon observed in only 0.23% of cases. The most frequently mentioned factors leading to spontaneous regression of MM are operative trauma, infection, vaccination (BCG and rabies vaccines. Malignant melanoma is classified into 4 different clinical subtypes: superficial spreading melanoma, lentigo maligna melanoma, nodular melanoma, and acral lentiginous melanoma (McCourt et al., 2014). The incidence of melanoma is generally more common in people from higher socioeconomic class, thus the largest incidence occurs in Australia and USA, followed by western European countries. Most affected has been the Caucasian race, however melanoma occurs also in other races (Little, Eide, 2012). Not only skin phototype, but also duration of sunlight exposure without sun protection use as well as genetic factors are considered as main predisposing factors (Bataille, de Vries, 2008).

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The microbiota seems to play a crucial role in the development of tumour in mice and rat models where germ-free or antibiotics-treated rats or mice have usually fewer tumours, although the evidence has been found mainly for colorectal and liver cancer (Schwabe, Jobin, 2013). Grivennikov et al. (2012) also found that metabolism of certain bacterial species can also enhance tumour growth. The association between skin cancer and wound-caused inflammation is also known. Hoste et al. (2015) showed that tumour incidence correlates with wound size, bacteria and chronic inflammation, and that exposure to bacterial flagellin, the protein present in flagellated bacteria, promotes tumour formation in mice. Tran et al. (2013) discovered that tetanus–diphtheria–pertussis vaccination enhanced a spontaneous regression of metastatic melanoma.

The aim of our work was to evaluate if there is a correlation between the composition of bacterial species and incidence of melanoma on skin of pigs.

MATERIAL AND METHODS

The bacterial samples were collected by MTS-002 Swabs with Amies medium with activated charcoal (Med-Lab trade, Czech Republic) from four eight-week-old MeLiM (melanoblastoma-bearing Liběchov minipigs) piglets with hereditary melanoma (Horak et al., 1999). The animals were housed in the farrowing pen together with the sow and they had an unlimited approach to water and commercial feed mixtures according to their age. Melanoma and cutaneous smears were harvested from the defined area on the back or proximal part of the limbs. The cutaneous smears were sampled from the site 5 cm far from the edge of selected melanoma. By this procedure we obtained eight samples, four melanoma and four controls. The samples were immediately inoculated using spread-plate technique on 9 mm Petri dishes containing the following media (all Oxoid, UK): CM0003 Nutrient agar for isolation of total aerobic bacteria, CM0619 Wilkins-Chalgren anaerobe agar for isolation of total anaerobic bacteria, and CM0145 Staphylococcus medium No. 110 for isolation of staphylococci.

The plates were subsequently cultivated at 37°C for 24 h for total aerobes and staphylococci and at 37°C for 24 h in anaerobic atmosphere created by an AnaeroGen Atmosphere Generation System (Oxoid). After the cultivation, 56 colonies from Nutrient agar, 24 colonies from Wilkins-Chalgren anaerobe agar, and 24 colonies from Staphylococcus medium were isolated and propagated at 37°C for ٢٤ h in vials containing CM0067 Nutrient broth No. 2 (Oxoid). In addition, 88 colonies from Nutrient agar, 80 colonies from Wilkins-Chalgren anaerobe agar, and 24 colonies from Staphylococcus medium were directly used for MALDI TOF analysis.

After cultivation, the obtained well grown isolates underwent the identification by MALDI TOF mass spectrometry on an AutoFlex Speed mass spectrometer (Bruker Daltonik, Germany) using ethanol-formic acid extraction (Schulthess et al., 2013)the direct transfer-formic acid method with on-target formic acid treatment, and ethanol-formic acid extraction. At first, 1 ml of each sample was centrifuged at 14 500 g for 3 min, the supernatant was discarded, and the surface of pellet was washed by 70% ethanol in order to remove the residual growth medium. After another centrifugation, the ethanolic supernatant was discarded and the pellet was resuspended in 15 µl of 70% formic acid (Fisher Scientific, USA) and 15 µl of acetonitrile (LC-MS Chromasolv®), centrifuged, and supernatant was used for MALDI-TOF analysis. The 1 µl of supernatant was applied on MTP 384 polished steel target plate (Bruker Daltonik) and allowed to air dry. Then, all samples were overlaid by 1 µl of α-cyano-4-hydroxycinnamic acid dissolved in acetonitrile, water, and trifluoroacetic acid (50 : 47.5 : 2.5) as a MALDI matrix (Bruker Daltonik).

