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Supplemental information

The ratio of nicotinic acid to nicotinamide as a microbial biomarker for assessing cell therapy product sterility

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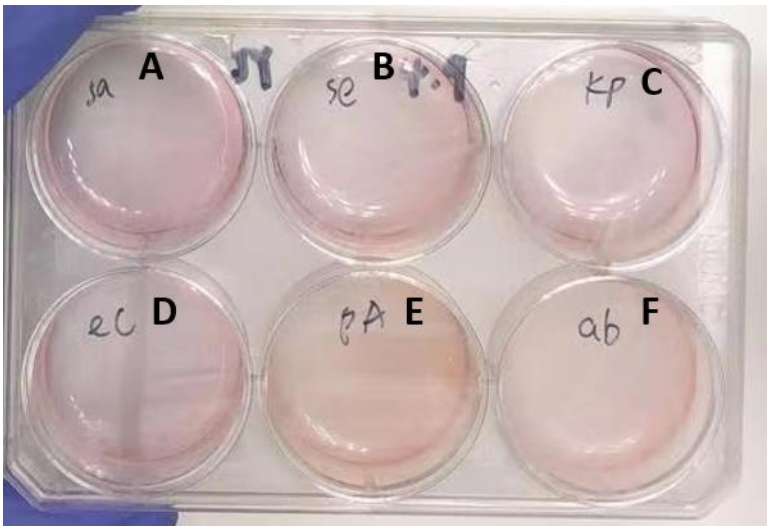

Figure S1. Medium colour changes after MSCs have been contaminated with 1×10^4 CFU/mL of (A) *S. aureus,* (B) *S. epidermidis*, (C) *K. pneumoniae* , (D) *E. coli,* (E) *P. aeruginosa,* and (F) *A. baumannii* for 24 h.

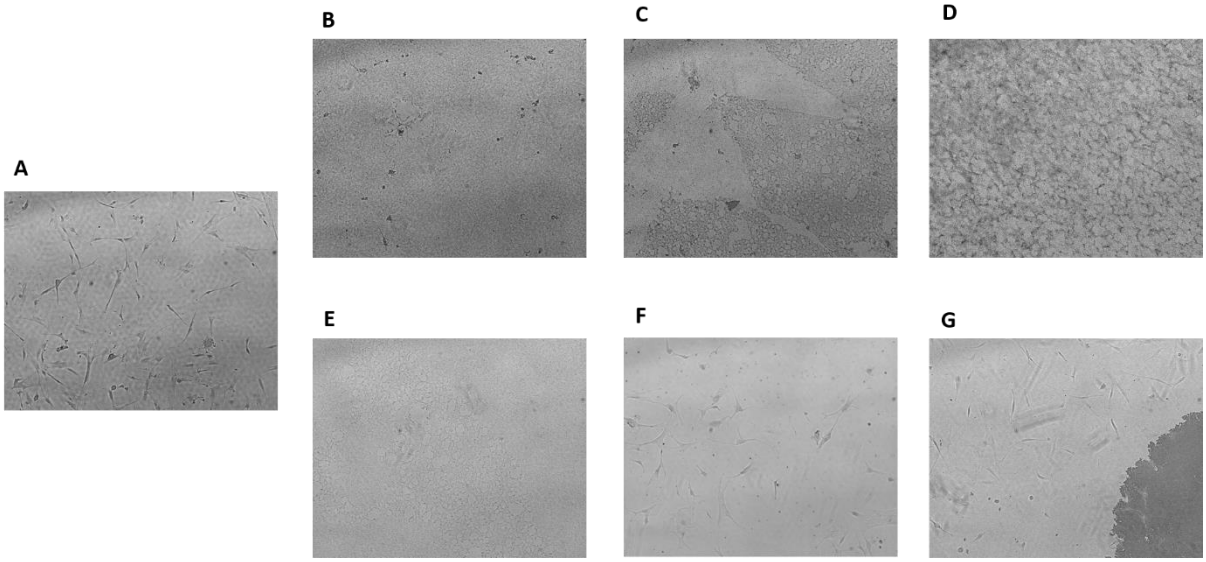

Figure S2. MSCs contaminated with six different bacteria for 24 h. 1×10^5 MSCs were contaminated with (A) no bacteria, (B) *A. baumannii,* (C) *E. coli,* (D) *K. pneumoniae,* (E) *P. aeruginosa,* (F) *S. aureus* and (G) *S. epidermidis.* The numbers of bacteria used for the contamination study was approximately 1×10^4 CFU/mL. Magnification was 4 X in all panels.

