Supplementary Information

MALDI-TOF-MS based identification and molecular characterization of food associated methicillin-resistant *Staphylococcus aureus*

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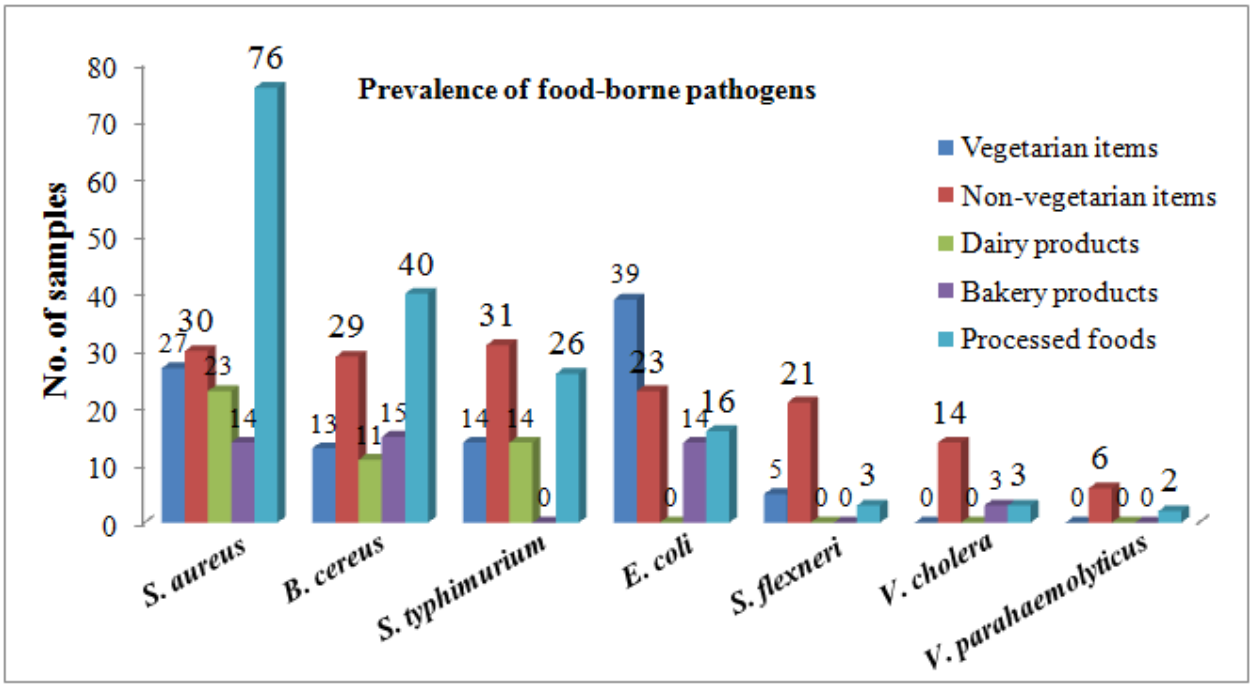
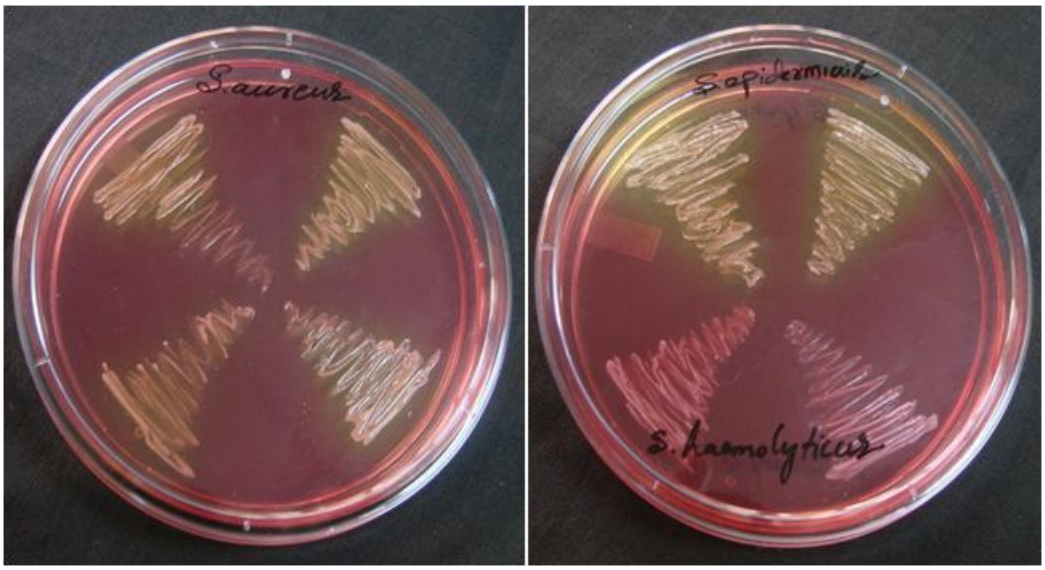
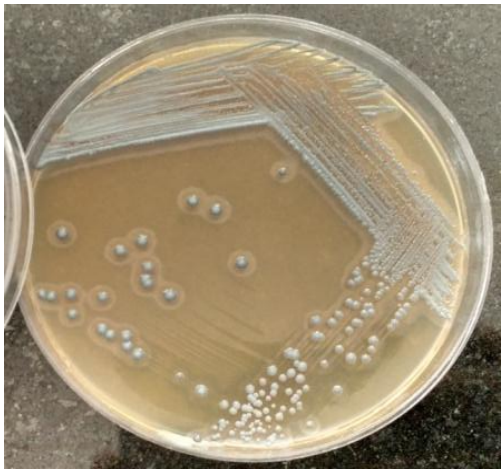


Supplementary Fig S1. Different food samples and associated food-borne pathogens. Varieties food samples collected from different regions of Mysuru, India and isolated diverse pathogens from food samples associated.



