SUPPLEMENTARY MATERIALS

Detection of antimicrobial resistance-related changes in biochemical composition of *Staphylococcus aureus* by means of Atomic Force Microscopy – Infrared Spectroscopy

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Table S1. List of all *S. aureus* strains used in this study.*

Strain	Antibiotic MIC[a] (µg/ml)			Pair	Clinical syndrome	Class
	Met[b]	Van[c]	Dap[d]			
A5937	> 16	1 - 1.5	0.12	]	SAB[e], IE[f]	VSSA
A5940		2 – 4	0.25	]		VISA
A6224	> 16	2	0.25	]	SAB	VSSA
A6226		3	2	]		VISA
A6300	> 16	2	0.25	]	SAB, PJI[g]	VSSA
A6298		4	2	]		VISA
A8090	> 16	1	0.5	]	SAB, IE	VSSA
A8094		8	2.0	]		VISA
A8796	> 16	1	0.5	]	SAB, Vertebral OM[h]	DpS
A8799		2	2	]		DpR
A8819	> 16	1	0.25	]	SAB, OM	DpS
A8817		1	2	]		DpR
A9299	0.5	1 – 2	0.25	]	septic arthritis	DpS
A9305		1	2	]		DpR
A9719	> 16	1 - 2	0.25	]	SAB, IE	DpS
A9744		1 - 2	1 - 2	]		DpR
A9754	> 16	2	0.5	]	SAB, IE	DpS
A9757		2	4	]		DpR
A9763	> 16	1	0.5	]	SAB, IE	DpS
A9764		2	4	]		DpR
A9765	> 16	1	0.5	]	SAB, OM, PJI	DpS
A9766		2	2	]		DpR
A9781	> 16	1	0.5	]	SAB, OM	DpS
A9794	> 16		0.5	]		DpS
A9792			2	]		DpR
A9798		2	2	]		DpR
A10102	> 16	1	0.5	]	SAB	DpS
A10103		1	2	]		DpR

[a] MIC, Minimum Inhibitory Concentration;

[b] Met, Methicillin;

[c] Van, Vancomycin;

[d] Dap, Daptomycin;

[e] SAB, *S. aureus* Bacteraemia;

[f] IE, Infective Endocarditis;

[g] PJI, Prosthetic Joint Infection;

[h] OM, Osteomyelitis

* The list of strains contain all strains used in the performed experiments (AFM-IR and ATR-FTIR). All of these strains are clinical pairs, as indicated in column 5 by brackets joining each clinical pair. Each clinical pair is obtained from the same patient prior to and after antibiotic therapy. Each clinical pair originates from different patient. All strains were obtained from the same source – Alfred Hospital.

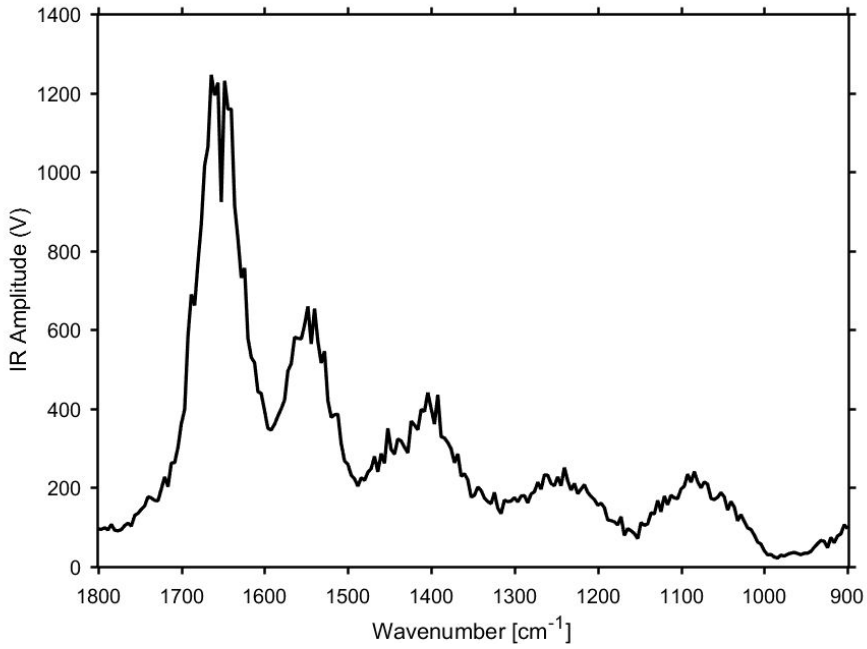

Figure S1. Example of a raw AFM-IR spectrum for VISA. No processing was applied to the spectrum.