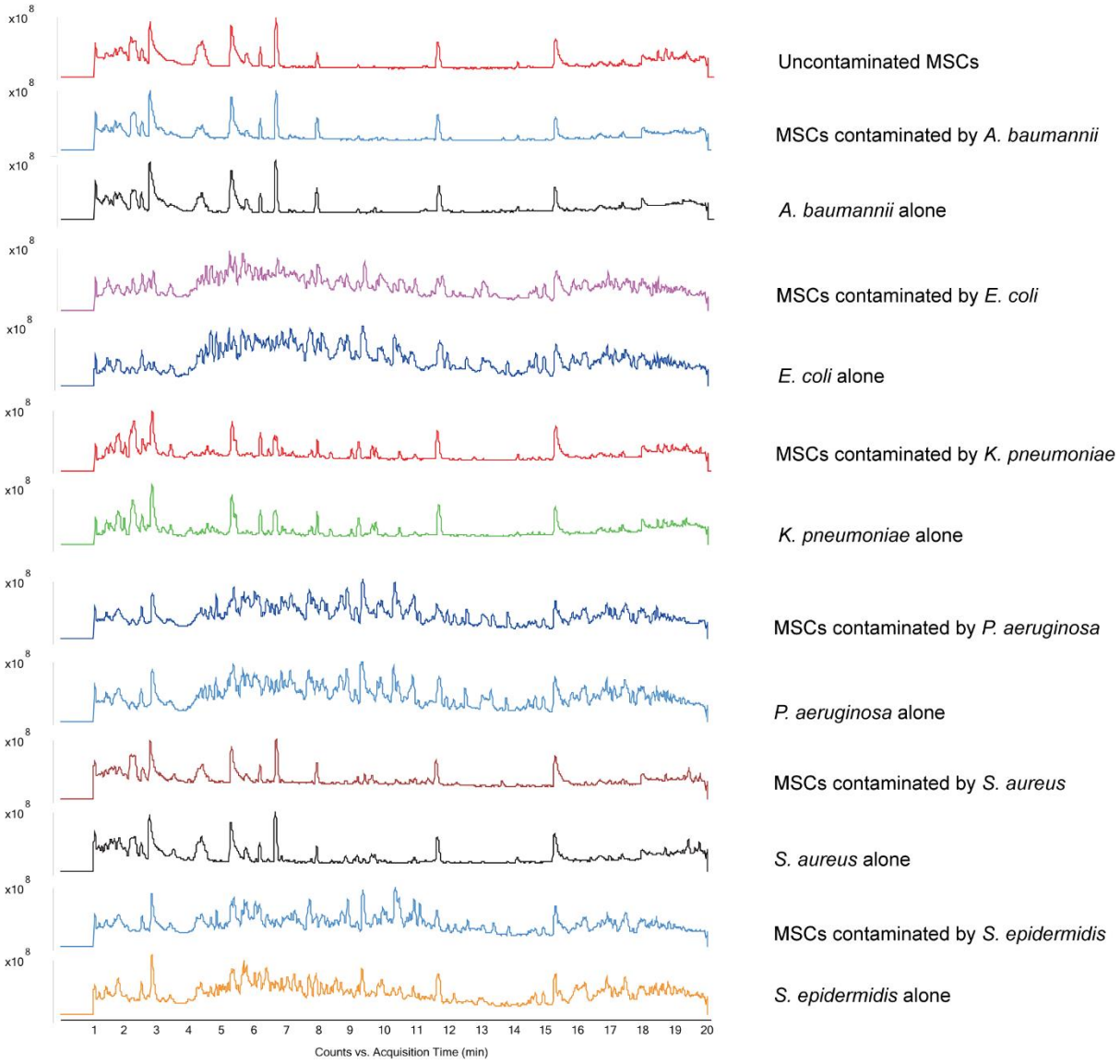

Figure S3. Exometabolomics chromatograms of contaminated and uncontaminated MSCs samples and cell medium contaminated with each bacterium alone.

