Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org
Original Research

Resistant/susceptible classification of respiratory tract pathogenic bacteria based on volatile organic compounds profiling

Najmeh Karami[1], Hassan Rezadoost[2], Fateme Mirzajani[3], Abdollah Karimi[1], Alireza Ghassempour[2], Atousa Aliahmadi[4*], Fatemeh Fallah[1*]

[1] Pediatric Infections Research Center, Research Institute for Children Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] Department of Phytochemistry, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, G.C., Tehran, Iran
[3] Protein Research Center, Shahid Beheshti University, G.C., Tehran, Iran
[4] Department of Biology, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, G.C., Tehran, Iran

Correspondence to: dr.fafallah@gmail.com, atousa.aliahmadi@gmail.com

Received March 28, 2018; **Accepted** June 26, 2018; **Published** June 30, 2018

Doi: http://dx.doi.org/10.14715/cmb/2018.64.9.2

Abstract: Resistance to antibiotics is an emerging and growing threat. To address this threat, attempts are being made by researchers to identify the Volatile Organic Compounds (VOCs) of bacteria. It is believed that unique combinations could be found among the VOCs produced by each microorganism. The current study aimed to identify and compare the VOCs of antibiotic-resistant and standard strains of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*. A polymer of divinylbenzene /carboxen /polydimethylsiloxane was applied for absorption of volatile compounds in headspace bacterial samples in form of a solid phase micro-extraction fiber holder. Gas chromatography-mass spectrometry technique was used for identification of volatile compounds. The analysis of the VOCs indicated that some VOCs appeared only in standard strains while others were common only among resistant strains. Exclusive VOCs to a specific strain were also detected. This study demonstrated that resistant strains of bacteria produced VOCs that were different from those of the standard strains. In addition, VOCs released by bacteria after passing the logarithmic growth phase showed no significant differences. The identification of VOCs can be a precise way to differentiate bacterial species, also it can be said that the VOCs produced by different pathogenic microorganisms can be the suitable biomarkers for their detection.

Key words: Resistant strains; Standard strains; Volatile organic compounds; Solid phase micro-extraction.

Introduction

The development of resistance to common antibiotics used for the treatment of pathogenic bacteria has caused important health care problems worldwide (1-3). Antimicrobial agents have become ineffective or less effective in treating microbial infections (4). Antibiotic resistance is a growing threat and the resulting mortality and morbidity are not limited to specific geographical locations (5). Resistant bacteria include both Gram-positive and Gram-negative types (4-6). The most important resistant Gram-negative bacteria are *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Escherichia coli* (7). *Staphylococcus aureus* is the most common resistant Gram-positive bacteria (8).

Diagnosis and differentiation of pathogenic bacteria in a short time is the most important step in the treatment of infections (9). Much effort has been devoted to designing accurate and rapid diagnostic methods that can differentiate the causative agent of infections (10). In recent years, researchers have endeavored to identify the volatile organic compounds (VOCs) of bacteria (11-14). It is believed that for each microorganism, a unique VOC profile can be found (15-16). Scientists are hoping to use this feature in designing a quick and accurate method for bacterial identification (11, 17). Among the various methods that have been used to identify VOCs (13, 18-20), Gas Chromatography-Mass Spectrometry (GC-MS) is used extensively (21-23).

The method of VOCs extraction differs by the technique used to identify the organic compounds (17, 24-26). Solid Phase Micro-Extraction (SPME) is an important method of collecting the VOCs of microorganisms for identification by GC-MS. The advantages of SPME include its quick extraction process, its simplicity and usually does not require solvents (27-28).

The current study aimed to identify and compare the VOCs of five prevalent resistant bacteria (*E. coli*, *S. aureus*, *P. aeruginosa*, *A. baumannii* and *K. pneumoniae*) with their standard strains using SPME for extraction and GC-MS for detection. In the end, the exclusive VOCs that can be used as biomarkers to distinguish resistant species from non-resistant bacteria will be introduced.

Materials and Methods

Bacterial strains

The standard strains of *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), *P. aeruginosa* (ATCC 27853), *A. baumannii* (ATCC 19606) and *K. pneumoniae* (ATCC 700683) and an antibiotic-resistant strain of each species were evaluated. All resistant strains were isolated

from clinical samples and identified by species-specific polymerase chain reaction (PCR) and routine bacteriological testing (data not shown). A stock of each strain was prepared using nutrient broth containing 15% (v/v) glycerol kept at -80 °C.

Susceptibility tests

The minimum inhibitory concentration (MIC) of different types of antibiotics (for non-standard strains or antibiotic-resistant strains) was determined according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (29). In brief, serial double dilutions of the antibiotics were prepared in 96-well trays using Mueller Hinton broth (MHB) medium as the diluents at a concentration of 0.25-512 µg/ml. The inoculants of the microbial strains were prepared in sterile normal saline from freshly-cultured bacteria and adjusted to 0.5 McFarland standard turbidity. Inoculants were further diluted (1:100) using MHB medium just before addition to the serial diluted samples. The trays were incubated for 20 h at 37 °C and the MIC values were recorded as the lowest concentrations that could inhibit visible growth of microorganisms. The sensitivity and resistance of studied non-standard species to antibiotics are presented in Table 1.

Extraction of headspace

All bacterial strains were cultured in nutrient agar plates and then one isolated colony was sub-cultured onto 30 ml tryptic soy broth (TSB) in 100 ml glass bottles and incubated at 37 °C, with agitation at 150 rpm (30). The headspace of each strain was extracted at 2, 4 and 24 h. A suspension of microorganisms at OD_{600}~0.5 was used for the initiation of each experiment (31) and the corresponding sterile broth mediums were assessed as blank samples (28). In the headspace experiments, volatile compounds were absorbed using A SPME fiber holder (57330-U; Sigma-Aldrich) with a fiber coated with divinylbenzene /carboxen /polydimethylsiloxane (DVB /CAR /PDMS) 50/30 µm (57328-U; Sigma-Aldrich). To improve the absorption of VOCs, after the incubation time, 2 ml of 36% NaCl was added to each sample. The DVB/CAR/PDMS fiber was suspended from the top of the bottle containing the culture and then placed on a magnetic stirrer hotplate at 70 °C for 30 min (30). After extraction, the SPME fiber was transferred to the injection part of the GC-MS and the extracted VOCs was desorbed from the fiber into a chromatography column. For thermal desorption, the SPME fiber remained in the injector for 2 min before it was exposed to the headspace of the bacterial samples (27). Each state was tested at least three times.

GC-MS analysis of volatile compounds

A trace GC-MS system (Thermo Quest-Finnigan) equipped with a DB-5 column (60 m in length, 0.25 mm inner diameter and 0.25 µm film thickness) was used to study the bacterial VOCs using helium carrier gas at a flow rate of 1.1 ml/min. The starting temperature was 50 °C which was increased at a rate of 10 °C/min to 250 °C. The GC-MS was set in split-less mode and a quadrupole ion trap with ionization energy of 70 eV was used in the filament.

