Diagnosing Staph Infections with VOC Biomarkers

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Staphylococci are broadly adaptable and their ability to grow in unique environments has been widely established, but the most common and clinically relevant staphylococcal niche is the skin and mucous membranes of mammals and birds. *S. aureus* causes severe infections in mammalian tissues and organs, with high morbidities, mortalities, and treatment costs. *S. epidermidis* is an important human commensal but is also capable of deadly infections. The development of volatile organic compound (VOC) profiles for the detection and identification of pathogens is an area of intensive research, with significant efforts toward establishing breath tests for infections.

Metabolomics | Volatile Organic Compounds | Breathprint | Breath-based Diagnostics | Staphylococcus aureus | Staphylococcus epidermidis | Coagulase Negative Staphylococci | Diagnosis | GC×GC-TOFMS | Bovine Mastitis

1. In Vitro and Animal Model Feasibility Studies

Volatile organic compound (VOC) biomarkers for *S. aureus* infections have been studied at all stages of biological and chemical translational development [1][2][3][4][5][6], demonstrating feasibility for diagnosing and characterizing staph infections in clinical and field settings. Based on the published analyses of *S. aureus* VOCs, ten analytes comprise a common *S. aureus* volatile suite (**Table 1**) [4][6][7][8][9][10][11][12][13][14][15]. All of these metabolites are produced by a broad diversity of fungi and bacteria, including coagulase-negative staphylococci, suggesting they may be produced by universal metabolic pathways [16]. However, combining the differences in the relative abundances of these common metabolites with suites of accessory metabolites yields VOC profiles that differentiate staph from other microbial taxa. In in vitro cultures, *S. aureus* has been successfully differentiated from *Acinetobacter spp.*, *Candida spp.*, *Clostridium perfringens*, *Enterobacter spp.*, *Enterococcus spp.*, *Proteus mirabilis, Klebsiella spp., P. aeruginosa, Streptococcus spp., E. coli, Burkholderia cepacia* complex, *H. influenzae*, *H. pylori*, *Citrobacter spp.*, *S. maltophilia*, *Salmonella enterica*, *Serratia marcescens*, *Moraxella catarrhalis*, *Neisseria meningitidis*, *S. epidermidis*, and *S. lugdunensis* based on their volatile profiles measured using GC, direct injection MS, and sensor array technologies (**Table 2**) [4][5][6][7][12][14][15][17][18][19][20][21][22][23][24][25][26]. The unique and shared VOC profiles of each taxon form the foundation of in vitro detection and identification technologies being developed for clinical use [27]. Differences in the volatile profiles have been extended to in vitro models of skin wound infection biofilms, where it has been shown that *S. aureus* can be differentiated from Gram-negative bacteria, such as *P. aeruginosa*, and also Gram-positive pathogens such as *Streptococcus pyogenes* [28]

[29][30]. *S. aureus* and *S. epidermidis* also have unique volatile profiles in vitro under a variety of growth conditions, indicating that infections caused by the former will be differentiable from non-infectious colonization by the latter [5][6][12][31][32].

Table 1. The canonical VOCs of the *S. aureus* volatilome.

IUPAC Name	Common Name	Molecular Formula	MW *	CAS *	KEGG *	References
acetaldehyde	ethanal	CH_3CHO	44	75-07-0	C00084	[4][6][9]
ethanol	ethyl alcohol	C_2H_6O	46	64-17-5	C00469	[4][6][9][11][15]
methanethiol	methyl mercaptan	CH_4S	48	74-93-1	C00409	[4][13]
propan-2-one	acetone	C_3H_6O	58	67-64-1	C00207	[4][6]
acetic acid	acetic acid	$C_2H_4O_2$	60	64-19-7	C00033	[4][6][12][14]
3-methylbutanal	isovaleraldehyde	$C_5H_{10}O$	86	590-86-3	C07329	[4][6][9]
3-hydroxybutan-2-one	acetoin	$C_4H_8O_2$	88	513-86-0	C00466	[7][8][11][12][14]
3-methylbutan-1-ol	isoamyl alcohol	$C_5H_{12}O$	88	123-51-3	C07328	[4][6][7][11][12][13][14][15]
(methyldisulfanyl)methane	dimethyl disulfide	$C_2H_6S_2$	94	624-92-0	C08371	[4][6][7][13]
3-methylbutanoic acid	isovaleric acid	$C_5H_{10}O_2$	102	503-74-2	C08262	[4][6][12][13][14]

* MW: Molecular weight; CAS: Chemical Abstracts Service; KEGG: Kyoto Encyclopedia of Genes and Genomes.