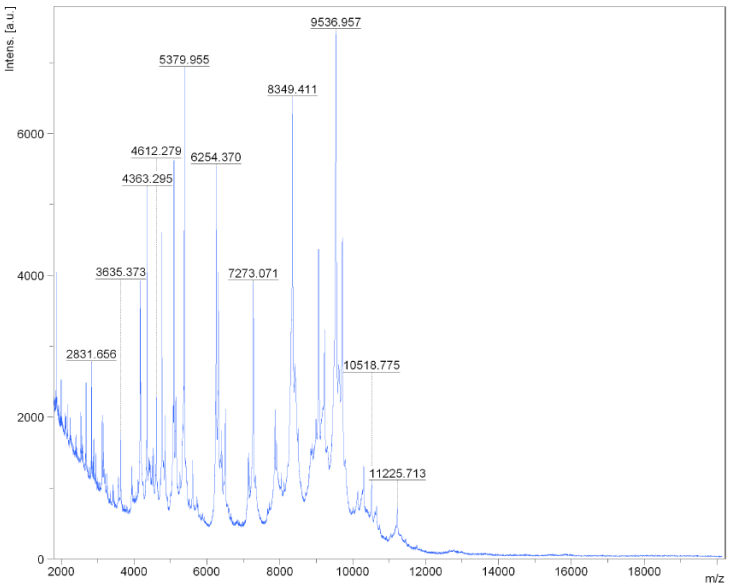
A.



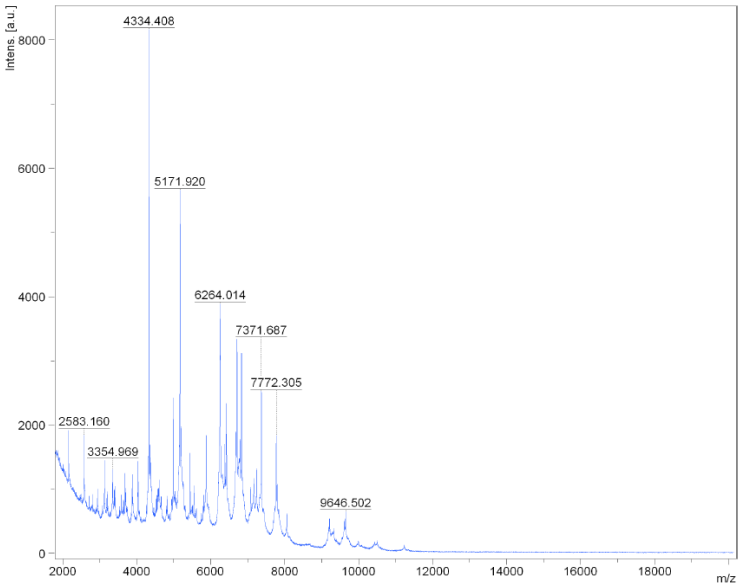
B.

Supplementary Fig S2. Food-borne *Staphylococcal* spp. on selective medium. **A.** This figure indicating the *S. aureus, S. epidermidis* and *S. haemolyticus* pathogens on selective Mannitol Salt Phenol-red Agar medium. **B.** Indicating the *S. aureus* on Baired Parker Agar medium