The VOCs were identified using the National Institute of Standards and Technology (NIST) reference library. To analyze the GC-MS data, Xcalibur 3.0 with Foundation 3.0 SP2 software (Thermo-Fisher Scientific) was used and the Kovats retention index (RI) was calculated for each chromatographic peak. The NIST 17 mass spectral library (NIST17/2017/EPA/NIH) was used to identify each compound according to its RI. Sample studies were conducted by a Phytochemistry specialist to define each of the detected compounds as organic.

Statistical analysis

All statistical analyses were performed using SPSS version 24.0. To evaluate the differences between groups, Mann-Whitney test was used. A p-value of less than 0.05 was considered statistically significant.

Results

GC-MS Chromatograms of volatile compound

The headspaces of all bacterial strains (five resistant and five standard strains) were extracted by SPME and injected into the GC-MS at 2, 4 and 24 hours after culturing. The chromatograms of extracted VOCs from 24 h cultures are presented in Figure 1. As shown in these chromatograms, after 24 h incubation, VOC profiles of all resistant strains were totally different from the corresponding standard strains.

Table 1. Antibiotic susceptibility pattern of assessed resistant bacteria.

	E. coli	*S. aureus*	*P. aeruginosa*	*A. baumannii*	*K. pneumoniae*
Resistant to	Cephalotin Cefazolin Ciprofloxacin Ofloxacin Tetracyclin Nalidixic acid Amikacin Sulfamethoxazole Trimethoprim Gentamicin Ceftazidime Ceftriaxon	OxacilinCefoxitin Ceftriaxon Ciprofloxacin Sulfamethoxazole Trimethoprim Tetracyclin Erythromycin Clotrimazol	Ciprofloxacin Sulfamethoxazole Trimethoprim Gentamicin Amikacin	Imipenem Ceftriaxone Sulfamethoxazole Trimethoprim Cefotaxime Ceftazidime Tetramycin Ciprofloxacin Gentamicin Amikacin Tobromycin	CephalotinCefazolin Ciprofloxacin Ofloxacin Tetracyclin Nalidixic acid Amikacin Sulfamethoxazole Trimethoprim Gentamicin Ceftazidime Ceftriaxon
Sensitive to	Nitrofurantoin	Rifampicin Chloramphenicol	Ceftazidime Colistin	Colistin	Colistin

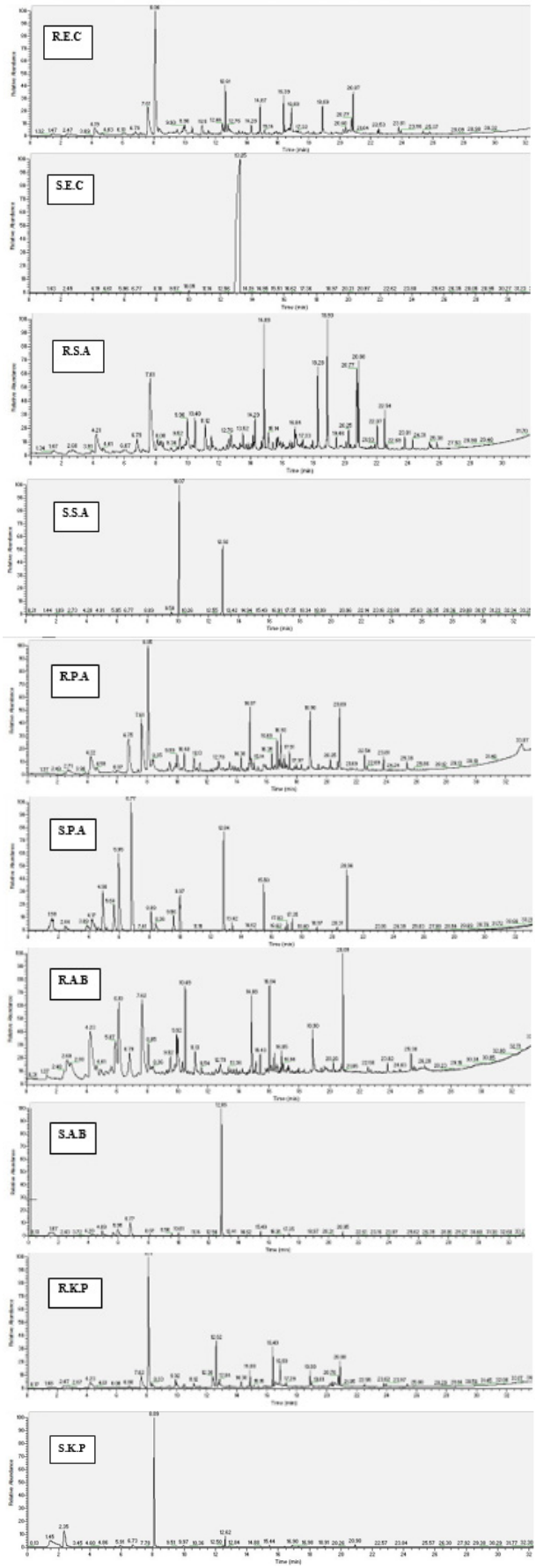

Figure 1. GC-MS Chromatograms of VOCs of 10 studied strains after 24h growth Distinct VOC profiles were obtained for each bacterial species belongs to resistant or standard groups. R.E.C: Resistant *E. coli*, S.E.C: Standard *E. coli*, R.S.A: Resistant *S. aureus*, S.S.A: Standard *S. aureus*, R.P.A: Resistant *P. aeruginosa*, S.P.A: Standard *P. aeruginosa*, R.A.B: Resistant *A. baumannii*, S.A.B: Standard *A. baumannii*, R.K.P: Resistant *K. pneumoniae* and S.K.P: Standard *K. pneumoniae*

The detected VOCs of studied strains

All identified VOCs from *E. coli*, *S. aureus*, *P. aeruginosa*, *A. baumannii* and *K. pneumoniae* (in both resistant and standard strains) are presented in Table 2. The mean of pick area (%) ±SD for each compound which has been produced by the assessed bacteria, regardless of the type of strain and the time of assay (2, 4 and 24 h), are shown in the separate columns. Also the frequency of producer strains of each mentioned volatile compound are shown. According to the data demonstrated in Table 2, the main differences in VOC profile between resistant and standard strains of assessed bacteria can be seen.