Table 2. Analyses of the in vitro volatilomes of *S. aureus* in comparison to other pathogens.

Ref.	VOC Detection Method	*Acinetobacter* spp.	*Acinetobacter baumannii*	*Burkholderia cepacia* complex	*Candida* spp.	*Candida albicans*	*Citrobacter* spp.	*Clostridium perfringens*	*Enterobacter* spp.	*Enterobacter cloacae*	*Enterococcus* spp.	*Escherichia coli*	*Haemophilus influenzae*	*Helicobacter pylori*	*Klebsiella* spp.	*Klebsiella pneumoniae*	*Moraxella catarrhalis*	*Neisseria meningitidis*	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Staphylococcus epidermidis*	*Stenotrophomonas maltophilia*	*Streptococcus* spp.	*Streptococcus pyogenes*
[12]	PRT-MS						x					x		x	x					x				
[19]	E-Nose		x			x				x		x	x				x			x		x		
[4]	GC-MS																			x				

Ref.	VOC Detection Method	*Acinetobacter spp.*	*Acinetobacter baumannii*	*Burkholderia cepacia complex*	*Candida spp.*	*Candida albicans*	*Citrobacter spp.*	*Clostridium perfringens*	*Enterobacter spp.*	*Enterobacter cloacae*	*Enterococcus spp.*	*Escherichia coli*	*Haemophilus influenzae*	*Helicobacter pylori*	*Klebsiella spp.*	*Klebsiella pneumoniae*	*Moraxella catarrhalis*	*Neisseria meningitidis*	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Staphylococcus epidermidis*	*Stenotrophomonas maltophilia*	*Streptococcus spp.*	*Streptococcus pyogenes*
[9]	GC×GC-MS																				x			
[8]	GC×GC-MS																				x			
[7]	GC-MS															x			x	x				
[12]	GC-MS											x								x	x				
[14]	GC-MS											x								x	x				
[13]	GC-IMS											x								x					
[19]	SIFT-MS											x						x		x			x		
[20]	SIFT-MS			x																x		x			
[21]	GC×GC-MS	x				x			x		x	x			x				x	x					
[22]	E-Nose											x	x				x			x			x		
[23]	E-Nose							x				x								x	x				x
[24]	SESI-HRMS											x	x							x		x	x		
[25]	GC-MS											x				x				x					
[26]	GC-MS					x						x													
[28]	GC-MS																			x					
[29]	GC-MS																			x					x
[30]	SIFT-MS																			x	x				x
[31]	IMR-MS +										x										x				
[32]	CSA **		x			x			x		x	x			x				x	x	x		x		

* IMR-MS: ion-molecule reaction mass spectrometry ** CSA: Colorimetric Sensor Array.

[2] ies have athogen. ancerous at within produced by each of the three infection etiologies and the uninfected controls when analyzed by SPME-GC-MS. The breath volatiles from the rabbit infection models also showed significant differences when analyzed at 24 h post-inoculation, with six discriminatory VOCs translating from the in vitro to in vivo models. Mouse model studies by Zhu, Hill, and colleagues determined that SESI-MS breathprinting distinguished between seven of the most common causes of human bacterial lung infections: *S. aureus, H. influenzae, K. pneumoniae, Legionella pneumophila, M. catarrhalis, P. aeruginosa,* and *S. pneumoniae* [34]. They also demonstrated that breath VOCs could discriminate between infections caused by *P. aeruginosa* strains PAO1 vs. FRD1, and *S. aureus* RN450 [2] and that the etiology of bacterial lung infections can be correctly classified from early infection to clearance (from 6–120 h post-infection) [35]. In studies that exposed mice to live *S. aureus* and *P. aeruginosa*, non-infectious but immunogenic lysates of the bacteria, or saline controls, they found that breathprints of infections are the combination of bacterial metabolites, host metabolites that are correlated to immune response, and novel biomarkers that are created by the feedback between pathogen and host during active infection [3]. The involvement of the host immune system in generating VOC biomarkers during staph infections lends further support for the feasibility of differentiating between infections vs. asymptomatic colonization in humans and animals.