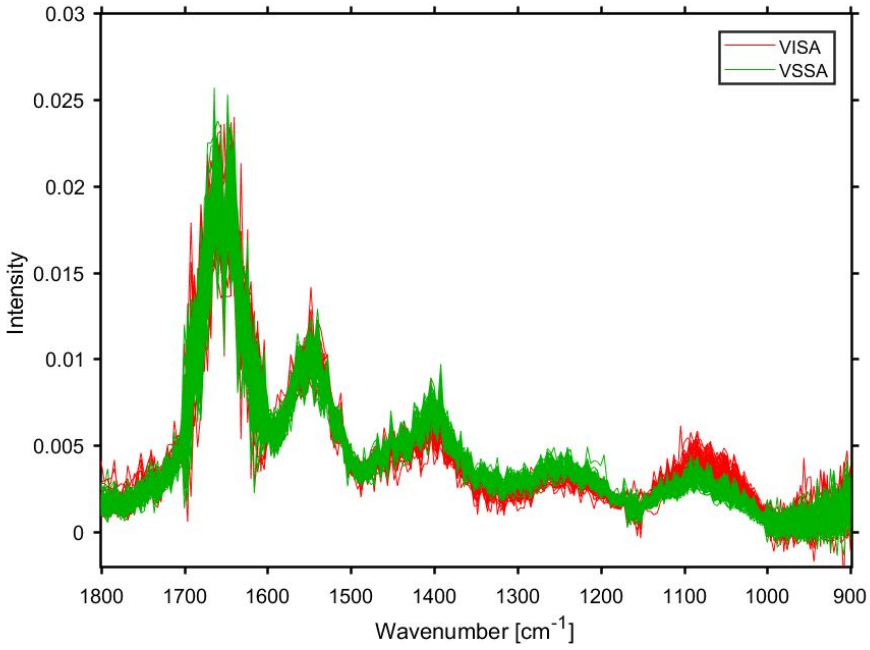

Figure S2. All collected AFM-IR spectra (VSSA/VISA dataset) after SNV normalization (no smoothing applied). For comparison, the average spectra for VISA and VSSA after smoothing are presented in the manuscript in Figure 2 c and d.

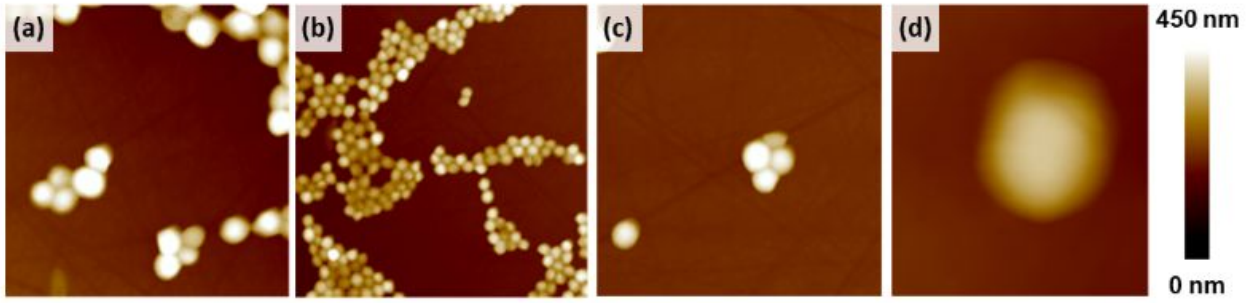

Figure S3. AFM heights images collected prior to AFM-IR data collection from various replicates of VSSA parent strain. Size of the images areas: (**a**), 8.46 × 4.66 µm, (**b**) 20 × 20 µm, (**c**) 8.54 × 4.38 µm and (**d**) 2.48 × 2.19 µm.