46 ***E. coli***

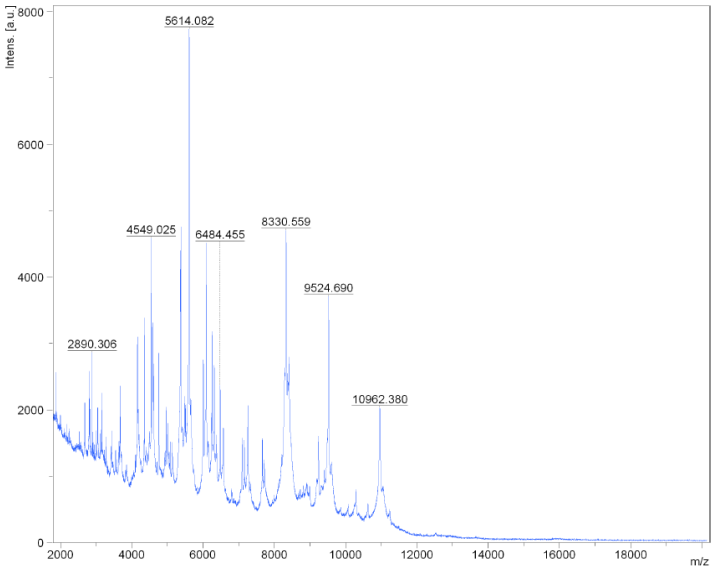


B. cereus

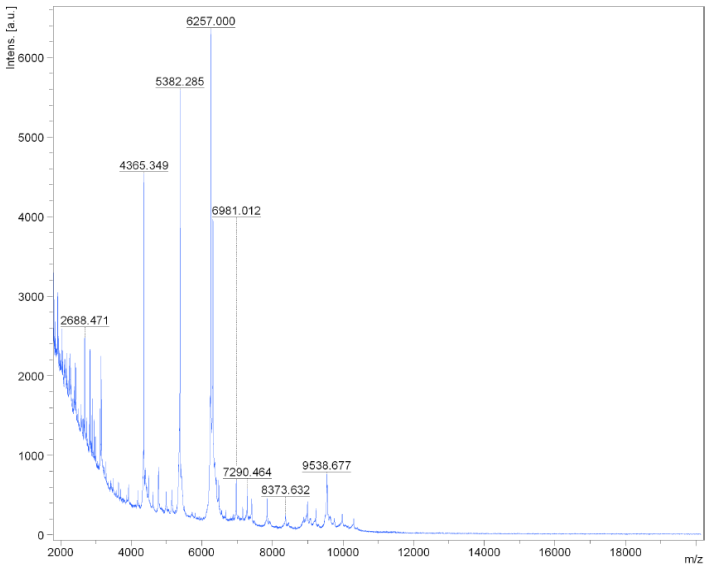


47

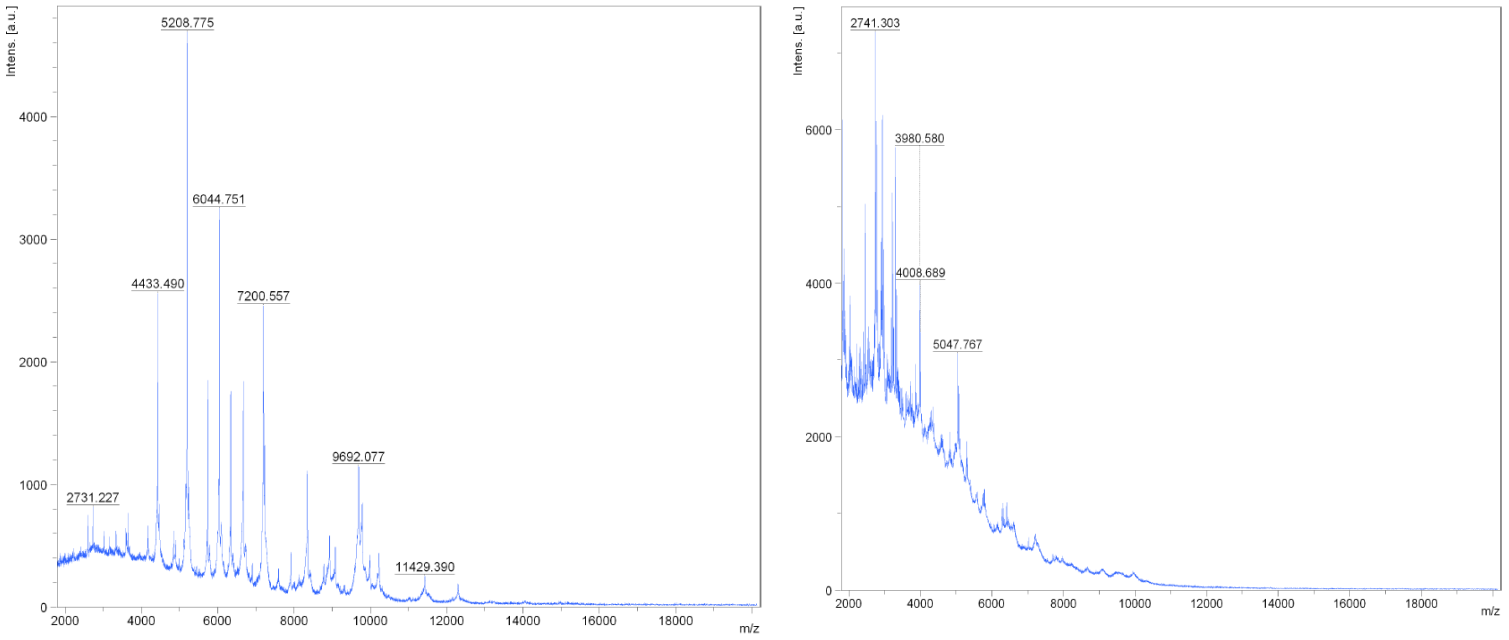
48 ***S. typhimurium***



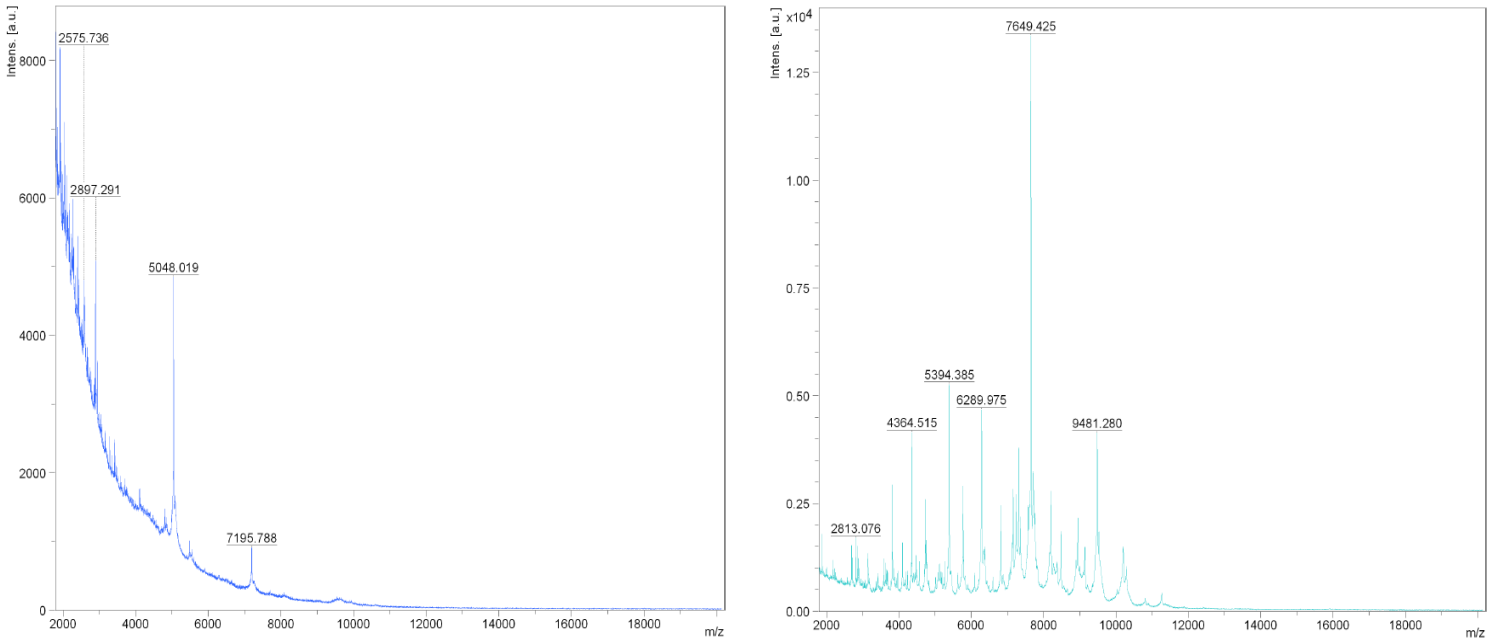
S. flexneri