VOC biomarkers are also being developed to identify clinically important staph strains, such as MRSA and toxigenic isolates. In 2010, Jia and colleagues performed a proof-of-concept study of methicillin-sensitive *S. aureus* ATCC 29213 (MSSA) and methicillin-resistant *S. aureus* NRS 382 (MRSA) cultivated in vitro and analyzed via SPME-GC-MS [9], concluding that VOC analysis by GC-MS was suitable for differentiating MRSA and MSSA and that it may form the basis for an innovative and non-invasive diagnostic platform. These initial findings were strengthened by a SESI-MS/MS analysis of VOCs produced by isogenic MRSA and MSSA *S. aureus* strains-RN450 and 450M, respectively–that genetically differ only by the presence/absence of the SCC*mec* genes that confer methicillin resistance [36]. In this study, Li and colleagues evaluated the in vitro bacterial metabolic perturbations caused by antibiotic treatment with ampicillin. They showed that the MRSA and MSSA strains exhibited discriminately different metabolic profiles under the same growth conditions both before and after exposure to antibiotics. Further, Bean and colleagues showed that the volatilome differences between *S. aureus* RN450 and 450M are also detectable in the breathprints of mouse lung infection models caused by these strains, even without antibiotic exposure [37]. Combined, these studies suggest that VOCs may be used to both detect

MRSA infections in situ prior to antibiotic treatment failure, and to subsequently monitor antibiotic treatment efficacy. VOCs have also shown promise for the detection and differentiation of enterotoxic and non-enterotoxic *S. aureus* strains [13] –an important issue for food safety–broadening the potential utility of VOC-based diagnostics for staph.

2. Diagnosing Human Infections

VOC signatures detected in human biospecimens can differentiate infected vs. non-infected individuals in conditions where *S. aureus* is a prevalent etiology, with new diagnostics for Ventilator-Associated Pneumonia (VAP) being a common target for volatile biomarkers. An investigation by Schnabel and colleagues of 100 patients with a clinical suspicion of VAP sampled exhaled breath from the expiratory limb of the ventilators and analyzed the VOCs using GC-TOFMS [38]. Bronchoalveolar lavage (BAL) diagnostic criteria confirmed VAP in 32 patients and ruled out VAP in 68. Subsequent multivariate statistical analysis of the breath VOC profiles enabled the identification of 12 compounds that discriminate against VAP+ and VAP- patients with sensitivity and specificity of approximately 76% and 73%, respectively [38]. The BreathDx Consortium recently published results from a study of 93 breath samples from ventilated patients who were enrolled upon clinical suspicion of VAP [39]. They identified a panel of 10 VOCs that had a 96% negative predictive value for differentiating subjects with VAP (diagnosed via positive BAL cultures) versus those who are culture negative, with potentially important implications for reducing the overprescription of antibiotics in ventilated patients. Staph-specific biomarkers for VAP are also under development. In a pilot study of 22 mechanically ventilated patients diagnosed with VAP, 17 of which were confirmed by positive cultures, Filipiak and colleagues found important overlaps between the in vitro VOCs produced by *S. aureus*, *E. coli*, *Candida* spp., and hemolytic *Streptococcus* and the VOCs detected in patients infected by those pathogens [40]. As observed in mouse model studies, they found that roughly a third of *S. aureus* VOCs they had previously detected in vitro were found in the breath of *S. aureus*-infected patients. The ventilated patients were sampled longitudinally over three to eight days, and several patients transitioned between infected and uninfected states during the analysis. Promisingly, several of the potential breath biomarkers for *S. aureus* were detected more frequently during periods of infection vs. resolution. Similar encouraging overlaps between *S. aureus* VOCs from in vitro bacterial cultures and ex vivo specimens were seen in the analysis of mucus from sinus infections [41]. However, neither study contained sufficient numbers of *S. aureus*-positive subjects and samples to confirm these correlations.