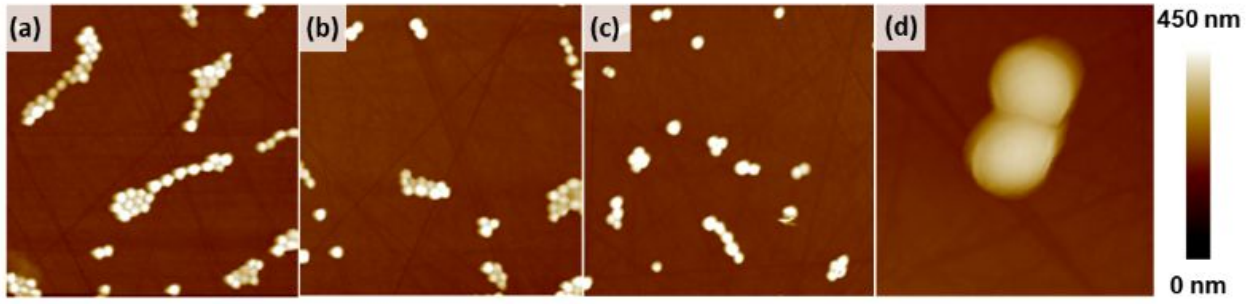

Figure S4. AFM heights images collected prior to AFM-IR data collection from various replicates of VISA daughter strain. Size of the images areas: (**a**) 18.9 × 18.3 µm, (**b**) 20 × 20 µm, (**c**) 20 × 20 µm and (**d**) 5.59 × 5.29 µm.

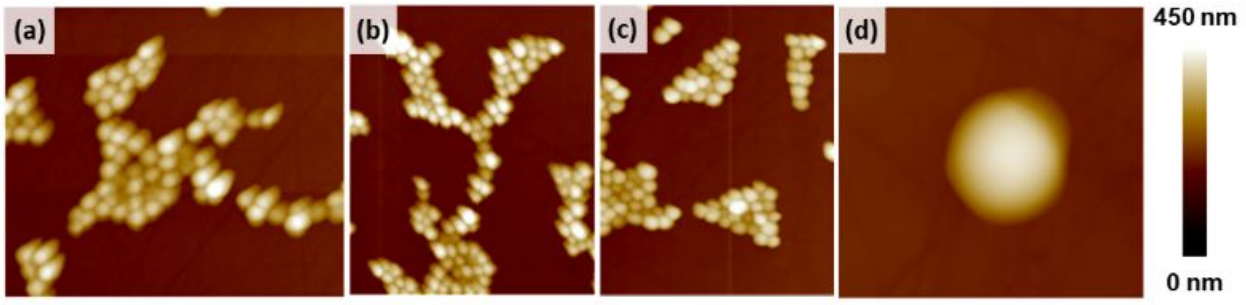

Figure S5. AFM heights images collected prior to AFM-IR data collection from various replicates of DpS parent strain. Size of the images areas: (**a**) 20 × 20 µm, (**b**) 19.1 × 16.55 µm, (**c**) 16.66 × 13.57 µm and (**d**) 3.21 × 2.99 µm.

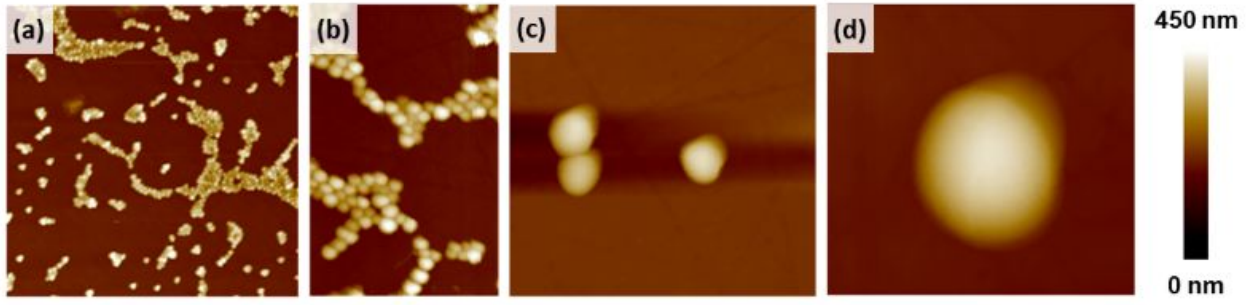

Figure S6. AFM heights images collected prior to AFM-IR data collection from various replicates of DpR daughter strain. Size of the images areas: (**a**) 50 × 50 µm, (**b**)12.99 × 20 µm, (**c**) 5.59 × 5.29 µm and (**d**) 1.92 × 1.77 µm.

Table S2. Assignment of vibrational modes for bands observed in AFM-IR and ATR-FTIR spectra.