Direct transfer method was carried out by the application of a selected colony directly from the agar plate to MALDI target and, overlaying by 1 µl of formic acid and 1 µl of acetonitrile. After drying out, the sample was overlaid by MALDI matrix as in the previous procedure.

After drying out at room temperature, the plate containing samples obtained by both described procedures was inserted into an AutoFlex Speed mass spectrometer (Bruker Daltonik) and measured in positive linear mode in the detection mass range 2–20 kDa. To optimise the experimental conditions, a solution of Bruker Bacterial Test Standard (BTS; Bruker Daltonik) containing *Escherichia coli* DH5 alpha extract was used to calibrate the system, according to manufacturer's instructions. The obtained data were evaluated by

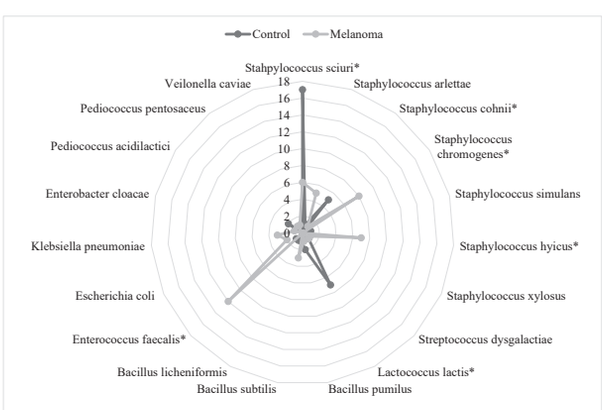

Fig. 1. Occurrence of species successfully identified by MALDI-TOF mass spectrometry
species with asterisk showed a significant difference ($P < 0.05$) between proportions of control and melanoma groups by Chi-squared test

MALDI Biotyper Real Time Classification software (Bruker Daltonik). For the statistical evaluation the samples that were not identified properly and samples reaching less than 1.800 MALDI Biotyper score value were excluded. The samples with more than 1.800 score value, but comprising other species identified by the score differing less than 0.200 were also excluded. This approach has been used based on manufacturer's instructions where the score between 1.700 and 1.999 indicates probable genus identification, score between 2.000 and 2.299 indicates secure genus and probable species identification, and score over 2.300 indicates secure species identification.

Statistical evaluation was performed by Statgraphics Centurion XV (StatPoint) using Chi-squared test at a significance level of $P = 0.05$. Differences in variability of bacterial species between melanoma and control group were evaluated for each growth media separately and altogether.

RESULTS

Bacterial colonies were formed on all three tested media. We managed to identify 92 isolates out of 290 in total where the analysis by ethanol-formic acid extraction has been much more effective as we successfully identified 56 out of 97 isolates giving 57.73% success rate compared to direct transfer method where we successfully identified only 36 out of 193 giving 18.65% success rate. Identified species are shown in Fig. 1.

In terms of groups we successfully identified to the species level 39 isolates from healthy epidermis (control group) and 53 isolates from melanoma.

The most abundant bacterium was *Staphylococcus sciuri* identified 23 times in total (17 times in control group and 6 times in melanoma group). This species falls with the proportion 0.26 beyond the limit ($P < 0.05$ confidence level). Other identified bacterium worth mentioning was *Lactococcus lactis* isolated in 7 cases exclusively from control group by Wilkins-Chalgren anaerobe agar. The 0.00 proportion of *L. lactis* is also beyond the limit ($P < 0.05$). On the contrary, the melanoma group showed exclusive abundance of *Staphylococcus chromogenes*, which was identified 8 times in this group and not even once in control group which means that the proportion 0.00 of this strain is also beyond the limit ($P < 0.05$). The same case displayed *Staphylococcus hyicus*, which was identified 7 times in melanoma group and not even once in control group and also lays beyond the limit (proportion 1.00, $P < 0.05$). *Enterococcus faecalis* was detected 12 times in melanoma group and only once in control group and with the proportion 0.92 falls beyond the limit ($P < 0.05$). Last significant proportion was displayed by *Staphylococcus cohnii* identified 5 times in control group and once in melanoma group which makes a proportion of 0.17 and falls beyond limit ($P < 0.05$).