The most significant progress in the development of *S. aureus* VOC biomarkers has come from studies of persons with cystic fibrosis (CF) lung infections. In an analysis of the VOCs detected in 154 BAL fluid samples from CF patients with a variety of lung infections, Nasir et al. built models to discriminate samples from *S. aureus* vs. *P. aeruginosa* infections (n = 59), as well as models that discriminate *S. aureus* positive vs. negative samples (n = 133) [1]. The former model included 11 VOC biomarkers that had an area under the receiver operator curve (AUROC) of 0.79, and the latter model was 8 VOCs that could discriminate staph infected vs. uninfected patients with an AUROC of 0.88. Neerincx and colleagues analyzed the breath of 18 CF patients, 13 of whom had *S. aureus* infections, and identified nine VOCs that differentiate infected and uninfected CF patients with sensitivity

and specificity of 1.00 and 0.80, respectively [42]. In both studies, the *S. aureus-infected* cohort included some subjects who had co-infections with other pathogens, such as *S. maltophilia*, *H. influenzae*, fungi/yeast, and nontuberculous mycobacteria, and the *S. aureus*-negative cohort included a mix of subjects who had no detected pathogens and subjects who had other infections. The predictive ability of VOCs to differentiate *S. aureus* infected versus uninfected patients in such a complex infection landscape as CF lung disease is notable.

Several studies have demonstrated the feasibility of detecting and characterizing non-respiratory infections by VOC analysis of ex vivo specimens or in vitro cultures. In a pilot study by Rogosch and colleagues, laboratory-confirmed bloodstream infections (n = 8) were detected with 100% diagnostic accuracy via E-Nose analysis of tracheal aspirates of 28 intubated preterm neonates [43]. The preclinical detection of late-onset sepsis caused by *S. aureus* and CoNS in preterm infants is possible up to three days prior to the onset of symptoms by the analysis of fecal VOCs using high-field asymmetric waveform ion mobility spectrometry [44]. Colorimetric sensor arrays (CSAs) have been developed to detect patterns of specific VOCs from in vitro cultures, enabling the direct identification of bacteria and yeast that cause bloodstream infections during blood culture enrichment [32][45]. Lim et al. showed that a blood culture cap modified to contain 73 VOC color indicators could differentiate *S. aureus*, *S. epidermidis*, and *S. lugdunensis* from 15 other bacterial taxa after 9 h of culturing with an overall sensitivity and specificity of 95.3% and 99.7%, respectively, using a CSA library based on more than a thousand blood culture analyses [32]. CSAs can also be used to perform rapid antibiotic susceptibility testing directly from blood cultures by growing aliquots of the cultures in an antibiotic array and monitoring for bacterial growth via VOC production. Kuil and colleagues analyzed the performance of the SPECIFIC REVEAL® CSA system for antibiotic susceptibility testing of 96 positive blood cultures [46]. They observed perfect agreement with the categorical results (susceptible, intermediate, or resistant) provided by the bioMérieux VITEK®2 system for infections caused by Gram-negative bacteria and 91% agreement for the Gram-positives, including *S. aureus*. The errors in the susceptibility results for the Gram-positive infections were due to the misclassification of CoNS, with five *S. epidermidis* and four other CoNS showing discrepancies for oxacillin, cefoxitin, or vancomycin.

Few research studies utilizing GC-MS analysis focus on identifying *S. epidermidis* volatiles [5][6][12][47], but as a skin commensal, there has been interest in how *S. epidermidis* VOCs contribute to the attraction of mosquitoes. Verhulst and colleagues demonstrate a profile of eight *S. epidermidis* VOCs, produced in the context of human skin, comprising a suite of semiochemicals that attract *Anopheles gambiae*, a mosquito known to carry malaria [47]. These compounds include dimethyl disulfide, butyl acetate, butyl 2-methylbutanoate, 2-pentadecanone, dimethyl tetrasulfide, dimethyl pentasulfide, hexathiepane, and the inorganic compound octasulfur. With the universality of *S. epidermidis* as a human colonizer but the paucity of information on *S. epidermidis* VOCs, much work remains to characterize this bacterium (including strain-to-strain variations) and to determine the similarities and differences of its volatilome compared to its aggressively pathogenic relative, *S. aureus.*