Band position [cm^{-1}]	Assignment	Compound
928	ν(CO), ν(CC),	carbohydrates
1025	ν(CO), ν(CC) and δ(COH)	carbohydrates
1082/1084/1088	$\nu_s(PO_2^-)$, ν(CO), ν(CC), δ(COH)	Phosphodiester containing compounds: phospholipids, DNA, carbohydrates
1118	ν(CO),	carbohydrates
1152	ν(CC), ν(CO), δ(COH)	carbohydrates
1228/1238	$\nu_{as}(PO_2^-)$, amide III (δ(N-H) coupled with ν(C-N)), & C-O-C & C-O ring vibrations	Phosphodiester containing compounds: phospholipids, DNA, carbohydrates
1398	δ_s (CH_3)	mainly protein, lipids, carbohydrates
1494	in plane δ(C-H)	protein, lipids, carbohydrates
1547/1548	Amide II (δ(N-H) coupled with ν(C-N))	proteins
1640	Amide I δ (H-O-H)	proteins, water
1656	Amide I (α-helix structure)	proteins
1688	Amide I (antiparallel β-sheet)	proteins,
1728 – 1740	ν (C = O)	lipids, phospholipids carbohydrates

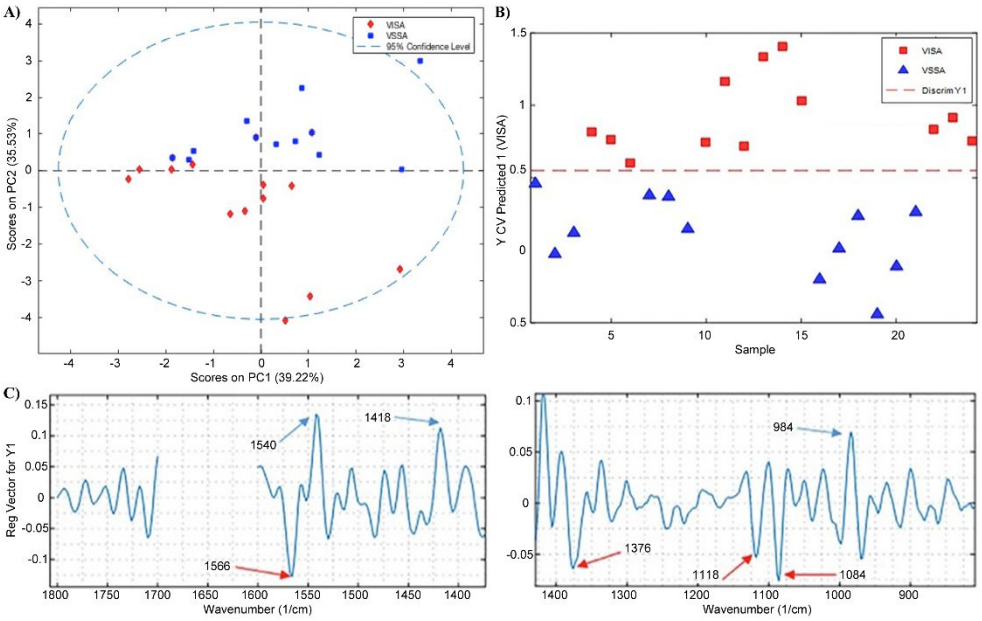

Figure S7. (**A**) PCA of the ATR-FTIR dataset showed discrimination between VSSA and VISA strains with 39.22% variance attributed to PC1 and 35.53% variance attributed to PC2. (**B**) PLSDA showed greater discrimination between VSSA and VISA. The dotted line represents the threshold of discrimination. (**C**) A PLS regression vector captured the bands responsible for the discrimination between VSSA and VISA. Maxima bands represent changes in VISA strains compared with VSSA, whereas minima bands represent changes in VSSA compared with VISA. Raw ATR-FTIR data (prior to any pre-processing) used for the presented PCA and PLS-DA is shown in Figure S7.