Comparison using Chi-square test showed a clear significant difference between the proportions of bacteria in control and melanoma group ($P < 0.0001$). A significant difference was observed even when comparing identified bacteria from Nutrient agar ($P = 0.006$) and Wilkins-Chalgren agar ($P = 0.01$) alone. Comparison of proportions in Staphylococcus medium did not find significant differences ($P = 0.17$) due to small amount of isolates.

DISCUSSION

Schulthess et al. (2013)the direct transfer-formic acid method with on-target formic acid treatment, and ethanol-formic acid extraction reported that the direct transfer preparation method with the use of formic acid and without the use of ethanol for MALDI-TOF identification was equally successful for species identification as the ethanol-formic acid extraction. Our experiment does not confirm this claim as 53.85% of successfully identified species is much more than 18.75% obtained by the direct transfer method. The authors cited identified Gram-positive cocci from their strain collection which can be the cause of our different findings whereas we identified more heterogeneous samples containing not only Gram-positive cocci, but also bacilli and Gram-negative bacteria from *Enterobacteriaceae* family. Wilson et al. (2017) also performed multiple extraction methods for MALDI-TOF identification and they achieved much a more successful identification (89.9%) by the direct transfer than in our work. However, they identified bacteria from collection as did Schulthess et al. (2013) in their study and not samples originating directly from the environment. With regard to our findings, we recommend to perform the ethanol-formic acid extraction to identify unknown bacteria from the piglet skin or a similar environment.

McIntyre et al. (2016)normal cutaneous flora likely has been selected for because it potentiates or, at minimum, does not impede wound healing. While pigs are the gold standard model for wound healing studies, the porcine skin microbiome has not been studied in detail. Herein, we performed 16S rDNA sequencing to characterize the pig skin bacteriome at several anatomical locations. Additionally, we used bacterial conditioned-media with in vitro techniques to examine the paracrine effects of bacterial-derived proteins on human keratinocytes (NHEK reported that more than 50% of bacteria identified on pig skin belong to the *Firmicutes* phyla. We observed that all bacteria identified from healthy skin belong to *Firmicutes* phyla which could be probably due to different method of identification considering that McIntyre et al. (2016) used 16s rDNA sequencing which is a method able to identify non-cultivable strains of bacteria.

Regarding the isolated bacteria, the *Staphylococcus sciuri* that has been found significantly more frequently on healthy skin is commensal bacterium frequently isolated from humans and animals, but its presence has also been reported in human and animal infections. Some members of the species have been shown to be pathogenic and could carry multiple virulence and resistance genes (Nemeghaire et al., 2014). Chen et al. (2007) isolated *S. sciuri* even from pericardial fluid from piglets diseased with exudative epidermitis. In our case we found *S. sciuri* mainly on healthy skin so we assume that this species has no involvement in melanoma proliferation or it has protective effect against melanoma formation.

Lactococcus lactis is another bacterium present significantly more often on healthy skin of examined piglets. Its role in melanoma regression is up to date unknown, but *L. lactis* is a generally known probiotic bacterium and it produces lactic acid that has been investigated as an inhibitor on melanin synthesis in melanoma cells (Usuki et al., 2003). On the contrary, Brand et al. (2016) found out that lactic acid suppressed T and NK cells and thus promoted tumor growth in their study, although it is not known how does lactic acid contribute to activation or deactivation of other immune cells. Generally, lactic acid suppresses the growth of pathogenic bacteria (Coates et al., 2014; Hor, Liong, 2014), so more investigation is needed to reveal the role of lactococci in skin cancer.