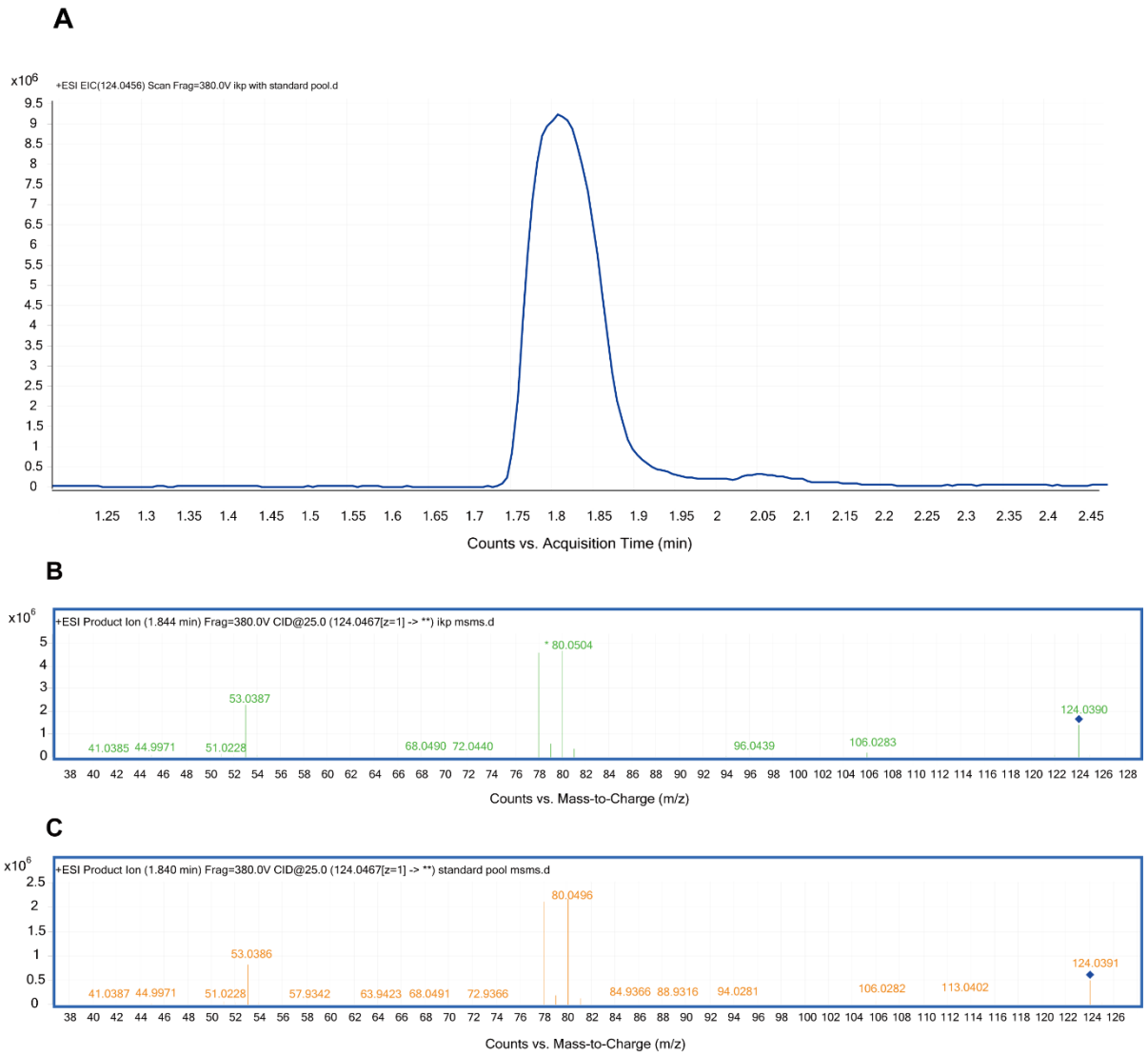

Figure S4. Nicotinic acid identification. A. Co-elution of nicotinic acid standard compound and representative contaminated MSC samples. B. MS/MS spectrum of metabolite *m/z* 123.0366 in representative contaminated MSC sample. C. MS/MS spectrum standard compound nicotinic acid. MS/MS fragment masses for both compounds are the same (*m/z* 80.0496, *m/z* 78.0833, *m/z* 53.0386).