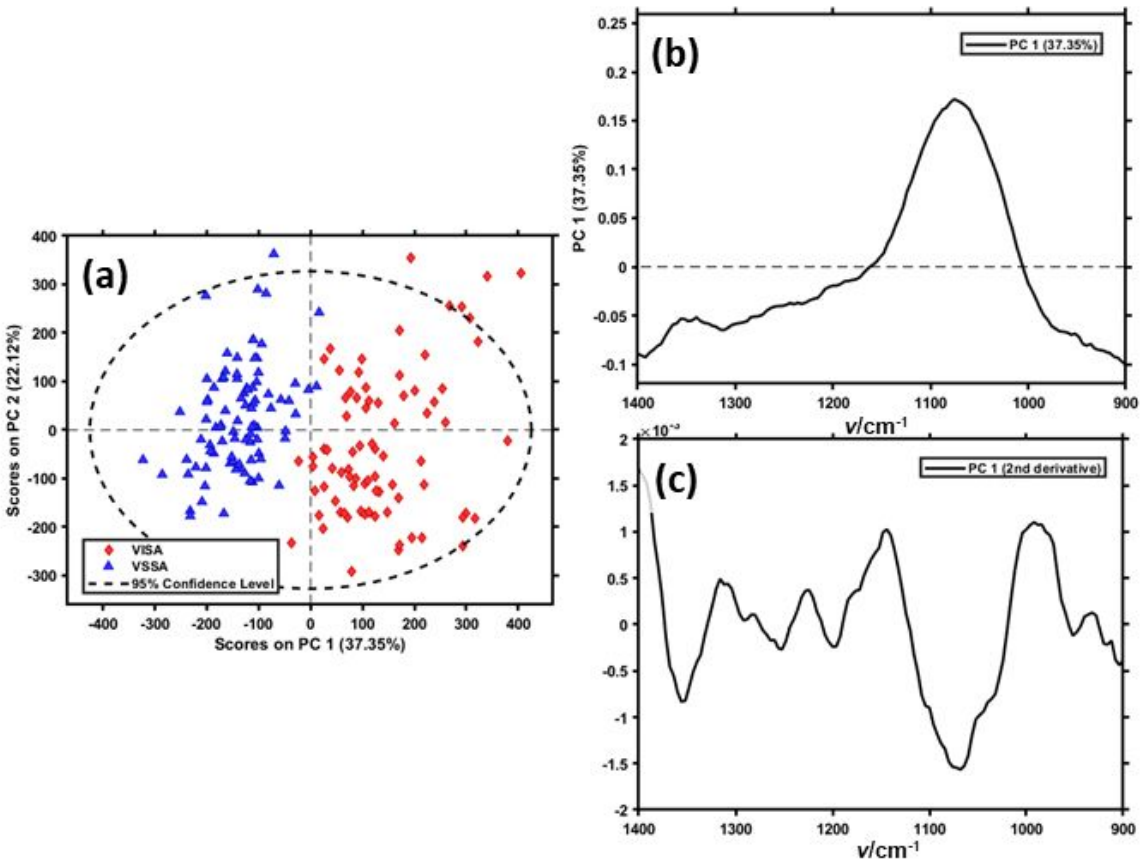

Figure S8. Results of PCA analysis of AFM-IR spectra of Vancomycin Intermediate *S. aureus* (VISA) and Vancomycin Susceptible *S. aureus* (VSSA): (**a**) scores plot showing the grouping between VISA and VSSA occurring along PC1, which explains 37.35 % of variance together with corresponding to PC1 (**b**) loading plot and (**c**) its 2nd derivative. Spectral features identified by PCA as characteristic for VISA (positive values of PC1 in (a)) as shown as positive values of the loading plot in (b) and negative values in its 2nd derivative (shown in (c)). PCA was performed on pre-processed data, using the standard normal variate, 2nd derivatives and mean centring as pre-processing. Raw data, prior to pre-processing are presented in Figure S10.