Staphylococcus cohnii has also occurred in a significantly higher rate in healthy skin. The occurrence of *S. cohnii* has mostly been reported in animals (Kloos et al., 1976), but it has been shown that it is able to produce a biofilm similarly as *S. sciuri* (Garza-Gonzalez et al., 2011). Just like in the case of *S. sciuri*, there are no data that *S. cohnii* would play a role in melanoma incidence in pigs or in humans.

Exudative epidermitis associated with the abundance of *S. sciuri* has been a result of *Staphylococcus chromogenes* strain in the study carried out by Andresen et al. (2005)which is recognised as the causative agent of exudative epidermitis (EE. Other authors do not consider *S. chromogenes* as a an etiological agent of disease, especially in pigs (Sato et al., 2004), although it can develop mastitis in cattle (Devriese et al., 2002; Enger et al., 2015)and (2. *S. chromogenes* has been isolated exclusively from melanomas in our study. However, due to the lack of data regarding its toxicity in pigs we assume that its presence on melanoma could be a consequence, not a cause.

When we consider the role of *Staphylococcus hyicus* that has been also isolated exclusively from melanomas, we get a different finding. Multiple authors blame this bacterium as a causative agent of exudative epidermitis in pigs (Andresen, 2005; Park et al., 2013) due to its production of exfoliative toxins by some strains. The fact that *S. hyicus* is able to trigger the skin disease induces the idea that it can participate on the melanoma progression although a lot more investigation needs to be carried out to prove this claim.

Enterococcus faecalis has been significantly more abundant on melanoma. This species is a member of commensal flora of animals, but it can also cause infections, e.g. in wounds (Hwang et al., 2011). Its role in melanoma is, as well as in bacteria mentioned above, unknown.

Staphylococci are normal inhabitants in healthy animals (Skalka, 1991)in pigs and in domestic fowl, using the method of selective isolation of strains. A total of 6066 samples was examined; 4567 strains were revealed and they included all known species, except Staphylococcus caseolyticus, S. saccharolyticus, S. schleiferi and S. lugdunensis. 3.8% of strains failed to be identified with any species. The test samples were taken from slaughtered animals, only in calves intravital smears of tonsils were examined. The species most frequently isolated from the tonsil tissue in adult cattle were as follows: S. aureus (19.9%, but apparently some strains can switch to detrimental mode if favorable conditions occur. The question needed to be answered is if they can be cause or consequence of melanoma. This article demonstrates that several staphylococcal species appear on melanoma more than on healthy skin and other species *vice versa*. The same goes for *Enterococcus faecalis* and *Lactococcus lactis*, which are both lactic acid bacteria but meanwhile *E. faecalis* occurred mostly on melanoma, *L. lactis* occurred only on healthy skin. *E. faecalis* is able to survive and thrive in various niches (Nallapareddy, Murray, 2008)growth in 40% serum, a biological cue with potential clinical relevance, elicited adherence of all 46 E. faecalis strains tested to fibronectin and fibrinogen, but not to elastin; the adherence levels were independent of strain source and was eliminated by treating cells with trypsin. As previously reported, serum also elicited collagen adherence. While prolonged exposure to serum during growth was needed for enhancement of adherence to fibrinogen, brief exposure (<5 min and can cause diseases, so its role on skin could be detrimental.

CONCLUSION

In this work, we managed to isolate and describe various bacteria from melanoma and healthy skin of piglets. Our results indicate that ethanol-formic acid extraction should be used to identify bacteria from piglet skin by MALDI-TOF.

More research needs to be carried out to verify the significance of bacteria in influencing melanoma regression or promotion. Nevertheless, we managed to find the differences in the microbial composition of healthy piglet skin and melanoma.

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REFERENCES

Andresen LO (2005): Production of exfoliative toxin by isolates of *Staphylococcus hyicus* from different countries. Veterinary Record, 157, 376–378.

Andresen LO, Ahrens P, Daugaard L, Bille-Hansen V (2005): Exudative epidermitis in pigs caused by toxigenic *Staphylococcus chromogenes*. Veterinary microbiology, 105, 291–300. doi: 10.1016/j.vetmic.2004.12.006.