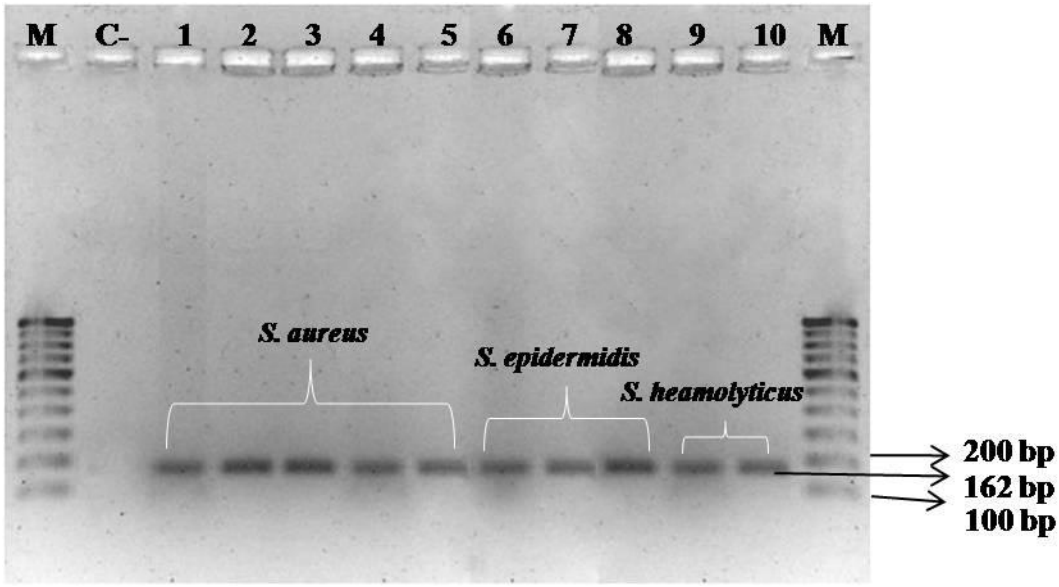
P. aeruginosa ***V. cholera***



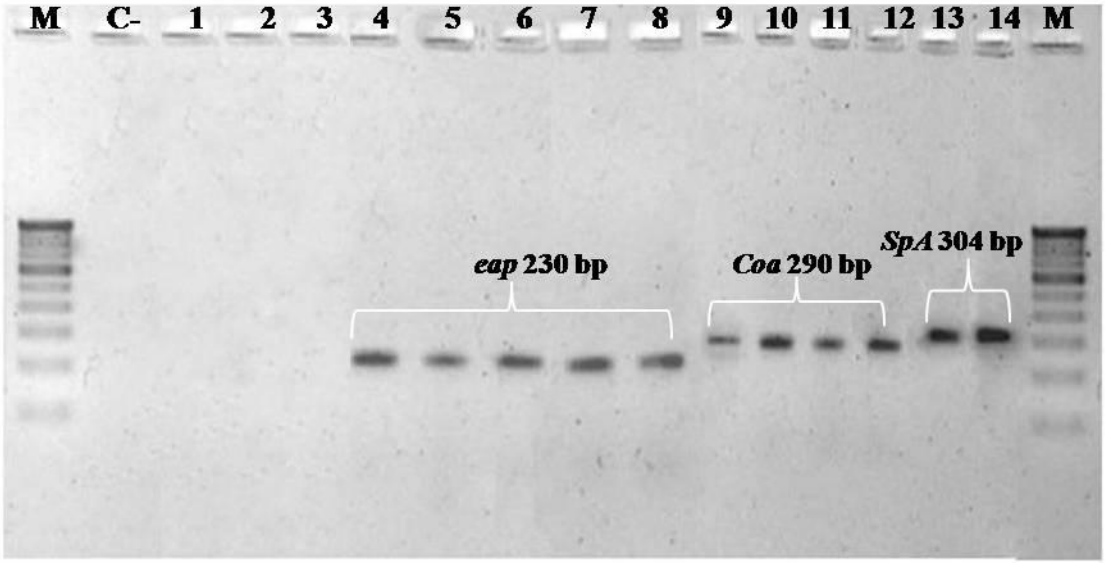
V. parahaemolyticus ***E. aerogenes***



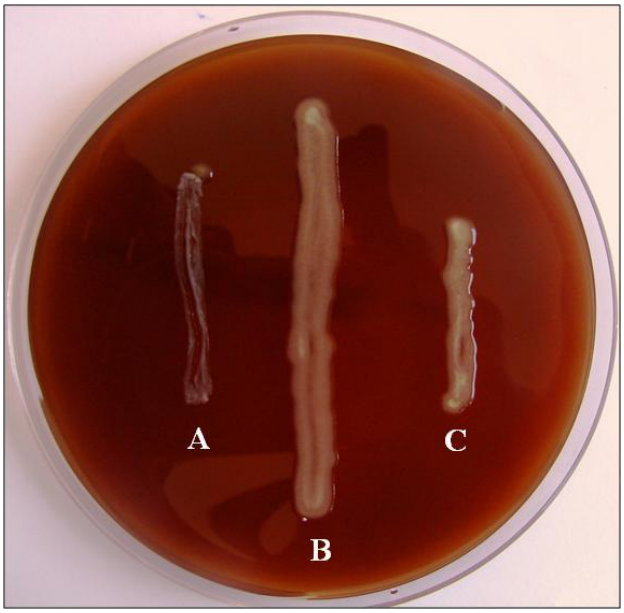
Supplementary Fig S3. Different food-borne pathogens MALDI-TOF-MS spectra.