Common VOCs produced by different strains

From the results of analysis of the recognized VOCs, some were produced by both standard and resistant strains and were common among some bacteria. These included for example 4-*t-butyl-2-(1-methyl-2-nitroethyl) cyclohexane*, *2,5-(1,1-dimethylethyl)-phenol* and *2,3-pentandione*. Some detected VOCs were produced only by assessed standard strains. These included *1-(1,5-dimethyl-4-hexyl-4-methyl-benzene*, *1,2-butadiyene*, *1,3-heptadiene-3-yne*, *2,6-dibutyl-2,5-cyclohexadiene-1,4-dione*, *2h-tetrazole-5-carboxylicacid2-phenyl*, *2-methyl tetradecane*, *3-propionyloxypentadecane*, *caryophyllene*, *cis-dihydro-α-terpinyl acetate*, *cyclohexene, 4-ethenyl*, *decene*, *dimethyl sulfone*, *ethyl butanoate*, *levomenthol*, *methyl isopropyl hexenal* and *α-acetoxydihydrocoumarin*. In addition, some identified VOCs were produced only by resistant strains. These included *1-(1-hexyl)-cyclohexanol*, *1,2-benzenedicarboxulic acid*, *1,8-cineole*, *10-methylnonadecane*, *1-naphtalenol*, *2,4,5-trimethyl benzeneamine*, *2-methyl naphtalene*, *2-methyy-5-(1-methylethyl)-phenol*, *2-piperidinone*, *2-undecanol*, *2-undecanone*, *3-methyl-pentadecane*, *4-butoxy-1-butene*, *5-methyl-pentadecane*, *7-methoxy-6-ethoxy-2,2-dimethyl-2-chromene*, *acetophenone*, *caryolan-8-ol*, *curcumene*, *cyclohexane propanol*, *decyl-cyclohexane*, *hexadecane*, *indolizine*, *isocyanomethyl benzene*, *nonadecane*, *o-isopropylanisol*, *selinene*, *styrene* and *tetradecanal* (Table 2).

Uncommon VOCs produced by different strains

The VOCs exclusively produced by resistant *E. coli* were *1-(1,1-dimethylethyl)-cyclohexane*, *1-dodecane*, *2-nonanone*, *4-decene*, *5-decene-1-ol*, *6-dodecane*, *alloaromadendrene*, *docosane* and *sesquiesabinene*. The VOCs exclusively produced by standard *E. coli* were *decanol*, *2-acetyl-1-pyrroline*, *dodecanol*, *indole* and *phenyl ethyl pyrrole* (Table 2). Although *indole* was produced by other studied bacteria, it was released in much higher amounts by standard *E. coli* than by *P. aeruginosa*, *A. baumannii*, *K. pneumoniae* and *S. aureus*.

While the VOCs exclusively produced by resistant *S. aureus* were *benzaldehyde*, *dimethyl octenal*, *epicedrol*, *decane* and *nerylacetone*, particular VOCs of standard *S. aureus* were *1-decyne*, *1-penten-3-ol*, *2,5-dimethyl pyrazine*, *2-ethyl hexanol*, *allylbutylhydroquinone* and *benzene acetaldehyde*. *Dodecane* was produced by both the resistant and standard *S. aureus* (Table 2).

The VOCs produced exclusively by resistant *P. aeruginosa* were *1-phenyl-ethanone*, 1-*undecenone*, *cedrene*, *limonene*, *sesquiphellandrene* and *α-terphenyl*

Table 2. The identified VOCs in studied bacteria (standard and resistant strains).

	Resistant Strains			Standard Strains		
VOCs	**Mean ± SD[1]**	**N[2]**	**Strain[3]**	**Mean ± SD[4]**	**N[5]**	**Strain[6]**
(E)-2-hexyl ester- Butanoic acid	2.76 ± 2.18	3	E.c (24h), P.a (2h), A.b (2h)	3.06 ± 2.29	5	E.c(2h), A.b (2h), K.p (2,4,24h)
(z)-2-Octene-1-ol	1.39 ± 1.25	4	P.a (4,24h), A.b(4h), K.p (24h)	1.69 ± 1.47	2	K.p (2,4h)
(z)-4-Decan-1-ol	1.81 ± 1.5	3	E.c (2,4,24h)	3.18 ± 0.1	2	K.p (4,24h)
1-(1-hexyl)-cyclohexanol	1.13 ± 0.9	3	S.a (2,24h), A.b (2h)	-	0	-
1-(1,1-dimethylethyl)-cyclohexane	3.52 ± 0.7	3	E.c (2,4,24h)	-	0	-
1-(1,5-dieethyl-4-hexyl)-4-methyl-Benzene	2.62	1	A.b (4h)	-	0	-
1-(1,5-dimethyl-4-hexyl-4-methyl-Benzene	-	0	-	2.08 ± 2.02	3	E.c (2h), P.a (2h), A.b (2h)
1-Decene	13.71 ± 21.76	3	K.p (2,4,24h)	-	0	-
1-Dodecane	3.58 ± 3.32	2	E.c (4,24h)	-	0	-
1-Methoxy-2-propanol	-	0	-	2.65	1	P.a (4h)
1-methyl-4-(1-methylethyl)-Cyclohexanol	1.93 ± 0.88	2	K.p (2h,4h)	-	0	-
1-Naphtalenol	3.11 ± 0.48	4	E.c (4,24h),	-	0	-
1-Penten-3-ol	-	0	-	6.14	1	S.a (2h)
1-phenyl ethanone	16.97 ± 4.61	2	E.c (2h), P.a (2h)	-	0	-
1-Undecenone	14.53 ± 8.08	2	P.a (4,24h)	-	0	-
1,2-Benzenedicarboxulic acid	1.61	1	P.a (4h)	-	0	-
1,2-Butadiyene	-	0	-	3.96 ± 1.89	7	S.a (2,4,24h), P.a (4,24h), A.b (4,24h)
1,3-Butadiyene	1.74 ± 1.81	7	S.a (24h), P.a (2,4,24h), K.p (2,4,24h)	1.33 ± 0.74	2	K.p (2,4h)
1,3-Heptadiene-3-yne	-	0	-	8.17 ± 8.25	5	K.p (2,24h), S.a (2h), P.a (2h), A.b(2h)
1,5-Decadiene	0.93 ± 0.58	5	E.c (2h), P.a (4,24h), A.b (2h), K.p (2h)	1.86 ± 0.66	2	K.p (4,24h)
1,8-cineole	6.83 ± 4.46	11	E.c (2,4,24h), S.a (2,42h), P.a (2), A.b (4,24h), K.p (2,4h)	-	0	-
1,9-Decadiene	0.93 ± 0.46	7	E.c (4h), S.a (4,24h), P.a (2,24h), A.b (24h), K.p (4h)	1.77	1	K.p (4h)
10-Methylnonadecane	0.3	1	A.b (2h)	-	0	-
2-(phenylmethylene)-Octanal	1.1 ± 0.63	3	A.b (2h), K.p (4h), S.a (24h)	0.36	1	K.p (4h)
2-Acetyl-1-pyrroline	-	0	-	14.39 ± 12.23	5	P.a (4h), A.b (4,24h), E.c (4,24h)
2-Decanone	1.73 ± 1.9	9	E.c (2,4,24h), S.a (2,24h), A.b(2,4,24h), K.p (24h)	-	0	-
2-Decenal	-	0	-	1.26	1	A.b (24h)
2-ethenyl-6-methyl-Pyrazine	2.01 ± 1.7	7	K.p (24h), S.a (4,24h), P.a (4h), A.b (2,4,24h)	4.96 ± 4.79	7	K.p (2,4,24h), E.c (2h), S.a (2h), P.a (2h), A.b (2h)
2-Ethyl hexanol	-	0	-	1.69 ± 0.9	2	S.a (2,4h)
2-Heptanone	0.95 ± 0.42	8	A.b (4,24h), K.p (2,24h), E.c (2,24h), S.a (2,4h)	1.02 ± 0.97	4	P.a (2h), E.c (4,24h), S.a (2h)
2-Hexan-1-ol	1.98 ± 1.75	7	S.a (4,24h), A.b (4,24h), K.p (2,4h), E.c (24h)	-	0	-
2-methyl Naphtalene	0.94 ± 0.36	6	S.a (4,24h), P.a (4,24h), A.b (24h), K.p (24h)	-	0	-
2-Methyl tetradecane	-	0	-	1.74 ± 0.95	4	P.a (4,24h), A.b (4,24h)
2-methyl-1-propanol	0.96 ± 0.46	3	K.p (24h), E.c (24h), A.b (24h)	2.66 ± 2.02	3	P.a (4,24h), A.b (24h)
2-methyl-2-Undecanethiol	-	0	-	6.06	1	K.p (24h)
2-methyl-5-(1-methylethyl)-Phenol	2.23 ± 0.88	2	S.a (4h), A.b (4h)	-	0	-