Bataille V, de Vries E (2008): Melanoma – Part 1: epidemiology, risk factors, and prevention. BMJ, 337: a2249.

Brand A, Singer K, Koehl GE, Kolitzus M, Schoenhammer G, Thiel A, Matos C, Bruss C, Klobuch S, Peter K, Kastenberger M, Bogdan C, Schleicher U, Mackensen A, Ullrich E, Fichtner-Feigl S, Kesselring R, Mack M, Ritter U, Schmid M, Blank C, Dettmer K, Oefner PJ, Hoffmann P, Walenta S, Geissler EK, Pouyssegur J, Villunger A, Steven A, Seliger B, Schreml S, Haferkamp S, Kohl E, Karrer S, Berneburg M, Herr W, Mueller-Klieser W, Renner K, Kreutz M (2016): LDHA-Associated lactic acid production blunts tumor immunosurveillance by T and NK cells. Cell Metabolism, 24, 657–671. doi: 10.1016/j.cmet.2016.08.011.

Cervinkova M, Kucerova P, Cizkova J (2017): Spontaneous regression of malignant melanoma – is it based on the interplay between host immune system and melanoma antigens? Anticancer Drugs, 28, 819–830. doi: 10.1097/CAD.0000000000000526.

Chen S, Wang Y, Chen F, Yang H, Gan M, Zheng SJ (2007): A highly pathogenic strain of *Staphylococcus sciuri* caused fatal exudative epidermitis in piglets. PLoS ONE, 2, e147. doi: 10.1371/journal.pone.0000147.

Coates R, Moran J, Horsburgh MJ (2014): Staphylococci: colonizers and pathogens of human skin. Future Microbiology, 9, 75–91. doi: 10.2217/fmb.13.145.

Devriese LA, Baele M, Vaneechoutte M, Martel A, Haesebrouck F (2002): Identification and antimicrobial susceptibility of *Staphylococcus chromogenes* isolates from intramammary infections of dairy cows. Veterinary Microbiology, 87, 175–182. doi: 10.1016/S0378-1135(02)00047-0.

Enger BD, Fox LK, Gay JM, Johnson KA (2015): Reduction of teat skin mastitis pathogen loads: differences between strains, dips, and contact times. Journal of Dairy Science, 98, 1354–1361. doi: 10.3168/jds.2014-8622.

Garza-Gonzalez E, Morfin-Otero R, Martinez-Vazquez MA, Gonzalez-Diaz E, Gonzalez-Santiago O, Rodriguez-Noriega E (2011): Microbiological and molecular characterization of human clinical isolates of *Staphylococcus cohnii*, *Staphylococcus hominis*, and *Staphylococcus sciuri*. Scandinavian Journal of Infectious Diseases, 43, 930–936. doi: 10.3109/00365548.2011.598873.

Grivennikov SI, Wang K, Mucida D, Stewart CA, Schnabl B, Jauch D, Taniguchi K, Yu G-Y, Osterreicher CH, Hung KE, Datz C, Feng Y, Fearon ER, Oukka M, Tessarollo L, Coppola V, Yarovinsky F, Cheroutre H, Eckmann L, Trinchieri G, Karin M (2012): Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. Nature, 491, 254–258. doi: 10.1038/nature11465.

Hor YY, Liong MT (2014): Use of extracellular extracts of lactic acid bacteria and bifidobacteria for the inhibition of dermatological pathogen *Staphylococcus aureus*. Dermatologica Sinica, 32, 141–147. doi: 10.1016/j.dsi.2014.03.001.

Horak V, Fortyn K, Hruban V, Klaudy J (1999): Hereditary melanoblastoma in miniature pigs and its successful therapy by devitalization technique. Cellular and Molecular Biology (Noisy-le-Grand, France), 45, 1119—1129.

Hoste E, Arwert EN, Lal R, South AP, Salas-Alanis JC, Murrell DF, Donati G, Watt FM (2015): Innate sensing of microbial products promotes wound-induced skin cancer. Nature Communications, 6: 5932. doi: 10.1038/ncomms6932.