3. Diagnosing Animal Infections

Several research groups have been exploring the use of VOCs to detect *S. aureus* in symptomatic and sub-clinical mastitis in cattle [48][49][50][51][52], as well as the antibiotic resistance status of the infections. By adapting the VOC

detection to E-Nose and other field-deployable detection devices [53][54], livestock can be routinely monitored to reduce the transmission of unnoticed infections and to advance antibiotic stewardship activities through an enhanced empirical selection of appropriate medications for MRSA. In response to the urgent need to proactively monitor livestock for antimicrobial-resistant pathogens, Yuan and colleagues propose the implementation of high-resolution visual and olfactory sensing for enhanced perception of contagious disease among dairy cattle [52]. Their goal is to engineer a novel and heterogeneous digital intelligence structure that exploits the combination of visual and olfactory data of individual animals during milking. Asymptomatically infected udders potentially exhibit elevated temperatures that can be recorded by thermal imaging cameras networked to milking robots. During milking, VOC patterns can also be detected by E-Nose. By constructing high-performance machine learning models, these artificial intelligence systems may lead the way to innovative precision livestock management. With the potential to surveil and identify early infectious disease in single animals, this technology could interrupt the commonplace asymptomatic transmission of *S. aureus* throughout the herd. This novel approach using artificial intelligence for perception in uncovering underlying diseases enhances the One Health Antimicrobial Resistance initiative goals regarding antimicrobial stewardship while diminishing economic losses due to unanticipated infectious outbreaks.

References

1. Nasir, M.; Bean, H.D.; Smolinska, A.; Rees, C.A.; Zemanick, E.T.; Hill, J.E. Volatile molecules from bronchoalveolar lavage fluid can ‘rule-in’ Pseudomonas aeruginosa and ‘rule-out’ Staphylococcus aureus infections in cystic fibrosis patients. Sci. Rep. 2018, 8, 826.
2. Zhu, J.; Bean, H.D.; Wargo, M.J.; Leclair, L.W.; Hill, J.E. Detecting bacterial lung infections: In vivo evaluation of in vitro volatile fingerprints. J. Breath. Res. 2013, 7, 016003.
3. Bean, H.D.; Jimenez-Diaz, J.; Zhu, J.; Hill, J.E. Breathprints of model murine bacterial lung infections are linked with immune response. Eur. Respir. J. 2015, 45, 181–190.
4. Filipiak, W.; Sponring, A.; Baur, M.M.; Filipiak, A.; Ager, C.; Wiesenhofer, H.; Nagl, M.; Troppmair, J.; Amann, A. Molecular analysis of volatile metabolites released specifically by Staphylococcus aureus and Pseudomonas aeruginosa. BMC Microbiol. 2012, 12, 113.
5. Jenkins, C.L.; Bean, H.D. Influence of media on the differentiation of Staphylococcus spp. by volatile compounds. J. Breath. Res. 2020, 14, 016007.
6. Jenkins, C.L.; Bean, H.D. Dependence of the staphylococcal volatilome composition on microbial nutrition. Metabolites 2020, 10, 347.
7. Zechman, J.M.; Aldinger, S.; Labows, J.N., Jr. Characterization of pathogenic bacteria by automated headspace concentration-gas chromatography. J. Chromatogr. 1986, 377, 49–57.