2-nonanone	22.37 ± 12.61	5	K.p (2,4h), E.c (2,4,24h)	-	0	-
2-octyl-1-ol	0.55	1	E.c (24h)	1.69	1	K.p (24h)
2-Piperidinone	2.54 ± 1.48	2	S.a (2h), A.b (2h)	-	0	-
2-tridecanone	3.8 ± 0.3	2	K.p (2,4h)	-	0	-
2-Undecanol	1.94 ± 1.58	7	K.p (24h), S.a (4, 24h), P.a (4,24h), A.b (4h,24)	-	0	-
2-Undecanone	3.23 ± 1.9	7	A.b (2,24h),K.p (2,24), E.c (2,4,24h)	-	0	-
2,3-Hexandione	0.94 ± 0.46	9	A.b (2,4h), K.p (2,4h), E.c (2,4h), S.a (2,4h), P.a (24h)	1.46	1	K.p (4h)
2,3-Pentandione	1.3 ± 0.73	9	K.p (24h), E.c (24h), S.a (2,4,24h), P.a (2,4,24h), A.b (2h)	12.05 ± 10.48	11	K.p (4,24h), E.c (2,24h), S.a (4,24h), P.a (4,24h), A.b (2,4,24h)
2,4,5-trimethyl Benzeneamine	1.09 ± 0.36	4	A.b (4,24h), K.p (2,4h)	-	0	-
2,5-(1,1-dimethylethyl)-Phenol	1.64 ± 1.09	6	K.p (24h), S.a (4,24h), P.a (24h), A.b (2,4h)	1.15 ± 0.77	6	K.p (2,4,24h), E.c (2h), P.a (2h), A.b (2h)
2,5-dimethyl Pyrazine	-	0	-	8.65 ± 8.82	4	K.p (2,4h), S.a (2), A.b (2h),
2,6-bis(1,1-dimethylethyl)-4-methyl-phenol	-	0	-	18.37 ± 10.68	2	K.p (2, 24h)
2,6-dibutyl-2,5-cyclohexadiene-1,4-dione	-	0	-	1.44 ± 1.34	2	P.a (2h), A.b (2h)
2,6,10-trimethyl-Pentadecane	2.03 ± 0.27	2	A.b (4,24h)	5.48	1	K.p (24h)
2H-Tetrazole-5-carboxylicacid, 2-phenyl	-	0	-	2.54 ± 1.39	3	P.a (24h), A.b (24h), K.p (4h)
3-(3,3-dimethylbutyl)-Cyclohexanone	0.83	1	A.b (24h)	-	0	-
3-decen-1-ol	6.47 ± 3.37	2	K.p (2,4h)	-	0	-
3-Methyl-1,5-heptadiene	2.3 ± 1.19	7	E.c (2h), S.a (2,4h), P.a (2h), A.b (2,4,24h)	2.54 ± 0.72	4	K.p (2,4,24h), A.b (2h)
3-methyl-Pentadecane	0.92	1	P.a (4h)	-	0	-
3-Propionyloxypentadecane	-	0	-	1.24 ± 0.78	7	A.b (2h), K.p (2,4,24h), E.c (2h), S.a (2h), P.a (2h)
3-Undecanone	0.17	1	K.p (2h)	-	0	-
3-Undecene-2-one	2.15 ± 0.6	2	K.p (2,4h)	-	0	-
4-Butoxy-1-Butene	1.16 ± 0.73	5	K.p (4h), S.a (4h), A.b (4,24h), K.p (2h)	-	0	-
4-Decene	4.8	1	E.c (4h)	-	0	-
4-t-butyl-2-(1-methyl-2-nitroethyl) cyclohexane	3.17 ± 2.45	4	S.a (24h), P.a (2h), A.b (2,4h)	4.5 ± 2.4	7	S.a (2h), P.a (2h), E.c (2h), A.b (2h), K.p (2,4,24h)
5-Decene-1-ol	3	1	E.c (2h)	-	0	-
5-methyl-Pentadecane	1.46 ± 0.37	2	P.a (2h), E.c (2h)	-	0	-
5,8-Diethyl-6-dodecanol	1.09 ± 0.32	6	E.c (24h), P.a (2,4,24h), K.p(4,24h)	-	0	-
5.5-Dodecadinyl-1, 12-diol	-	0	-	15.72	1	K.p (4h)
6-Dodecane	2.46 ± 1.54	2	E.c (4,24h)	-	0	-
6-Methyl-5-hepten-2-one	0.61 ± 0.21	7	P.a (4,24h), K.p (2,4,24h), E.c (2,4h)	2.43	1	K.p (2h)
7-methoxy-6-ethoxy-2,2-dimethyl-2-chromene	1.13 ± 0.56	5	S.a (4h), A.b (2,4h), K.p (2,24h)	-	0	-
Acetophenone	16.21 ± 4.41	12	E.c (4,24h), S.a (2,4,24h), P.a (4,24h), A.b (2,4,24h), K.p (2,4h)	-	0	-
Alloaromadendrene	1.1	1	E.c (2h)	-	0	-
Anisol	-	0	-	1.19	1	S.a (2h)
Aromadendrene	5.12	1	A.b (24h)	-	0	-
Benzaldehyde	9.33 ± 11.39	6	S.a (2,4,24h), A.b (2,4,24h)	6.9 ± 5.54	2	S.a (2h), A.b (2h)
Benzene acetaldehyde	-	0	-	7.04	1	S.a (2h)