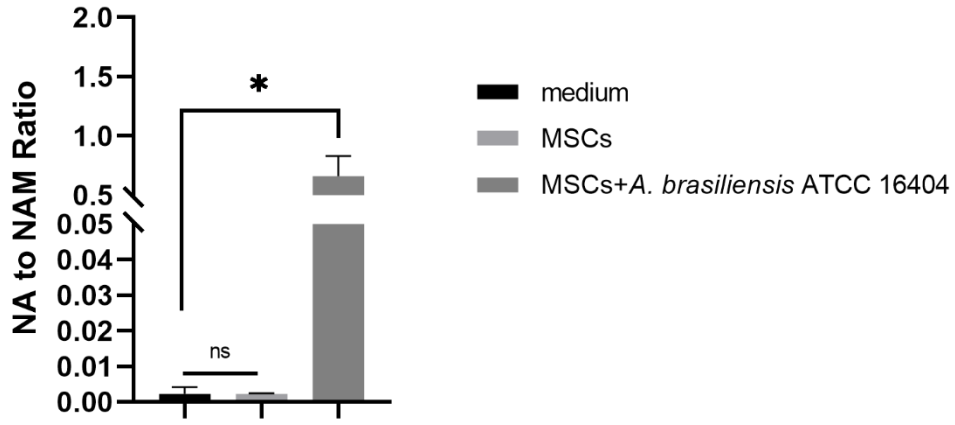

Figure S5. NA to NAM ratio for uncontaminated and contaminated MSC culture medium by *A. brasiliensis***.** With an inoculation condition of 10 CFU/mL for 72 h, the ratio of NA to NAM in contaminated MSC culture medium was 200 times higher than the uncontaminated MSC culture medium. *$p < 0.05$ compared to uncontaminated MSCs group using t-test.

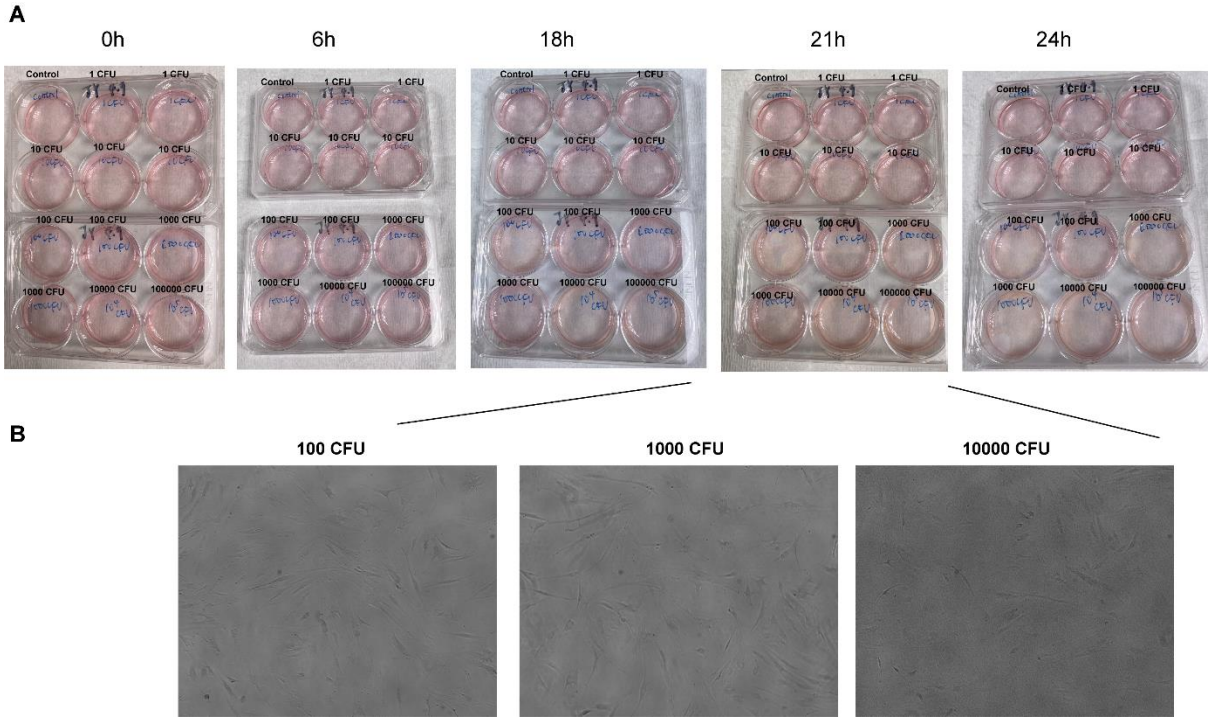

Figure S6. (A) Medium changes after MSCs have been contaminated with different numbers of *E. coli* at 0 h, 6 h, 18 h, 21 h and 24 h. (B) Cell morphology observation after 21 h incubation. Magnification was 4 X in all panels.