8. Dörries, K.; Lalk, M. Metabolic footprint analysis uncovers strain specific overflow metabolism and d-isoleucine production of Staphylococcus aureus COL and HG001. PLoS ONE 2013, 8, e81500.
9. Jia, B.; Sohnlein, B.; Mortelmans, K.; Coggiola, M.; Oser, H. Distinguishing methicillin-resistant and sensitive Staphylococcus aureus using volatile headspace metabolites. IEEE Sens. J. 2010, 10, 71–75.
10. Allardyce, R.A.; Langford, V.S.; Hill, A.L.; Murdoch, D.R. Detection of volatile metabolites produced by bacterial growth in blood culture media by selected ion flow tube mass spectrometry (SIFT-MS). J. Microbiol. Methods 2006, 65, 361–365.
11. Liebeke, M.; Dörries, K.; Zühlke, D.; Bernhardt, J.; Fuchs, S.; Pane-Farre, J.; Engelmann, S.; Völker, U.; Bode, R.; Dandekar, T. A metabolomics and proteomics study of the adaptation of Staphylococcus aureus to glucose starvation. Mol. Biosyst. 2011, 7, 1241–1253.
12. Fitzgerald, S.; Duffy, E.; Holland, L.; Morrin, A. Multi-strain volatile profiling of pathogenic and commensal cutaneous bacteria. Sci. Rep. 2020, 10, 17971.
13. Baptista, I.; Santos, M.; Rudnitskaya, A.; Saraiva, J.A.; Almeida, A.; Rocha, S.M. A comprehensive look into the volatile exometabolome of enteroxic and non-enterotoxic Staphylococcus aureus strains. Int. J. Biochem. Cell Biol. 2019, 108, 40–50.
14. Fitzgerald, S.; Holland, L.; Morrin, A. An investigation of stability and species and strain-level specificity in bacterial volatilomes. Front. Microbiol. 2021, 12, 693075.
15. Lu, Y.; Zeng, L.; Li, M.; Yan, B.; Gao, D.; Zhou, B.; Lu, W.; He, Q. Use of GC-IMS for detection of volatile organic compounds to identify mixed bacterial culture medium. AMB Express 2022, 12, 31.
16. Muchowska, K.B.; Varma, S.J.; Moran, J. Synthesis and breakdown of universal metabolic precursors promoted by iron. Nature 2019, 569, 104–107.
17. Lechner, M.; Fille, M.; Hausdorfer, J.; Dierich, M.P.; Rieder, J. Diagnosis of bacteria in vitro by mass spectrometric fingerprinting:A pilot study. Curr. Microbiol. 2005, 51, 267–269.
18. Chen, C.-Y.; Lin, W.-C.; Yang, H.-Y. Diagnosis of ventilator-associated pneumonia using electronic nose sensor array signals: Solutions to improve the application of machine learning in respiratory research. Respir. Res. 2020, 21, 45.
19. Allardyce, R.A.; Hill, A.L.; Murdoch, D.R. The rapid evaluation of bacterial growth and antibiotic susceptibility in blood cultures by selected ion flow tube mass spectrometry. Diagn. Microbiol. Infect. Dis. 2006, 55, 255–261.
20. Dryahina, K.; Sovova, K.; Nemec, A.; Spanel, P. Differentiation of pulmonary bacterial pathogens in cystic fibrosis by volatile metabolites emitted by their in vitro cultures: Pseudomonas

aeruginosa, Staphylococcus aureus, Stenotrophomonas maltophilia and the Burkholderia cepacia complex. J. Breath. Res. 2016, 10, 037102.

21. Rees, C.A.; Burklund, A.; Stefanuto, P.H.; Schwartzman, J.D.; Hill, J.E. Comprehensive volatile metabolic fingerprinting of bacterial and fungal pathogen groups. J. Breath. Res. 2018, 12, 026001.
22. Dutta, R.; Hines, E.L.; Gardner, J.W.; Boilot, P. Bacteria classification using Cyranose 320 electronic nose. Biomed. Eng. Online 2002, 1, 4.
23. Saviauk, T.; Kiiski, J.P.; Nieminen, M.K.; Tamminen, N.N.; Roine, A.N.; Kumpulainen, P.S.; Hokkinen, L.J.; Karjalainen, M.T.; Vuento, R.E.; Aittoniemi, J.J. Electronic nose in the detection of wound infection bacteria from bacterial cultures: A proof-of-principle study. European Surgical Research 2018, 59, 1–11.
24. Kaeslin, J.; Micic, S.; Weber, R.; Müller, S.; Perkins, N.; Berger, C.; Zenobi, R.; Bruderer, T.; Moeller, A. Differentiation of cystic fibrosis-related pathogens by volatile organic compound analysis with secondary electrospray ionization mass spectrometry. Metabolites 2021, 11, 773.
25. Lawal, O.; Muhamadali, H.; Ahmed, W.M.; White, I.R.; Nijsen, T.M.E.; Goodacre, R.; Fowler, S.J. Headspace volatile organic compounds from bacteria implicated in ventilator-associated pneumonia analysed by TD-GC/MS. J. Breath. Res. 2018, 12, 026002.
26. Karami, N.; Mirzajani, F.; Rezadoost, H.; Karimi, A.; Fallah, F.; Ghassempour, A.; Aliahmadi, A. Initial study of three different pathogenic microorganisms by gas chromatography-mass spectrometry. F1000research 2017, 6, 1415.
27. Jenkins, Carrie L.; Bean, Heather D. Current Limitations of Staph Infection Diagnostics, and the Role for VOCs in Achieving Culture-Independent Detection. *Pathogens* **2023**, *12*, 2.
28. Ashrafi, M.; Novak-Frazer, L.; Morris, J.; Baguneid, M.; Rautemaa-Richardson, R.; Bayat, A. Electrical stimulation disrupts biofilms in a human wound model and reveals the potential for monitoring treatment response with volatile biomarkers. Wound Repair Regen. 2019, 27, 5–18.
29. Ashrafi, M.; Novak-Frazer, L.; Bates, M.; Baguneid, M.; Alonso-Rasgado, T.; Xia, G.; Rautemaa-Richardson, R.; Bayat, A. Validation of biofilm formation on human skin wound models and demonstration of clinically translatable bacteria-specific volatile signatures. Sci. Rep. 2018, 8, 9431.
30. Slade, E.A.; Thorn, R.M.; Young, A.E.; Reynolds, D.M. Real-time detection of volatile metabolites enabling species-level discrimination of bacterial biofilms associated with wound infection. J. Appl. Microbiol. 2022, 132, 1558–1572.
31. Dolch, M.E.; Hornuss, C.; Klocke, C.; Praun, S.; Villinger, J.; Denzer, W.; Schelling, G.; Schubert, S. Volatile organic compound analysis by ion molecule reaction mass spectrometry for Gram-positive bacteria differentiation. Eur. J. Clin. Microbiol. Infect. Dis. 2012, 31, 3007–3013.