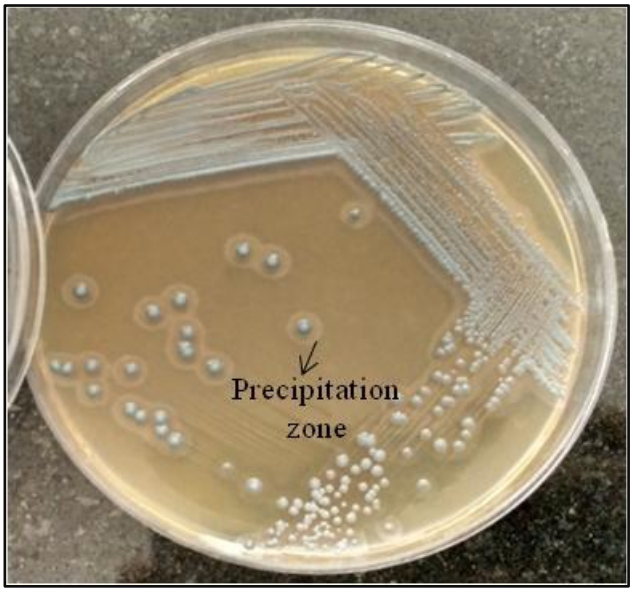
Supplementary Fig S4. Multi drug resistant (MDR) *Staphylococcal* isolates conserved *mecA* gene. All the *S. aureus* (1-5), *S. epidermidis* (6-8) and *S. haemolyticus* (9 and 10) amplified by *mecA* gene and amplified *mecA* gene product of 163 bp on agarose gel, along with 100 bp ladder (M) with negative control (C-).



Supplementary Fig S5. The specific identification of *Staphylococcus aureus* was assessed by species specific primers *Eap* (Lane 4-8), *Coa* (Lane 9-12) and *SpA* (Lane 13&14) genes and amplified product confirmed 230, 290 and 304 bp size on agarose gel electrophoresis respectively. Lane 1-3 *E. coli*, *S. typhimurium,* and *S. flexneri* respectively; C- indicates negative control and Lane M represent 100 bp ladder.



Supplementary Fig S6. Hemolytic activity of *Staphylococcal* species. Blood Agar medium exhibiting hemolytic activity: *S. haemolyticus* (A) showing γ-hemolytic, *S. epidermidis* (B) and *S. aureus* (C) shows α -hemolytic property.



Supplementary Fig S7. Lecithinase activity of *Staphylococcus aureus* on BPA medium.

Supplementary Table S1. Different vegetarian samples, locations and specific pathogen isolation.

Sl. No.	Samples	Location	Total No. of samples collected	Observations						
				***Staphylococcal* spp.**	***B. cereus***	***S. typhimurium***	***E. coli***	***S. flexneri***	***V. cholera***	***V. parahemolyticus***
1	Carrot	A, B, E	16	9	1	0	9	0	0	0
2	Beans	A, B	8	4	0	0	4	0	0	0
3	Capsicum	A, E	2	0	1	2	1	0	0	0
4	Cauliflower	C	2	1	0	0	1	0	0	0
5	Brinjal	A, B, D	10	2	2	0	5	0	0	0
6	Cabbage	A, C, E	3	1	1	0	1	0	0	0
7	Tomato	A, B, C	15	2	2	0	0	2	0	0
8	Radish	A, D	3	0	0	0	0	0	0	0
9	Ridge gourd	A, D	4	1	0	0	0	0	0	0
10	Bitter gourd	A, B	5	0	0	0	0	0	0	0
11	Cucumber	A, E	3	0	0	0	0	0	0	0
12	Chilli	A, B, C	9	1	2	1	2	0	0	0
13	Beet root	A, E	3	0	0	0	0	0	0	0
14	Ladies finger	A, B, D	4	0	0	1	1	0	0	0
15	Onion	A, B, C	13	0	1	0	5	3	0	0
16	Potato	B, C	8	2	1	0	2	0	0	0
17	Amaranth	A, C	2	0	0	0	0	0	0	0
18	Apple	A, C	7	3	1	0	2	0	0	0
19	Pineapple	B, C, E	3	1	0	0	1	0	0	0
20	Banana	A, B	4	0	0	0	1	0	0	0
21	Grapes	A, B	4	0	0	0	1	0	0	0
22	Orange	A, B	7	0	1	0	2	0	0	0
23	Gooseberry	C, E	2	0	0	0	1	0	0	0
Total			**137**	**27**	**13**	**4**	**39**	**5**	**0**	**0**

Supplementary Table S2. Different Non-vegetarian samples, locations and specific pathogen isolation.

Sl. No	Samples	Location	No. of samples collected	Observation						
				***Staphylococcal* spp.**	***B. cereus***	***S. typhimurium***	***E. coli***	***S. flexineri***	***V. cholera***	***V. parahemolyticus***
1	Chicken	A,C, D	20	6	4	3	5	2	2	1
2	Mutton	A, B, C	15	4	8	2	4	3	3	0
3	Fish	A, D, E	14	7	5	4	6	5	1	0
4	Crab	D	4	3	4	6	3	3	5	2
5	Prawn	D	4	2	3	7	2	3	3	1
6	Pork	D, E	5	3	2	5	2	4	0	2
7	Beef	D	7	5	3	4	1	1	0	0
Total			**69**	**30**	**29**	**31**	**23**	**21**	**14**	**6**

Supplementary Table S3. Different dairy samples, locations and specific pathogen isolation.