Benzophenone	1.75 ± 1.33	8	S.a (4,24h), P.a (24h), A.b (2,24h), K.p (4,24h), E.c (4h)	-	0	-
Butyraldehyde	0.42 ± 0.1	2	S.a (2,24h)	2.92 ± 1.52	5	S.a (24h), P.a (4h), K.p (2,4,24h)
Cadinene	1.14 ± 0.97	6	K.p (24h), E.c (24h), S.a (24h), P.a (4h), A.b (4,24h)	0.63	1	K.p (24h)
Carbamic acid	2.42 ± 1.4	7	K.p (24h), S.a (4,24h),P.a (2,4h), A.b (2,24h)	0.98	1	K.p (4h)
Caryolan-8-ol	0.95 ± 0.52	3	S.a (4h), A.b (2,4h)	-	0	-
Caryophyllene	-	0	-	3.02 ± 2.65	7	A.b (4,24h), E.c (24h),S.a (4,24h), P.a (4,24h)
Cedran-1,8-diol	1.51 ± 0.58	6	K.p (2,4,24h), S.a (2,24h), P.a (2h)	1.28 ± 0.27	5	K.p (2,4h), S.a (2h), P.a (2h), A.b (2h)
Cedrene	0.67	1	P.a (24h)	-	0	-
Cedrol	1.96 ± 1.5	9	P.a (24h), A.b (2h), K.p (2,4,24h), E.c (4,24h), S.a (2,24h)	1.4	1	S.a (2h)
cis-Dihydro-α-terpinyl acetate	-	0	-	17.41 ± 15.52	7	P.a (4,24h), A.b (4,24h), E.c (24h), S.a (4,24h)
Curcumene	1.9 ± 0.54	2	E.c (2h), P.a (24h)	-	0	-
Cyclohexane propanol	1.69 ± 1.25	6	A.b (4,24h), E.c (4h), P.a (2,4,24h)	-	0	-
Cyclohexene 4-ethenyl-	-	0	-	4.44 ± 3.21	6	S.a (2,4,24h), A.b (4,24h), K.p (2h)
Decane	6.07 ± 3.33	3	S.a (2,4h), A.b (24h)	-	0	-
Decanol	-	0	-	0.93	1	E.c (2h)
Decyl-Cyclohexane	1.19 ± 1.44	10	E.c (2,24h), S.a (24h), P.a (2,4,24h), A.b (4h), K.p (2,4,24h)	-	0	-
Dibutylphatalate	0.58 ± 0.27	3	P.a (24h), S.a (4h), A.b (4h)	1.57 ± 1.11	6	S.a (2h), P.a (2h), E.c (2h), A.b (2h), K.p (4,24h)
Dimethyl cyclo hexa-1,3 dine	0.73	1	A.b (24h)	-	0	-
Dimethyl Octenal	3.09 ± 2.71	2	S.a (2,4h)	2.39	1	S.a (2h)
Dimethyl sulfone	-	0	-	8.39 ± 3.12	3	P.a (4,24h), A.b (4h)
DimethylethylCyclohexanol	1.15 ± 0.4	6	P.a (4h), A.b (2,24h), K.p (24h), S.a (4,24h)	1.42 ± 0.99	3	K.p (2,4h), S.a (2h)
Docosane	1.5 ± 0.21	3	E.c (2,4,24h)	-	0	-
Dodecane	2.85	1	S.a (24h)	1.96	1	S.a (2h)
Dodecanol	-	0	-	0.27	1	E.c (2h)
Dodecenal	0.56	1	S.a (24h)	8.29 ± 10.87	3	K.p (2,4,24h)
Dodecenol	4.71 ± 3.61	3	A.b (2,4,24h)	-	0	-
Eicosane	1.35	1	P.a (2h)	-	0	-
Epicedrol	8.16	1	S.a (24h)	-	0	-
Ethyl butanoate	-	0	-	5.53 ± 6.07	9	E.c (24h), S.a (4,24h) , P.a (4,24h), A.b (4,24h), K.p (2,4h)
Heptadecane	4.04 ± 2.13	2	E.c (2,4h)	7.47 ± 4.15	3	S.a (2h), P.a (2h), A.b (2h)
hexadecane	5.13 ± 2.81	8	A.b (2,4h), K.p (2,4h), S.a (2,4,24h), P.a (24h)	-	0	-
Indole	1.53 ± 1.28	8	A.b (4,24h), K.p (2,24h), E.c (2,4h), S.a (4,24h)	28.51 ± 44.65	10	A.b (2,24h), P.a (24h), K.p (4,24h), E.c (2,4,24h), S.a (2,4h)
Indolizine	1.15 ± 0.65	7	P.a (24h), A.b (4,24h), K.p (2h), E.c (24h), P.a(2,4h)	-	0	-
Iisocyanomethyl Benzene	1.21 ± 0.79	3	A.b (4h), S.a (4h), P.a (2h)	-	0	-

Isopentyl acetate	-	0	-	1.47	1	P.a (24h)
Levomenthol	-	0	-	2.3 ± 1	5	P.a (24h), A.b (4,24h), S.a (4,24h)
limonene	1.88 ± 0.64	3	P.a (2,4,24h)	-	0	-
Longifolene	0.66 ± 0.33	6	P.a (4h), A.b (2,4h), K.p (2,4,24h)	2.29 ± 1.06	4	K.p (2,4,24h), S.a (2h)
Menthone	1.91 ± 1.39	2	K.p (2,4h)	-	0	-
Methyl isopropyl Hexenal	-	0	-	1.71	1	K.p (24h)
Myrcene	1.94	1	P.a (2h)	-	0	-
Naphthalenol	1.3 ± 0.83	5	P.a (4,24h), A.b (2h), K.p (24h), S.a (24h)	4.89 ± 3.67	3	A.b (2h), E.c (2h), P.a (2h)
Nonadecane	1.44 ± 0.84	6	P.a (2,4h), A.b (2h), E.c (2,4h), S.a (4h)	-	0	-
o-isopropylanisol	1.07 ± 0.54	8	S.a (4,24h), P.a (24h), A.b (2,24h), K.p (2,4,24h),	-	0	-
Ocimene	-	0	-	2.29	1	K.p (2h)
Octacosane	0.59 ± 0.1	2	E.c (2h), P.a (2h)	1.34 ± 0.53	6	K.p (2,4,24h), S.a (2h), P.a (2h), A.b (2h)
Octyl acetate	2.86 ± 2.8	4	A.b (2,4h), K.p (24h), S.a (4h)	4.43 ± 1.78	3	K.p (2,4,24h)
P-cymene	2.46 ± 0.92	2	K.p (2,4h)	-	0	-
Pentadecane	0.74 ± 0.19	2	E.c (24h), P.a (4h)	0.81 ± 0.72	3	S.a (2,4h), K.p (2h)
Pentylhexyl Benzene	-	0	-	0.46	1	A.b (2h)
Phthalic acid, butyl ester	1.54 ± 1.32	5	A.b (2,4h), S.a (2,4h), P.a (4h)	0.82 ± 0.75	4	K.p (4h), E.c (2h), S.a (2h), P.a (2h)
Selinene	1.42 ± 0.66	8	P.a (4,24h), A.b (4h), K.p (4,24h), E.c (24h), S.a (2h)	-	0	-
Sesquiesabinene	4.59 ± 0.45	2	E.c (4,24h)	-	0	-
Sesquiphellandrene	1.36	1	P.a (24h)	-	0	-
Styrene	10.19 ± 7.13	15	A.b (2,4,24h), K.p (2,4,24h), E.c (2,4,24h), S.a (2,4,24h), P.a (2,4,24h)	-	0	-
Tetradecanal	0.86 ± 0.66	3	P.a (24h), A.b (4h), K.p (24h)	-	0	-
Tetradecane	3.52 ± 1.91	10	S.a (2,4,24h), P.a (4,24h), A.b (2,4h), K.p (2,4h), E.c (2h)	-	0	-
Tetradecanol	0.88 ± 0.66	4	S.a (24h), P.a (24h), A.b (2h), K.p (4h)	0.45	1	K.p (4h)
Thiophene	-	0	-	8.84 ± 0.24	2	P.a (4,24h)
trans-Caryophyllene	8.48	1	K.p (24h)	-	0	-
Tridecanol	2.32 ± 2.45	6	P.a (4h), E.c (2,4h), S.a (2,4,24h)	4.1 ± 5.5	3	K.p (2,4,24h)
α-Acetoxydihydrocoumarin	-	0	-	1.31 ± 0.69	3	E.c (2h), P.a (2h), A.b (2h)
α-Methyl ionone	1.35	1	K.p (2h)	-	0	-
α-Terphenyl acetate	8.6 ± 1.61	2	P.a (4,24h)	-	0	-
β-Santalol	0.93 ± 0.76	2	S.a (24h), P.a (4h)	2.85 ± 2.56	10	P.a (4,24h), A.b (4,24h), K.p (2,4h), E.c (4,24h), S.a (4,24h)
β-Sesquiphellandrene	-	0	-	1.18	1	K.p (2h)