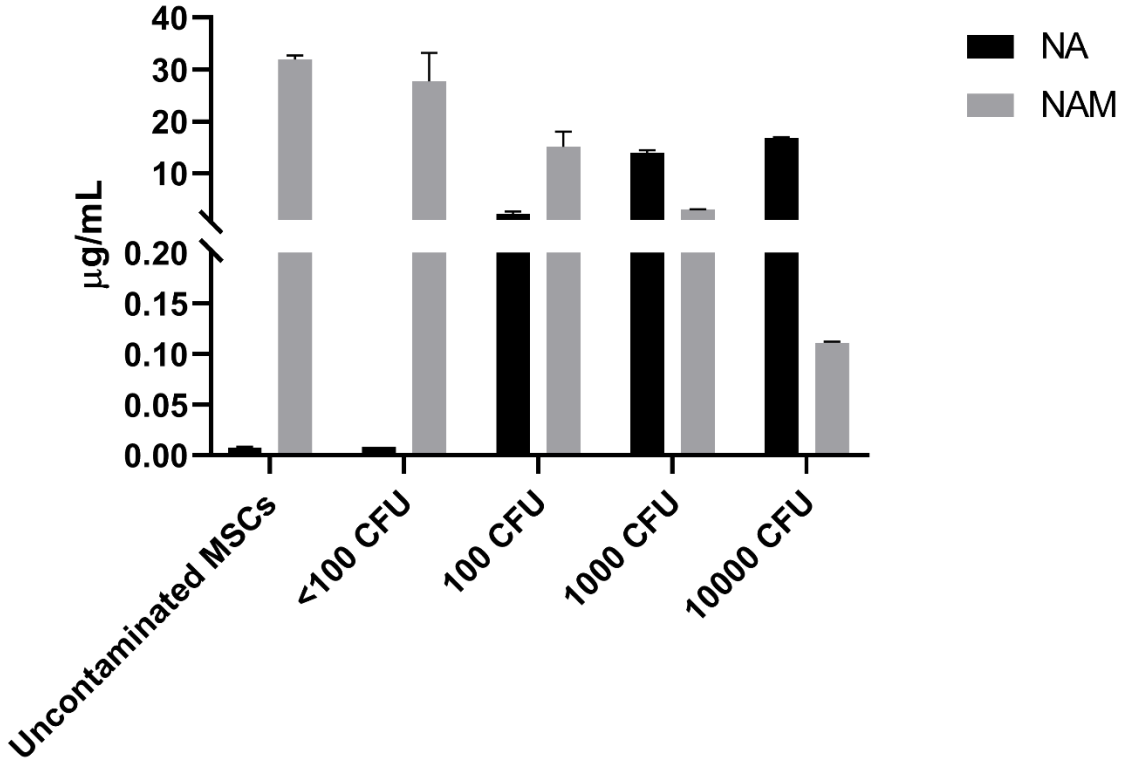

Figure S7. Absolute quantification of NA and NAM amount in uncontaminated and *E. coli*-contaminated MSCs. In uncontaminated MSCs or MSCs contaminated by <100 CFU/mL of *E. coli* for 24 h, the amount of NA was lower than 0.0002 µg/mL. After the cells were contaminated with 100, 1000 and 10000 CFU/mL of *E. coli*, the amount of NA increased to a highest amount of 16.60 µg/mL while NAM decreased from 31.93 in uncontaminated MSCs to a lowest amount of 0.12 µg/mL.