Sl. No	Samples	Location	No. of samples collected	Observation						
				***Staphylococcal* spp.**	***B. cereus***	***S. typhimurium***	***E. coli***	***S. flexineri***	***V. cholera***	***V. parahemolyticus***
1	Milk	A,B, C, D	65	16	5	2	0	0	0	0
2	Curd	A, D, E	4	2	3	0	0	0	0	0
3	Ice cream	A,G	8	1	2	0	0	0	0	0
4	Ghee	A,B	5	1	1	0	0	0	0	0
5	Paneer	A,C, D	5	3	0	2	0	0	0	0
Total			**87**	**23**	**11**	**4**	**0**	**0**	**0**	**0**

Supplementary Table S4. Different bakery samples, locations and specific pathogen isolation.

Sl. No	Samples	Location	No. of samples collected	Observation						
				***Staphylococcal* spp.**	***B. cereus***	***S. typhimurium***	***E. coli***	***S. flexineri***	***V. cholera***	***V. parahemolyticus***
1	Cake	A,B	6	2	1	0	1	0	0	0
2	Mysore pak	A,B, D	6	1	1	0	1	0	0	0
3	Bread	A,B	7	2	2	0	0	0	0	0
4	Rusk	A,B	8	0	2	0	2	0	0	0
5	Jamun	A, B, C	3	0	0	0	1	0	0	0
6	Rasgulla	A,D	8	0	0	0	0	0	0	0
7	Chips	A,B	16	3	4	0	4	0	0	0
8	Fried ground nut	A,D	4	4	3	0	2	0	2	0
9	Fried dhal	A, C, E	5	2	2	0	2	0	1	0
10	Saunpapdi	A, B, E	8	0	0	0	1	0	0	0
11	Biscuit	A	1	0	0	0	0	0	0	0
Total			**72**	**14**	**15**	**0**	**14**	**0**	**3**	**0**

Supplementary Table S5. Different processed samples, locations and specific pathogen isolation.

Sl. No	Samples	Location	No. of samples collected	Observation						
				***Staphylococcal* spp.**	***B. cereus***	***S. typhimurium***	***E. coli***	***S. flexineri***	***V. cholera***	***V. parahemolyticus***
1	Color rice	A, D, E	34	14	10	1	3	0	0	0
2	Idly	A, D	43	12	3	0	1	0	0	0
3	Chapathi	A, B	8	9	2	2	2	0	0	0
4	Chicken	C, D, E	16	12	8	9	3	1	0	0
5	Mutton	A, B	5	3	5	1	2	0	0	2
6	Kabab	A,A	15	13	9	8	1	2	3	0
7	Paulav	A	3	0	1	2	1	0	0	0
8	Sambar	A	4	1	1	2	2	0	0	0
9	Vada	A,C	5	1	1	1	1	0	0	0
10	Egg	A,B	2	1	0	0	0	0	0	0
Total			**135**	**66**	**40**	**26**	**16**	**3**	**3**	**2**

A: Devaraj Urs market, Mysore, B: T. K. Layout, Mysore, C: K. D. Road, Mysore, D: Bamboo bazaar, Mysore, and E: Nanjanagud.

Supplementary Table S6. MDR *Staphylococcal* species associated with different food items.

Food items	**Areas/Source**	***S. aureus***	***S. epidermidis***	***S. haemolyticus***
Vegetarian	Hospital	22	4	1
	Street vended	54	1	0
	Bakery	14	0	0
Non-vegetarian	Market	30	1	2
	Street vended	12	1	1
	Packed	6	1	0
Dairy	Milk	12	0	0
	Milk products	11	1	0
Vegetables and fruits	Market	27	0	0
	City	8	0	0

Supplementary Table S7. Different pathogens and confirmed at species level.

Sl. No.	Microorganisms	Standard	Isolated isolates		% of incidence	Specific genes							
			Total	Confirmed		***Eap***	***Emitic toxin***	***phoP***	***stx***	***mpl***	***Scaffolding protein***	***ompW***	***toxR***
1	*Staphylococcus aureus*	1	160	152	94.37	+	-	-	-	-	-	-	-
2	*Bacillus cereus*	1	108	97	89.81	-	+	-	-	-	-	-	-
3	*Salmonella typhimurium*	1	85	60	70.58	-	-	+	-	-	-	-	-
4	*Escherichia coli*	1	92	78	84.78	-	-	-	+	-	-	-	-
5	*Shigella flexneri*	1	29	20	68.96	-	-	-	-	-	+	-	-
6	*Vibrio cholera*	1	20	8	40.00	-	-	-	-	-	-	+	-
7	*Vibrio parahemolyticus*	1	8	2	25.00	-	-	-	-	-	-	-	+
8	*Pseudomonas aeruginosa*	1				-	-	-	-	-	-	-	-
9	*Bacillus subtilis*	1				-	-	-	-	-	-	-	-
10	*Enterobacter aerogenes*	1				-	-	-	-	-	-	-	-

Supplementary Table S8: Biochemical characterization of food-borne *Staphylococcal* isolates.

Biochemical tests [a]	**Results**		
	Sa	**Se**	**Sh**
Gram's reaction	+	+	+
Catalase test	+	+	+
Kovac's oxidase	-	-	-
KOH solubility	+	+	+
Starch hydrolysis	+	-	-
Gelatin hydrolysis	+	w	w
Coagulase	+	-	-
Lipase	+	-	+
DNase	+	-	-
Pigment	+	-	-

Sa: *S. aureus,* Se: *S. epidermidis* and Sh: *S. haemolyticus.* [a]All the test results are performed in five replicates and were repeated thrice. '+' indicates positive reaction, '-' indicates negative reaction and 'w' indicates weak reaction.