[1] The mean of pick area% ±SD of VOCs in resistant strains. [2] It shows the frequency of VOCs producer among five-studied resistant strain in three periods of time (the maximum is 15 and the minimum is zero). [3] Resistant strains: E. coli (E.c), S. aureus (S.a), P. aeruginosa (P.a), A. baumannii (A.b) and K. pneumoniae (K.p). The production of VOCs in each one of these strain investigated in three periods of time (2, 4 and 24 hours). [4] The mean of pick area% ±SD of VOCs in standard strain. [5] It shows the frequency of VOCs producer among five-studied standard strain in three periods of time (the maximum is 15 and the minimum is zero). [6] Standard strains: E. coli (E.c), S. aureus (S.a), P. aeruginosa (P.a), A. baumannii (A.b) and K. pneumoniae (K.p). The production of VOCs in each one of these strain investigated in three periods of time (2, 4 and 24 hours).

acetate. The VOCs released solely by standard *P. aeruginosa* were *2-acetyl-1-pyrroline*, *isopentyl acetate*, *myrcene* and *thiophene* (Table 2). Some compounds such as *2-ethenyl-6-methyl-pyrazine* and *heptadecane*

were produced in greater amounts by standard *P. aeruginosa* than by the other studied bacteria such as *A. baumannii* and *E. coli*.

The VOCs produced exclusively by resistant *A. baumannii* were *1-(phenylethyl)-pyrrol*, *3-(3,3-dimethylbutyl)-cyclohexanone*, *aromadendrene*, *benzaldehyde*, *decane*, *2,5-dimethyl pyrazine*, *dimethyl cyclo hexa-1,3 dine* and *dodecenol*. The VOCs produced exclusively by standard *A. baumannii* were *neryl acetate*, *2-acetyl-1-pyrroline*, *pentylethylpyrol* and *pentylhexyl benzene* (Table 2).

The VOCs produced exclusively by resistant *K. pneumoniae* were *1-decene*, *1-decyne*, *1-methyl-4-(1-methylethyl)-cyclohexanol*, *2-nonanone*, *2-tridecanone*, *2,5-dimethyl pyrazine*, *3-decen-1-ol*, *3-undecanone*, *3-undecene-2-one*, *menthone*, *p-cymene*, *trans-caryophyllene* and *α-methyl ionone*. The VOCs produced only by standard *K. pneumoniae* were *2,6-bis(1,1-dimethylethyl)-4-methyl-phenol*, *2-methyl-2-undecanethiol*, *5.5-dodecadinyl-1,12-diol*, *ocimene* and *β-sesquiphellandrene* (Table 2).

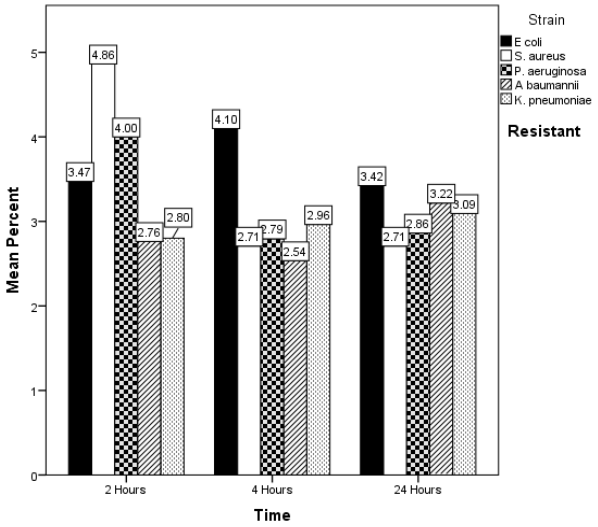

Figure 2. The comparison (Mann-Whitney test) among resistant strains of *E. coli*, *S. aureus*, *P. aeruginosa*, *A. baumannii* and *K. pneumoniae* in VOCs production at 2, 4, and 24 hours intervals. The Production of VOCs had no significant difference among these five resistant bacteria at three periods of time (P-value>0.05).

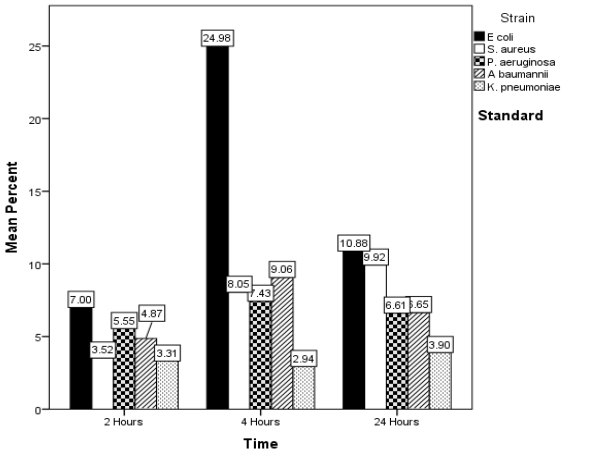

Figure 3. The comparison (Mann-Whitney test) among standard strains of *E. coli*, *S. aureus*, *P. aeruginosa*, *A. baumannii* and *K. pneumoniae* in VOCs production at 2, 4, and 24 hours intervals. The Production of VOCs had no significant difference among these five standard bacteria at three-time intervals (P-value>0.05).