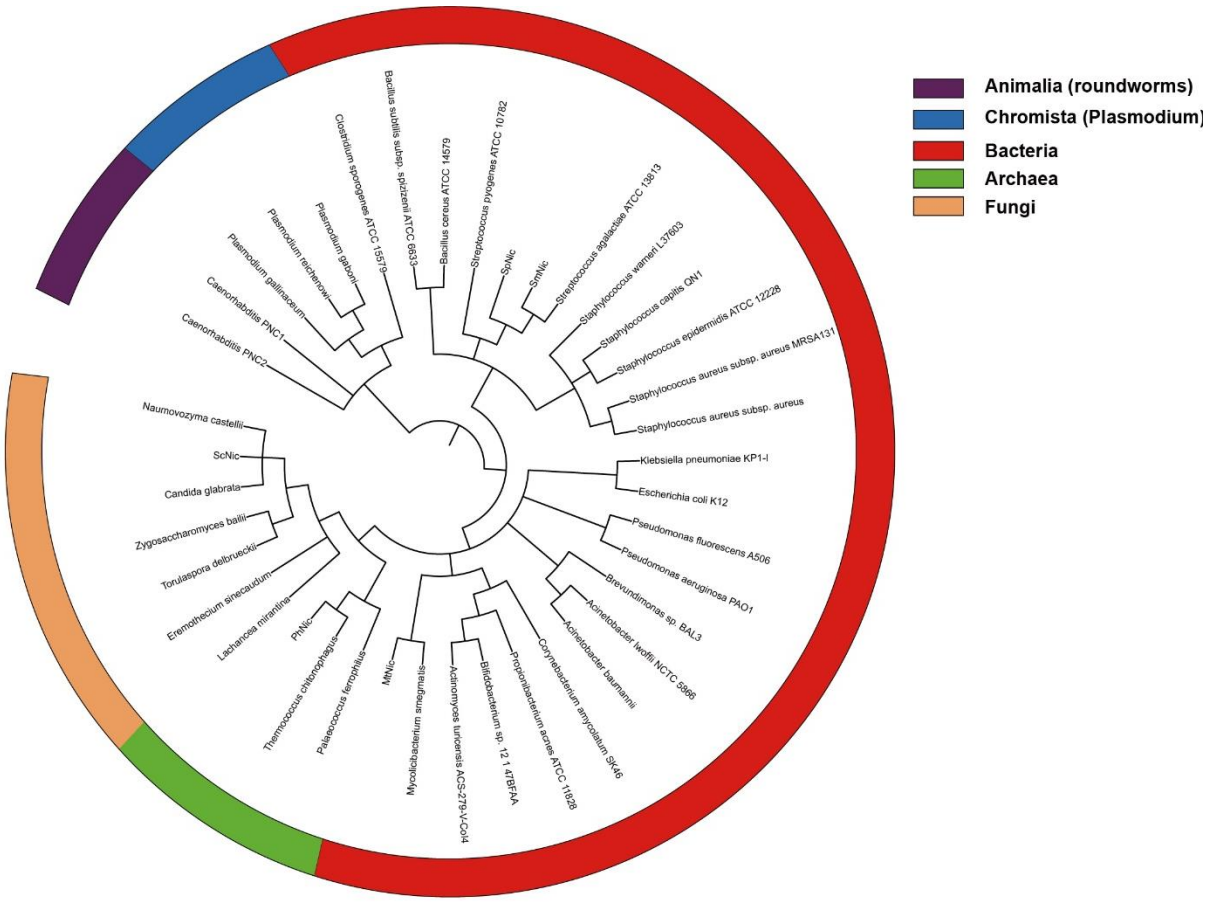

Figure S8. Phylogenetic analysis of nicotinamidases distributed in different kingdoms.