Supplementary Table S9: DNA sequence results of pathogens involved in this study.

Sl. No.	Pathogen	Strain	Gene	GenBank Accession no
1	*S. aureus*	UOM012	*16S rRNA*	KX641603
2	*S. aureus*	UOM018	*16S rRNA*	KX431901
3	*S. aureus*	UOM048	*16S rRNA*	KX431902
4	*S. aureus*	UOM051	*16S rRNA*	KX431903
5	*S. aureus*	UOM069	*16S rRNA*	KX641604
6	*S. aureus*	UOM082	*16S rRNA*	KX431904
7	*S. aureus*	UOM090	*16S rRNA*	KX355577
8	*S. aureus*	UOM114	*16S rRNA*	KX431905
9	*S. aureus*	UOM136	*16S rRNA*	KX641605
10	*S. aureus*	UOM157	*16S rRNA*	KX431906
11	*S. aureus*	UOM160	*16S rRNA*	KX431907
12	*S. aureus*	UOM090	*mecA*	KX371863
13	*S. epidermidis*	UOM036	*16S rRNA*	KX371861
14	*S. epidermidis*	UOM057	*16S rRNA*	KX431908
15	*S. epidermidis*	UOM080	*16S rRNA*	KX641606
16	*S. epidermidis*	UOM097	*16S rRNA*	KX431909
17	*S. epidermidis*	UOM036	*mecA*	KX387571
18	*S. haemolyticus*	UOM142	*16S rRNA*	KX371862
19	*S. haemolyticus*	UOM150	*16S rRNA*	KX431910
20	*S. haemolyticus*	UOM142	*mecA*	KX387572
21	*V. cholera*	UOM210	*16S rRNA*	KX452112
22	*V. parahaemolyticus*	UOM300	*16S rRNA*	KX452113
23	*E. coli*	UOM352	*16S rRNA*	KX452114
24	*S. flexneri*	UOM398	*16S rRNA*	KX452115
25	*S. typhimurium*	UOM400	*16S rRNA*	KX452116
26	*E. aerogenes*	UOM447	*16S rRNA*	KX452117
27	*B. cereus*	UOM380	*16S rRNA*	KX641607
28	*P. aeruginosa*	UOM219	*16S rRNA*	KX641608

Supplementary Table S10: Comparison of methods involved in pathogen identification.

	Standard culture	PCR	Sequencing	PCR-SSCP	MALDI-Biotyper
Assay time (sample processing to detection)	2-7 days	> 3 hr	Within a day	< 5 hr	< 10-30 min
Instrument	Incubator, Biosafety Laminar hood Rs. 50,000-2,00,000	Standard PCR machine Rs. 3,00,000 – 4,00,000	Automated sequencer Rs. 7,00,000	Electrophoresis unit Rs.25,000	Bruker Daltonics MALDI-TOF-MS ~Rs. 1,20,00,000
Cost (per assay)	~ Rs. 50 (media, plates, chemicals)	~ Rs. 60 (polymerases, dyes, primers, agarose)	~ Rs. 500 (PCR, purification, chemicals)	~ Rs. 90 (chemicals)	~ Rs. 2,500 (chemicals and instrumentation)
POC use	Difficult to apply	Easily applicable	Difficult to use	Easily applicable in the future	Might be applicable in the future, currently using in facilitated diagnostic labs
Care	Given at highest priority	Maintain hygiene	Maintain hygiene	Care should be taken	Work with sterile condition
Sensitivity	>80-85%	>90-94%	>90-96%	>95-97%%	>99%

Supplementary Table S11: Reference identification of pathogenic isolates obtained by MALDI-Biotyper and biochemical assays.

Sl.No.	Original biochemical identification	Biotyper ID (score)	Repeated biochemical identification	16S rDNA identification
1	*S. aureus*	*S. aureus* (2.28)	*S. aureus*	*S. aureus*
2	*S. epidermidis*	*S. epidermidis* (2.10)	*S. epidermidis*	*S. epidermidis*
3	*S. haemolyticus*	*S. haemolyticus* (2.01)	*S. haemolyticus*	*S. haemolyticus*
4	*S. typhimurium*	*S. typhimurium* (2.18)	*S. typhimurium*	*S. typhimurium*
5	*S. flexneri*	*S. flexneri* (2.03)	*S. flexneri*	*S. flexneri*
6	*E. coli*	*E. coli* (2.32)	*E. coli*	*E. coli*
7	*B. cereus*	*B. cereus* (2.00)	*B. cereus*	*B. cereus*
8	*P. aeruginosa*	*P. aeruginosa* (2.10)	*P. aeruginosa*	*P. aeruginosa*
9	*V. cholera*	*V. cholera* (2.01)	*V. cholera*	*V. cholera*
10	*V. parahaemolyticus*	*V. parahaemolyticus* (2.00)	*V. parahaemolyticus*	*V. parahaemolyticus*
11	*E. coli*	*E. aerogenes* (2.44)	*E. coli*	*E. aerogenes*

Supplementary Table S12: Molecular mechanism of antibacterial resistance identified in CoPS.