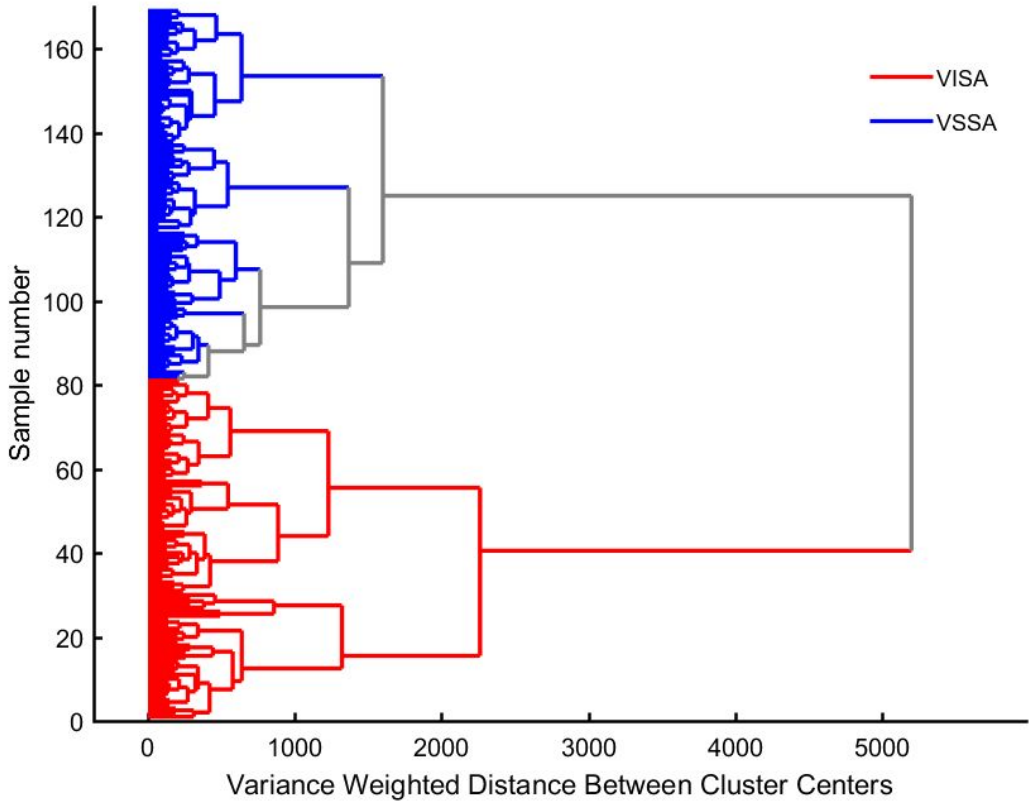

Figure S9. The result of HCA analysis performed on the VSSA (blue) and VISA (red) dataset after SNV normalization, but without Savitzky-Golay smoothing. The discrimination between groups is clearly visible, as in the case of HCA of dataset after Savitzky-Golay smoothing (presented in the main text, Fig. 2g).

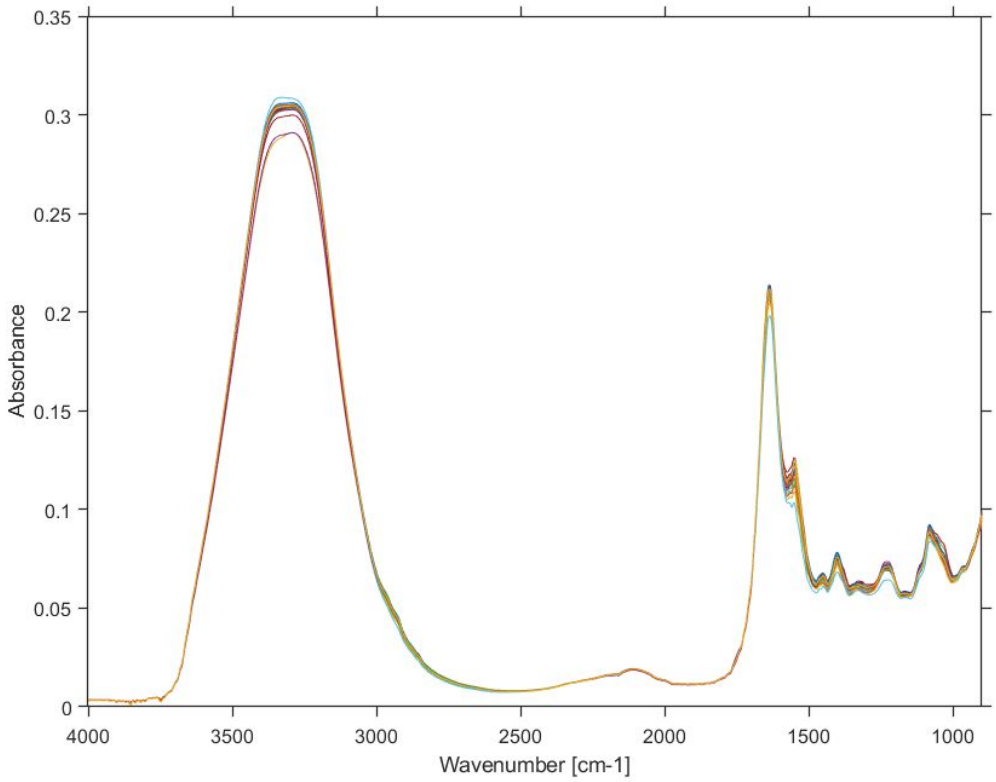

Figure S10. Raw ATR-FTIR data used for the PCA and PLS-DA analysis shown in Figure S8. Spectra are shown prior to implementation of any pre-processing procedures (except the ATR correction) used before PCA and PLS-DA.