Differences between standard and resistant strains of each species

Comparison of all volatile compounds produced by the resistant and standard strains of each species was done using the Mann-Whitney test. The results presented a significant difference ($p < 0.05$) between resistant and standard strains of *E. coli*, *P. aeruginosa*, *A. baumannii* and *K. pneumoniae* for VOC production. No significant difference ($p > 0.05$) occurred between resistant and standard strains of *S. aureus* (Table 3).

Total VOCs amounts produced by resistant or standard bacterial groups in different times

Comparison of the total VOCs production by two groups was done at three-time intervals (2, 4 and 24 h) using Mann-Whitney test (resistant strains are presented in Figure 2 and standard strains are presented in Figure 3). No significant difference ($p > 0.05$) existed in the total amount of released VOCs at three time periods by resistant (Figure 2) and standard bacteria (Figure 3).

Discussion

While different types of bacteria have different metabolisms (32) and various VOCs are produced by them (33-34), also they have some common VOCs due to the presence of some common biochemical cycles (21). Changes in metabolisms could alter VOCs and it may be possible that modification of susceptibility to antibiotics could result in some changes in bacterial metabolism (35) and VOC profile of bacteria. In order to discriminate between standard and resistant strains of some pathogenic bacteria, in the present study, the VOCs of *E. coli*, *S. aureus*, *P. aeruginosa*, *A. baumannii* and *K. pneumoniae* were studied.

Table 3. The comparison between resistant and standard bacteria in VOCs production.

	Groups				
	Resistant		Standard		P-value[3]
Bacteria	Mean ± SD[1]	N[2]	Mean ± SD[1]	N[2]	
E. coli	3.64 ± 5.28	77	10.96 ± 29.66	27	<0.001
S. aureus	3.18 ± 4.58	92	5.88 ± 10.29	50	0.128
P. aeruginosa	3.15 ± 4.83	92	6.43 ± 6.71	46	<0.001
A. baumannii	2.81 ± 3.84	104	6.45 ± 8.19	46	0.001
K. pneumoniae	2.95 ± 5.88	103	3.33 ± 4.27	87	0.003

[1] The mean of all VOCs produced in three time intervals ±SD. [2]All of identified compound types in 3 different culturing times. [3] P-value are based on Mann-Whitney Test.

Two criteria were chosen for discrimination between resistant and standard strains: uniqueness of produced VOCs or the abundance of some common compounds which have been produced in much higher amount in some strains. Also, comparison was made between the mean of total peak area percentage (for 2, 4 and 24 h cultures) of each resistant or standard strain using Mann-Whitney test. It was found that resistant strains of *E. coli*, *P. aeruginosa*, *A. baumannii* and *K. pneumoniae* produced significantly higher amount of VOCs than their corresponding standard strains. This difference was not significant between standard and resistant *S. aureus*. It is possible that by improving the extraction conditions of the VOCs or increasing culturing time, a significant difference between the standard and resistant strains of *S. aureus* could be obtained. In overall diversity of VOCs and average of VOC, the amounts were higher in the resistant group compared to the standard one. The VOCs produced exclusively by each strain are listed in the results section.

No significant difference existed in the total amount of VOCs production at different culturing time (2, 4 and 24 h) in the two studied groups of bacteria. It is most likely that all VOCs samples have been extracted and assessed after reaching logarithmic phase of growth. These results have been achieved *in vitro*, and *in vivo* study should be done using volunteers exhale samples for studying real bacterial infection VOCs.

According to the results of the current study, all of the assessed bacterial strains produced unique VOCs which could be applied as diagnostic markers. These results are in accordance with the other reported studies which mentioned the production of specific VOCs by bacterial species (18, 36-39).

Such data can be used to design an accurate diagnostic method. However there are some discrepancies in the reported studies regarding VOCs of even one bacterial species (10, 15, 40). Assessment of different bacterial strains of one species (23), application of different VOC extraction methods (30), and the use of different techniques for detection of the extracted VOCs can affect the quality and quantity of the identified VOCs (23). In this study, only two strains of 5 important human pathogenic bacteria were evaluated and it should be mentioned that for obtaining more accurate data, more strains must be assessed. Certainly, the examination of multiple species from prevalent pathogenic bacteria can provide the researchers with more precise information about the pattern of unique bacterial VOCs. On the other hand, evaluation and optimization of extraction methods of VOCs besides comparison of different analytical chemistry methods could be helpful in finding species specific biomarkers which could discriminate between standard and resistant bacteria.

To the best of our knowledge, this is the first study to compare the VOCs produced by resistant *A. baumannii* and *K. pneumoniae* with their standard strains. The study demonstrated that resistant strains of bacteria produced different VOCs than their standard strains.

Susceptible-resistant classification of bacteria by using VOC analysis could serve as a fast and non-invasive approach for the diagnosis of respiratory tract infections in humans. Moreover, the use of these biomarkers for the detection of pathogenic microorganism can be an excellent alternative to the present time consuming diagnostic methods.

Acknowledgments

This paper was extracted of Najmeh Karami Ph.D thesis that named: "Identification the volatile metabolic profiling of six respiratory pathogenic organisms". We would like to express our specific thanks to Shahid Beheshti University of Medical Sciences for the financial support. Also we appreciate all stuff of Pediatric Infections Research Center, Research Institute for Children Health, Shahid Beheshti University of Medical Sciences and Medicinal Plants and Drugs Research Institute, Shahid Beheshti University.

Interest conflict

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

1. Anderson DJ, Engemann JJ, Harrell LJ, Carmeli Y, Reller LB, Kaye KS. Predictors of mortality in patients with bloodstream infection due to ceftazidime-resistant Klebsiella pneumoniae. Antimicrobial agents and chemotherapy 2006; 50(5): 1715-1720.

2. Roberts RR, Hota B, Ahmad I et al. Hospital and societal costs of antimicrobial-resistant infections in a Chicago teaching hospital: implications for antibiotic stewardship. Clinical infectious diseases 2009; 49(8): 1175-1184.

3. McGowan JE. Resistance in nonfermenting gram-negative bacteria: multidrug resistance to the maximum. The American journal of medicine 2006; 119(6): S29-S36.

4. Magiorakos AP, Srinivasan A, Carey R et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical microbiology and infection 2012; 18(3): 268-281.

5. Boucher HW, Talbot GH, Bradley JS et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clinical infectious diseases 2009; 48(1): 1-12.

6. Cosgrove SE, Sakoulas G, Perencevich EN, Schwaber MJ, Karchmer AW, Carmeli Y. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible Staphylococcus aureus bacteremia: a meta-analysis. Clinical infectious diseases 2003; 36(1): 53-59.

7. Walker SS, Labroli M, Painter RE et al. Antibacterial small molecules targeting the conserved TOPRIM domain of DNA gyrase. PloS one 2017; 12(7): e0180965.

8. Pillar CM, Draghi DC, Sheehan DJ, Sahm DF. Prevalence of multidrug-resistant, methicillin-resistant Staphylococcus aureus in the United States: findings of the stratified analysis of the 2004 to 2005 LEADER Surveillance Programs. Diagnostic microbiology and infectious disease 2008; 60(2): 221-224.