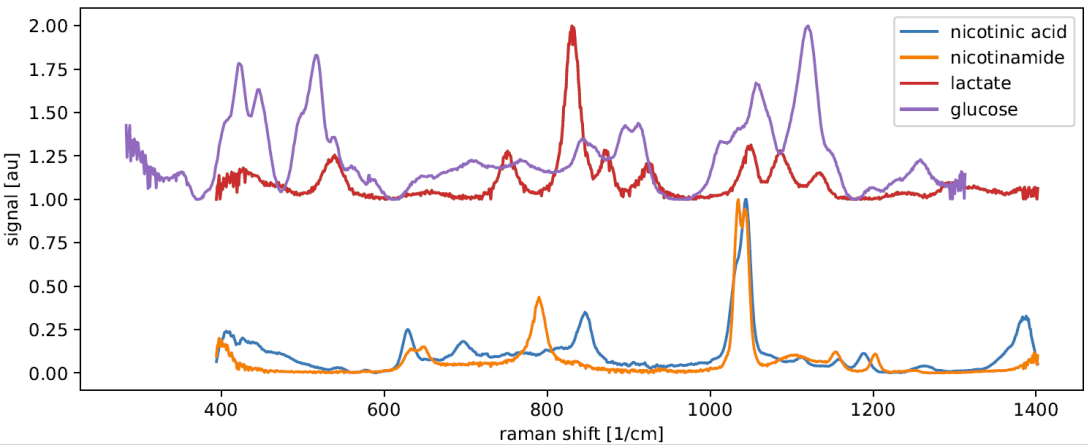

Figure S9. Raman spectra of nicotinic acid, nicotinamide, lactate and glucose with a concentration of 100 mM.

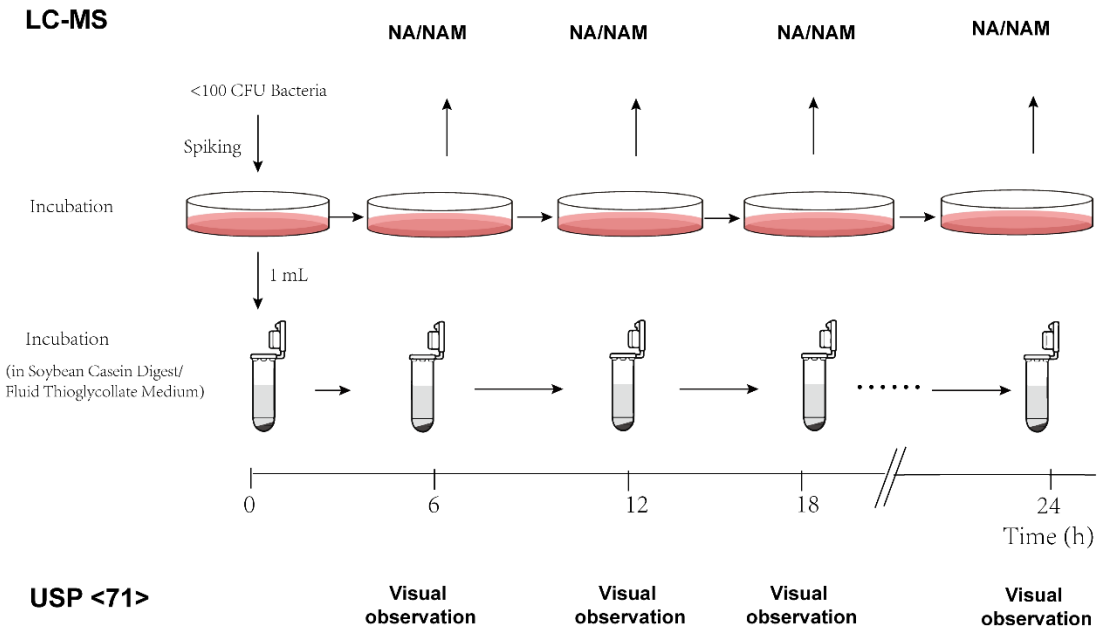

Figure S10. Experimental flow of the comparison study of USP <71> sterility test and NA/NAM ratio detection method.

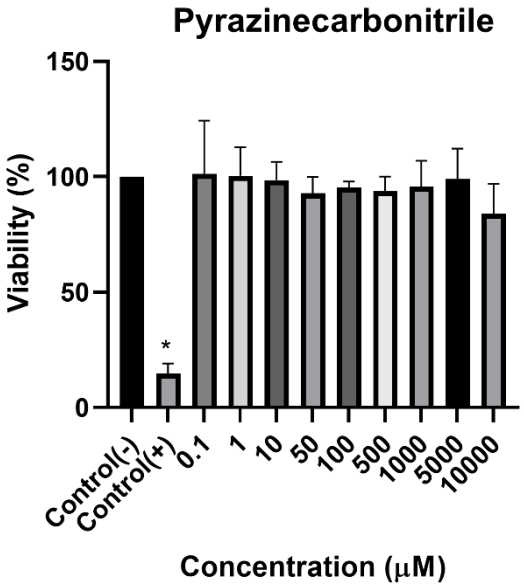

Figure S11. Cytotoxicity assay of pyrazinecarbonitrile using MTT. Cell viability in MSCs after incubating with different concentrations of pyrazinecarbonitrile (0.1- 10000 μM) were measured. 0.1% Triton X-100 and PBS were used as positive (+) and negative (-) controls, respectively. * p<0.05.