Original biochemical test identification		Phenotype	Resistant gene	Food source
S. aureus	**UOM012**	*AMP, AMC, CTX, OX, P, COT,TE*	*mecA*	*Tomato, Onion, Brinjal*
S. aureus	**UOM018**	*LZ, CIP, K, OX, P, C, CFM, B*	*mecA*	*Vada, Paulav*
S. aureus	**UOM048**	*AZM, B, CFM, E, OX, P, RIF, S*	*mecA*	*Colour rice,*
S. aureus	**UOM051**	*TE, NIT, K, TE, AZM*	*mecA*	*Chips,*
S. aureus	**UOM069**	*E, AMC*	*mecA*	*Chips, Milk*
S. aureus	**UOM082**	*S, TE, NIT, AMP, K, TOB, OX, P, CIP, C, AMC, AZM, B, CFM*	*mecA*	*Fish kabab, Chicken*
S. aureus	**UOM090**	*CD, NIT, K, TE, AZM, B, NX, CTX, P, OX, RIF, S, C, TOB*	*mecA*	*Idli, Vada, Paulav, Mutton, Chicken*
S. aureus	**UOM114**	*NIT, AMP, TOB, RIF,COT, OX, P, C, CFM, B*	*mecA*	
S. aureus	**UOM136**	*TOB, RIF,COT, AMP, B, K, OX, P*	*mecA*	*Apple, Bread*
S. aureus	**UOM157**	*TOB, CD, CFM*	*mecA*	*Colour rice, Paulav, Vada*
S. aureus	**UOM160**	*TE, OX, P, AMC*	-	*Grapes, Apple*
S. epidermidis	**UOM036**	*S, TE, B, CFM, OX, K, P, RIF, S, AMC, CTX*	*mecA*	*White rice (Street vender)*
S. epidermidis	**UOM057**	*S, TE, NIT, K, AMP, TOB, OX,COT, CIP, C, AMC, AZM, B, CFM*	*mecA*	*Soya milk* *Idli*
S. epidermidis	**UOM080**	*NIT, AMP, TOB, CFM, E, OX, S, TE*	*mecA*	
S. epidermidis	**UOM097**	*TE, AZM, RIF,COT, AMP, B, CFM*	*mecA*	*Carrot, Beet root*
S. haemolyticus	**UOM142**	*NIT, TE, AZM, K, B, CFM, CTX, AMP, TOB, CFM, E, OX*	*mecA*	*Pork, Fish, Mutton Chicken, Crab, Idli*
S. haemolyticus	**UOM150**	*TOB, COT, C, K, NIT, TE, AZM, E, AMC, OX, P*	*mecA*	*Idli, Tomato*

Abbreviations: AMP- amphicilin, AZM-azithromycin, AMC- amoxyclav, B- bacitracin, CFM- cefoxitin, CTX- cefotaxime, C- chloramphenicol, CIP- ciprofloxacin, CD- clindamycin, COT- co-Trimoxazole, E- erythromycin, GEN- gentamycin, K- kanamycin, LZ- linezolid, NIT- nitrofurantoin, NX- norfloxin, OX- oxacillin, P- pennicilin, RIF- rifampicin, S- streptomycin, TE- tetracycline, and TOB- tobramycin.

Supplementary Table S13: Proposed resistance genes conferred in our *Staphylococcal* species.

Drugs	Gene conferring resistance	Description
Amoxyclav	*amc*	Inhibiting bacterial cell wall synthesis
Amphicillin	*amr*	Inhibits the third and last stage of bacterial cell wall synthesis by binding to PBPs. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that Ampicillin interferes with an autolysin inhibitor
Azithromycin	*ermC*	Binds to the 23S rRNA of 50S subunit of the 70S bacterial ribosomes, and therefore inhibits RNA-dependent protein synthesis in bacterial cells
Bacitracin	*baca*	Undecaprenyl pyrophosphate phosphatase, which consists in the sequestration of Undecaprenyl pyrophosphate
Cefotaxime	*mecA*	Inhibition of cell wall synthesis via affinity for penicillin-binding proteins (PBPs). Cefotaxime shows high affinity for penicillin-binding proteins in the cell wall including PBP Ib and PBP III
Cefoxitime	*mecA*	Inhibition of cell wall synthesis
Chloramphenicol	cml_e4	Major facilitator superfamily transporter, chloramphenicol efflux pump
Ciprofloxacin	*norA*	Inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, strand supercoiling repair, and recombination.
Clindamycin **Clindamycin (inducible)**	*VGA(A)LC* *ermA/ermB,*	Bind to the 23S portion of the 50S subunit of bacterial ribosomes and inhibit early elongation of peptide chain by inhibiting transpeptidase reaction
Co-Trimoxazole	*dhfr*	Inhibits bacterial synthesis of dihydrofolic acid and inhibiting the enzyme dihydrofolate reductase by competing with PABA. This combination blocks 2 consecutive steps in bacterial biosynthesis of essential nucleic acids and proteins
Erythromycin	*msrA* *ermC/ermT*	Inhibits protein synthesis by reversibly binding to 50S ribosomal subunits of ribosomenear the 'P' site to stop the translation. Effective against actively dividing organisms.
Gentamicin	*aacA-aphD*	Works by irreversibly binding the 30S subunit of the bacterial ribosome, interrupting protein

		synthesis similar to Kannamycin
Kanamycin	*aacA-aphD*	Binds irreversibly to the bacterial 30S ribosomal subunit protein and 16S rRNA and prevents the formation of the initiation complex with messenger RNA intern inhibits bacterial protein synthesis
Linezolid	*Cfr*	Bacteriostatic action, which stops bacteria from reproducing
Methicillin	*mecA*	Penicillin binding protein, which has a low affinity for beta-lactams and catalyze a penicillin-insensitive transpeptidation
Nitrofurantoin		Damage bacterial DNA
Norfloxacin	*nfxC*	Extrusion of norfloxacin
Oxacillin	*mecA*	Binding to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall. Inhibits the third and last stage of bacterial cell wall synthesis
Pennicillin G	*blaZ/* bl2a_pc	Class A beta-lactamase. This enzyme breaks the beta-lactam antibiotic ring open and deactivates the molecule's antibacterial properties
Rifampicin	*Rif*	RNA polymerase with reduced affinity
Streptomycin	Str	Streptomycin resistance protein
Tetracycline	*tetK/tetL/tetM*	Major facilitator superfamily transporter, tetracycline efflux pump
Tobramycin	aac6ie/ *aacA-aphD*	Aminoglycoside N-acetyltransferase, which modifies aminoglycosides by acetylation.