Hwang IY, Lim SK, Ku HO, Park CK, Jung SC, Park YH, Nam HM (2011): Occurrence of virulence determinants in fecal *Enterococcus faecalis* isolated from pigs and chickens in Korea. Journal of Microbiology and Biotechnology, 21, 1352–1355.

Kloos WE, Zimmerman RJ, Smith RF (1976): Preliminary studies on the characterization and distribution of Staphylococcus and Micrococcus species on animal skin. Applied and Environmental Microbiology, 31, 53–59.

Little EG, Eide MJ (2012): Update on the current state of melanoma incidence. Dermatologic Clinics, 30, 355–361. doi: 10.1016/j.det.2012.04.001.

McCourt C, Dolan O, Gormley G (2014): Malignant melanoma: a pictorial review. The Ulster Medical Journal, 83, 103–110.

McIntyre MK, Peacock TJ, Akers KS, Burmeister DM (2016): Initial characterization of the pig skin bacteriome and its effect on *in vitro* models of wound healing. PLoS ONE, 11, e0166176. doi: 10.1371/journal.pone.0166176.

Nallapareddy SR, Murray BE (2008): Role of serum, a biological cue, in adherence of *Enterococcus faecalis* to extracellular matrix proteins, collagen, fibrinogen, and fibronectin. The Journal of Infectious Diseases, 197, 1728–1736. doi: 10.1086/588143.

Nemeghaire S, Argudin MA, Fessler AT, Hauschild T, Schwarz S, Butaye P (2014): The ecological importance of the *Staphylococcus sciuri* species group as a reservoir for resistance and virulence genes. Veterinary Microbiology, 171, 342–356. doi: 10.1016/j.vetmic.2014.02.005.

Park J, Friendship RM, Poljak Z, Weese JS, Dewey CE (2013): An investigation of exudative epidermitis (greasy pig disease) and antimicrobial resistance pat-

terns of *Staphylococcus hyicus* and *Staphylococcus aureus* isolated from clinical cases. The Canadian Veterinary Journal, 54, 139–144.

Sato H, Hirose K, Terauchi R, Abe S, Moromizato I, Kurokawa S, Maehara N (2004): Purification and characterization of a novel *Staphylococcus chromogenes* exfoliative toxin. Journal of Veterinary Medicine, Series B, 51, 116–122. doi: 10.1111/j.1439-0450.2004.00743.x.

Schulthess B, Brodner K, Bloemberg GV, Zbinden R, Bottger EC, Hombach M (2013): Identification of Gram-positive cocci by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry: comparison of different preparation methods and implementation of a practical algorithm for routine diagnostics. Journal of Clinical Microbiology, 51, 1834–1840. doi: 10.1128/JCM.02654-12.

Schwabe RF, Jobin C (2013): The microbiome and cancer. Nature Reviews Cancer, 13, 800–812. doi: 10.1038/nrc3610.

Skalka B (1991): Occurrence of staphylococcal species in clinically healthy domestic animals. Veterinarni medicina, 36, 9–19. (in Czech)

Tran T, Burt D, Eapen L, Keller OR (2013): Spontaneous regression of metastatic melanoma after inoculation with tetanus–diphtheria–pertussis vaccine. Current Oncology, 20, e270-3. doi: 10.3747/co.20.1212.

Usuki A, Ohashi A, Sato H, Ochiai Y, Ichihashi M, Funasaka Y (2003): The inhibitory effect of glycolic acid and lactic acid on melanin synthesis in melanoma cells. Experimental Dermatology, 12 (Suppl. 2), 43–50.

Wilson DA, Young S, Timm K, Novak-Weekley S, Marlowe EM, Madisen N, Lillie JL, Ledeboer NA, Smith R, Hyke J, Griego-Fullbright C, Jim P, Granato PA, Faron ML, Cumpio J, Buchan BW, Procop GW (2017): Multicenter evaluation of the Bruker MALDI Biotyper CA System for the identification of clinically important bacteria and yeasts. American Journal of Clinical Pathology, 147, 623–631. doi: 10.1093/ajcp/aqw225.

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