Table S1. List of potential compounds that are found in five out of the six bacterial cultures

Compound (m/z) @ Retention Time (min)	*A. baumannii*	*S. epidermidis*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*
244.0765@1.6811303						
72.061@2.764208						
143.9923@2.765261						
223.0908@7.1428566						
119.0783@9.38						
188.0507@9.68885						
1381.2642@13.5265						
1380.7632@13.534416						
169.0904@4.4611306						
230.1693@1.4843045						
236.1232@6.0229588						
230.1698@7.2533593						
254.0969@1.4002401						
155.1359@5.602751						
120.0367@1.79728						
95.0408@1.8315998						
347.0718@1.8693602						
274.1351@2.31796						
541.071@3.4601786						
576.9808@3.4596999						
523.061@3.459862						
717.0418@3.4603913						
72.0609@3.4681602						
247.0762@3.9413202						
300.996@3.9413204						
612.1671@4.05836						
153.0834@4.145875						
271.1247@4.774696						
312.0972@4.8290434						
275.1086@5.4727197						
478.1901@5.771307						
434.1363@6.3728266						
126.0363@6.9101605						
446.1728@8.01948						
265.1021@9.9045						
214.1385@9.9626						
196.1275@9.963601						
154.0684@10.94016						
161.048@1.445739						

*The yellow highlight means that the compound is present in the corresponding bacterial culture.

Table S2. List of potential compounds that are found in four out of the six bacterial cultures

Compound (m/z) @ Retention Time (min)	*A. baumannii*	*S. epidermidis*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*
203.1333@3.128478						
54.0497@3.4657829						
296.1068@4.991041						
429.1376@9.892653						
582.2585@15.691308						
1712.8005@13.702173						
650.006@12.755184						
657.3338@12.756331						
649.6718@12.760821						
287.2033@2.2533479						

*The blue highlight means that the compound is present in the corresponding bacterial culture.

Table S3. The calibration curve parameters, LOD, LOQ of two internal standard compounds

	Linear range (µg/mL)	Correlation coefficient (r^2)	Slope	y-intercept	LOD (ng/mL)	LOQ (ng/mL)	Recovery (%)
NA-$^{13}C_6$	0.0001-0.5	0.9998	4×10^{-7}	87596	0.93	3.11	107.5
NAM-$^{13}C_6$	0.005-0.75	0.9943	3×10^{-7}	32587	5.89	19.65	102.5

*LOD: Limit-Of-Detection; LOQ: Limit-Of-Quantification

Table S4. Validation of the intra- and inter-day accuracies of two standard compounds at low, medium, and high concentrations.

Compounds	**Spiked concentration (ng/mL)**	**Intra-day (n=6)**			**Inter-day (n=18)**		
		Observed concentration (ng/mL)[a]	**Precision RSD (%)[b]**	**Accuracy (%)[c]**	**Observed concentration (ng/mL)[a]**	**Precision RSD (%)[b]**	**Accuracy (%)[c]**
Nicotinic acid-$^{13}C_6$	9	9.001 ± 0.050	0.553	0.007	9.259 ± 0.268	2.890	2.886
	60	60.433 ± 0.237	0.392	0.721	63.746 ± 2.703	4.241	6.244
	400	406.577 ± 1.641	0.403	1.644	418.0094 ± 12.298	2.942	4.502
Nicotinamide-$^{13}C_6$	6	6.003 ± 0.028	0.465	0.061	6.000 ± 0.060	1.000	0.001
	60	60.728 ± 0.453	0.747	1.213	61.264 ± 1.222	1.994	2.108
	600	596.854 ± 3.896	0.652	-0.524	591.102 ± 5.997	1.014	-1.483

[a] Mean ± Standard Deviation (SD). [b] Relative Standard Deviation (RSD) % = (SD/mean) × 100. [c] [(mean observed concentration – spiked concentration)/spiked concentration] × 100.