32. Lim, S.H.; Mix, S.; Xu, Z.; Taba, B.; Budvytiene, I.; Berliner, A.N.; Queralto, N.; Churi, Y.S.; Huang, R.S.; Eiden, M. Colorimetric sensor array allows fast detection and simultaneous identification of sepsis-causing bacteria in spiked blood culture. J. Clin. Microbiol. 2014, 52, 592–598.
33. Zhou, Y.; Chen, E.; Wu, X.; Hu, Y.; Ge, H.; Xu, P.; Zou, Y.; Jin, J.; Wang, P.; Ying, K. Rational lung tissue and animal models for rapid breath tests to determine pneumonia and pathogens. Am. J. Transl. Res. 2017, 9, 5116.
34. Zhu, J.; Bean, H.D.; Jiménez-Díaz, J.; Hill, J.E. Secondary electrospray ionization-mass spectrometry (SESI-MS) breathprinting of multiple bacterial lung pathogens, a mouse model study. J. Appl. Physiol. 2013, 114, 1544–1549.
35. Zhu, J.; Jimenez-Diaz, J.; Bean, H.D.; Daphtary, N.A.; Aliyeva, M.I.; Lundblad, L.K.; Hill, J.E. Robust detection of P. aeruginosa and S. aureus acute lung infections by secondary electrospray ionization-mass spectrometry (SESI-MS) breathprinting: From initial infection to clearance. J. Breath. Res. 2013, 7, 037106.
36. Li, H.; Zhu, J. Differentiating antibiotic-resistant Staphylococcus aureus using secondary electrospray ionization tandem mass spectrometry. Anal. Chem. 2018, 90, 12108–12115.
37. Bean, H.D.; Zhu, J.; Sengle, J.C.; Hill, J.E. Identifying methicillin-resistant Staphylococcus aureus (MRSA) lung infections in mice via breath analysis using secondary electrospray ionization-mass spectrometry (SESI-MS). J. Breath. Res. 2014, 8, 041001.
38. Schnabel, R.; Fijten, R.; Smolinska, A.; Dallinga, J.; Boumans, M.-L.; Stobberingh, E.; Boots, A.; Roekaerts, P.; Bergmans, D.; van Schooten, F.J. Analysis of volatile organic compounds in exhaled breath to diagnose ventilator-associated pneumonia. Sci. Rep. 2015, 5, 17179.
39. Van Oort, P.M.; Nijsen, T.M.; White, I.R.; Knobel, H.H.; Felton, T.; Rattray, N.; Lawal, O.; Bulut, M.; Ahmed, W.; Artigas, A. Untargeted molecular analysis of exhaled breath as a diagnostic test for ventilator-associated lower respiratory tract infections (BreathDx). Thorax 2022, 77, 79–81.
40. Filipiak, W.; Beer, R.; Sponring, A.; Filipiak, A.; Ager, C.; Schiefecker, A.; Lanthaler, S.; Helbok, R.; Nagl, M.; Troppmair, J.; et al. Breath analysis for in vivo detection of pathogens related to ventilator-associated pneumonia in intensive care patients: A prospective pilot study. J. Breath. Res. 2015, 9, 016004.
41. Preti, G.; Thaler, E.; Hanson, C.W.; Troy, M.; Eades, J.; Gelperin, A. Volatile compounds characteristic of sinus-related bacteria and infected sinus mucus: Analysis by solid-phase microextraction and gas chromatography-mass spectrometry. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 2009, 877, 2011–2018.
42. Neerincx, A.H.; Geurts, B.P.; van Loon, J.; Tiemes, V.; Jansen, J.J.; Harren, F.J.; Kluijtmans, L.A.; Merkus, P.J.; Cristescu, S.M.; Buydens, L.M.; et al. Detection of Staphylococcus aureus in cystic fibrosis patients using breath VOC profiles. J. Breath. Res. 2016, 10, 046014.