9. Carey JR, Suslick KS, Hulkower KI et al. Rapid identification of bacteria with a disposable colorimetric sensing array. Journal of the American Chemical Society 2011; 133(19): 7571-7576.

10. Karami N, Mirzajani F, Rezadoost H et al. Initial study of three different pathogenic microorganisms by gas chromatography-mass spectrometry. F1000Research 2017; 6.

11. Barker M, Hengst M, Schmid J et al. Volatile organic compounds in the exhaled breath of young patients with cystic fibrosis.

Eur Respir J 2006; 27(5): 929 - 936.
12. Carroll W, Lenney W, Wang T, Španěl P, Alcock A, Smith D. Detection of volatile compounds emitted by Pseudomonas aeruginosa using selected ion flow tube mass spectrometry. Pediatric pulmonology 2005; 39(5): 452-456.
13. Dolch M, Frey L, Hornuss C et al. Molecular breath-gas analysis by online mass spectrometry in mechanically ventilated patients: a new software-based method of CO2-controlled alveolar gas monitoring. Journal of Breath Research 2008; 2(3): 037010.
14. Dolch M, Hornuss C, Klocke C et al. Volatile compound profiling for the identification of Gram-negative bacteria by ion-molecule reaction–mass spectrometry. Journal of applied microbiology 2012; 113(5): 1097-1105.
15. Karami N, Karimi A, Aliahmadi A et al. Identification of bacteria using volatile organic compounds. Cellular and Molecular Biology 2017; 63(2): 112-121.
16. Jünger M, Vautz W, Kuhns M et al. Ion mobility spectrometry for microbial volatile organic compounds: a new identification tool for human pathogenic bacteria. Applied microbiology and biotechnology 2012; 93(6): 2603-2614.
17. Bean HD, Zhu J, Hill JE. Characterizing Bacterial Volatiles using Secondary Electrospray Ionization Mass Spectrometry (SESI-MS). 2011; (52): e2664.
18. Allardyce RA, Langford VS, Hill AL, Murdoch DR. Detection of volatile metabolites produced by bacterial growth in blood culture media by selected ion flow tube mass spectrometry (SIFT-MS). Journal of Microbiological Methods 2006; 65(2): 361-365.
19. De Heer K, van der Schee MP, Zwinderman K et al. Electronic nose technology for detection of invasive pulmonary aspergillosis in prolonged chemotherapy-induced neutropenia: a proof-of-principle study. Journal of clinical microbiology 2013; 51(5): 1490-1495.
20. Nizio K, Perrault K, Troobnikoff A et al. In vitro volatile organic compound profiling using GC× GC-TOFMS to differentiate bacteria associated with lung infections: a proof-of-concept study. Journal of Breath Research 2016; 10(2): 026008.
21. Boots AW, Smolinska A, Berkel JJBNv et al. Identification of microorganisms based on headspace analysis of volatile organic compounds by gas chromatography–mass spectrometry. Journal of Breath Research 2014; 8(2): 027106.
22. Filipiak W, Sponring A, Baur MM et al. Molecular analysis of volatile metabolites released specifically by staphylococcus aureus and pseudomonas aeruginosa. BMC microbiology 2012; 12(1): 113.
23. Bos LD, Sterk PJ, Schultz MJ. Volatile metabolites of pathogens: a systematic review. PLoS pathogens 2013; 9(5): e1003311.
24. Bean HD, Dimandja J-MD, Hill JE. Bacterial volatile discovery using solid phase microextraction and comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry. Journal of Chromatography B 2012; 901: 41-46.
25. Julák J, Stránská E, Rosová V, Geppert H, Španěl P, Smith D. Bronchoalveolar lavage examined by solid phase microextraction, gas chromatography–mass spectrometry and selected ion flow tube mass spectrometry. Journal of Microbiological Methods 2006; 65(1): 76-86.
26. Maddula S, Blank LM, Schmid A, Baumbach JI. Detection of volatile metabolites of Escherichia coli by multi capillary column coupled ion mobility spectrometry. Anal Bioanal Chem 2009; 394(3): 791-800.
27. Tait E, Hill K, Perry J, Stanforth S, Dean J. Development of a novel method for detection of Clostridium difficile using HS-SPME-GC-MS. Journal of applied microbiology 2014; 116(4): 1010-1019.
28. Tait E, Perry JD, Stanforth SP, Dean JR. Identification of volatile organic compounds produced by bacteria using HS-SPME-GC–MS. Journal of chromatographic science 2014; 52(4): 363-373.
29. Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature protocols 2008; 3(2): 163-175.
30. Schulz S, Fuhlendorff J, Reichenbach H. Identification and synthesis of volatiles released by the myxobacterium Chondromyces crocatus. Tetrahedron 2004; 60(17): 3863-3872.
31. Filipiak W, Sponring A, Baur MM et al. Characterization of volatile metabolites taken up by or released from Streptococcus pneumoniae and Haemophilus influenzae by using GC-MS. Microbiology 2012; 158(Pt 12): 3044-3053.
32. Landete J. Plant and mammalian lignans: a review of source, intake, metabolism, intestinal bacteria and health. Food Research International 2012; 46(1): 410-424.
33. Thorn RMS, Reynolds DM, Greenman J. Multivariate analysis of bacterial volatile compound profiles for discrimination between selected species and strains< i> in vitro</i>. Journal of Microbiological Methods 2011; 84(2): 258-264.
34. Wilson AD, Baietto M. Advances in electronic-nose technologies developed for biomedical applications. Sensors 2011; 11(1): 1105-1176.
35. Tenover FC. Mechanisms of antimicrobial resistance in bacteria. The American journal of medicine 2006; 119(6): S3-S10.
36. Preti G, Thaler E, Hanson CW, 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. Journal of Chromatography B 2009; 877(22): 2011-2018.
37. Storer MK, Hibbard-Melles K, Davis B, Scotter J. Detection of volatile compounds produced by microbial growth in urine by selected ion flow tube mass spectrometry (SIFT-MS). Journal of Microbiological Methods 2011; 87(1): 111-113.
38. Kai M, Haustein M, Molina F, Petri A, Scholz B, Piechulla B. Bacterial volatiles and their action potential. Applied microbiology and biotechnology 2009; 81(6): 1001-1012.
39. Zhu J, Bean HD, Kuo Y-M, Hill JE. Fast detection of volatile organic compounds from bacterial cultures by secondary electrospray ionization-mass spectrometry. Journal of clinical microbiology 2010; 48(12): 4426-4431.
40. Shestivska V, Nemec A, Drevinek P, Sovova K, Dryahina K, Spanel P. Quantification of methyl thiocyanate in the headspace of Pseudomonas aeruginosa cultures and in the breath of cystic fibrosis patients by selected ion flow tube mass spectrometry. Rapid Commun Mass Spectrom 2011; 25(17): 2459 - 2467.