43. Rogosch, T.; Herrmann, N.; Maier, R.F.; Domann, E.; Hattesohl, A.; Koczulla, A.R.; Zemlin, M. Detection of bloodstream infections and prediction of bronchopulmonary dysplasia in preterm neonates with an electronic nose. J. Pediatr. 2014, 165, 622–624.
44. Berkhout, D.J.; Van Keulen, B.J.; Niemarkt, H.J.; Bessem, J.R.; De Boode, W.P.; Cossey, V.; Hoogenes, N.; Hulzebos, C.V.; Klaver, E.; Andriessen, P. Late-onset sepsis in preterm infants can be detected preclinically by fecal volatile organic compound analysis: A prospective, multicenter cohort study. Clin. Infect. Dis. 2019, 68, 70–77.
45. Shrestha, N.K.; Lim, S.H.; Wilson, D.A.; SalasVargas, A.V.; Churi, Y.S.; Rhodes, P.A.; Mazzone, P.J.; Procop, G.W. The combined rapid detection and species-level identification of yeasts in simulated blood culture using a colorimetric sensor array. PLoS ONE 2017, 12, e0173130.
46. Kuil, S.D.; Hidad, S.; Schneeberger, C.; Singh, P.; Rhodes, P.; de Jong, M.D.; Visser, C.E. Susceptibility testing by volatile organic compound detection direct from positive blood cultures: A proof-of-principle laboratory study. Antibiotics 2022, 11, 705.
47. Verhulst, N.O.; Andriessen, R.; Groenhagen, U.; Bukovinszkine Kiss, G.; Schulz, S.; Takken, W.; van Loon, J.J.; Schraa, G.; Smallegange, R.C. Differential attraction of malaria mosquitoes to volatile blends produced by human skin bacteria. PLoS ONE 2010, 5, e15829.
48. Hettinga, K.A.; van Valenberg, H.J.; Lam, T.J.; van Hooijdonk, A.C. Detection of mastitis pathogens by analysis of volatile bacterial metabolites. J. Dairy Sci. 2008, 91, 3834–3839.
49. Hettinga, K.A.; van Valenberg, H.J.; Lam, T.J.; van Hooijdonk, A.C. The influence of incubation on the formation of volatile bacterial metabolites in mastitis milk. J. Dairy Sci. 2009, 92, 4901–4905.
50. Hettinga, K.A.; van Valenberg, H.J.; Lam, T.J.; van Hooijdonk, A.C. The origin of the volatile metabolites found in mastitis milk. Vet. Microbiol. 2009, 137, 384–387.
51. Shinga, M.H. Investigating Alternative Methods to Detect Bovine Mastitis in Milk. Ph.D. Thesis, University of KwaZulu-Natal, Durban, South Africa, 2018. Available online: https://ukzn-dspace.ukzn.ac.za/handle/10413/16792.
52. Yuan, B.; Nørstebø, H.; Whist, A.C.; Belbachir, N. Detection of Lameness and Mastitis Pathogens in Milk Using Visual and Olfactory Sensing. 2020. Available online: https://hdl.handle.net/11250/2680881 (accessed on 26 May 2022).
53. Gierschner, P.; Küntzel, A.; Reinhold, P.; Köhler, H.; Schubert, J.K.; Miekisch, W. Crowd monitoring in dairy cattle—Real-time VOC profiling by direct mass spectrometry. J. Breath. Res. 2019, 13, 046006.
54. Wilson, A.D. Applications of electronic-nose technologies for noninvasive early detection of plant, animal and human diseases. Chemosensors 2018, 6, 45.