Electronic Supplementary Information (ESI)

Paper-based analytical devices (PADs) for colourimetric detection of *S. aureus*, *E. coli* and their antibiotic resistant strains in milk

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(A) Supplementary information to Experimental Section

Enrichment broths for selective growth and enrichment of *E. coli*, *S. aureus* and β-lactam resistant bacteria are summarised in Table S1. Table S2 summarises the list of primers employed for polymerase chain reaction (PCR) for detection of pathogenic bacteria in real milk samples.

Table S1. Enrichment broth with supplements to encourage growth of desired bacteria

Enrichment broth	Favours growth of bacteria	References
BPW + Vancomycin HCl (8 mg L^{-1})	*E. coli*	Bisha et al., 2014
BPW + Sodium Chloride (75 g L^{-1}) + Deferoxamine (0.01 g L^{-1})	*S. aureus*	Bruins et al., 2007; Isenberg, H.D., 2010; Rambach, 2003
BPW + Cefotaxime (2 µg mL^{-1})	β- lactam resistant bacteria	Mir et al., 2016

Table S2. List of primers used for PCR based identification of pathogens.

Primer	Sequence 5′-3′	Annealing temp. (°C)	Amplicon size (bp)	Reference
Eco 223 (F)	ATCAACCGAGATTCCCCCAGT	64	232	Riffon *et al*., 2001
Eco 455 (R)	TCACTATCGGTCAGTCAGGAG			
Sau 234 (F)	CGATTCCCTTAGTAGCGGCG	70	1267	Riffon *et al.*, 2001
Sau 1501 (R)	CCAATCGCACGCTTCGCCTA			
TEM (F)	AGATCAGTTGGGTGCACGAG	62	618	Yazdi *et al.*, 2011
TEM (R)	CAGTGCTGCAATGATACCG			
TEM-1 (F)	CGGCATTTTGCCTTCCTGTTTTTGC	55	702	Abdullah *et al*., 2017
TEM-1 (R)	GGCCCCAGTGCTGCAATGATA CCG			

References

Abdullah, Z.H., Merza, N.S., Sulaiman, A.M. and Raziq, A.H., 2017. Molecular detection of methicillin resistant Staphylococcus aureus harbouring β-lactamase resistance genes isolated from different sources of infections. *Zanco Journal of Medical Sciences*, 21(3), pp.1889-1896.

Bisha, B., Adkins, J.A., Jokerst, J.C., Chandler, J.C., Pérez-Méndez, A., Coleman, S.M., Sbodio, A.O., Suslow, T.V., Danyluk, M.D., Henry, C.S. and Goodridge, L.D., 2014. Colorimetric paper-based detection of Escherichia coli, Salmonella spp., and Listeria monocytogenes from large volumes of agricultural water. *Journal of Visualized Experiments*, (88), p.e51414.

Bruins, M.J., Juffer, P., Wolfhagen, M.J., Ruijs, G.J., 2007. Salt tolerance of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus. Journal of clinical microbiology* 45(2), 682-683.

Isenberg, H.D., 2010. Clinical Microbiology Procedures Handbook, Vol. I, II & III. American Society for Microbiology, Washington, DC.

Mir, R.A., Weppelmann, T.A., Johnson, J.A., Archer, D., Morris Jr, J.G. and Jeong, K.C., 2016. Identification and characterization of cefotaxime resistant bacteria in beef cattle. *PloS one*, 11(9).

Rambach, A., 2003. Chromogenic medium for detecting *Staphylococcus aureus*. Google Patents.

Riffon, R., Sayasith, K., Khalil, H., Dubreuil, P., Drolet, M. and Lagacé, J., 2001. Development of a rapid and sensitive test for identification of major pathogens in bovine mastitis by PCR. *Journal of clinical microbiology*, 39(7), pp.2584-2589.

Yazdi, M.K.S., Dallal, M.M.S., Mirzael, H.M.A., Sabbaghi, A., Mehrabadi, J.F., Lari, A.R., Eshraghian, M.R., Saneiee, A.F., Abdar, M.H. and Bakhtiari, R., 2011. Molecular detection of TEM broad spectrum β-lactamase in clinical isolates of Escherichia coli. *African Journal of Biotechnology*, 10(46), pp. 9454-9458.

(B) Optimisation of colourimetric paper-based assays with enzymes

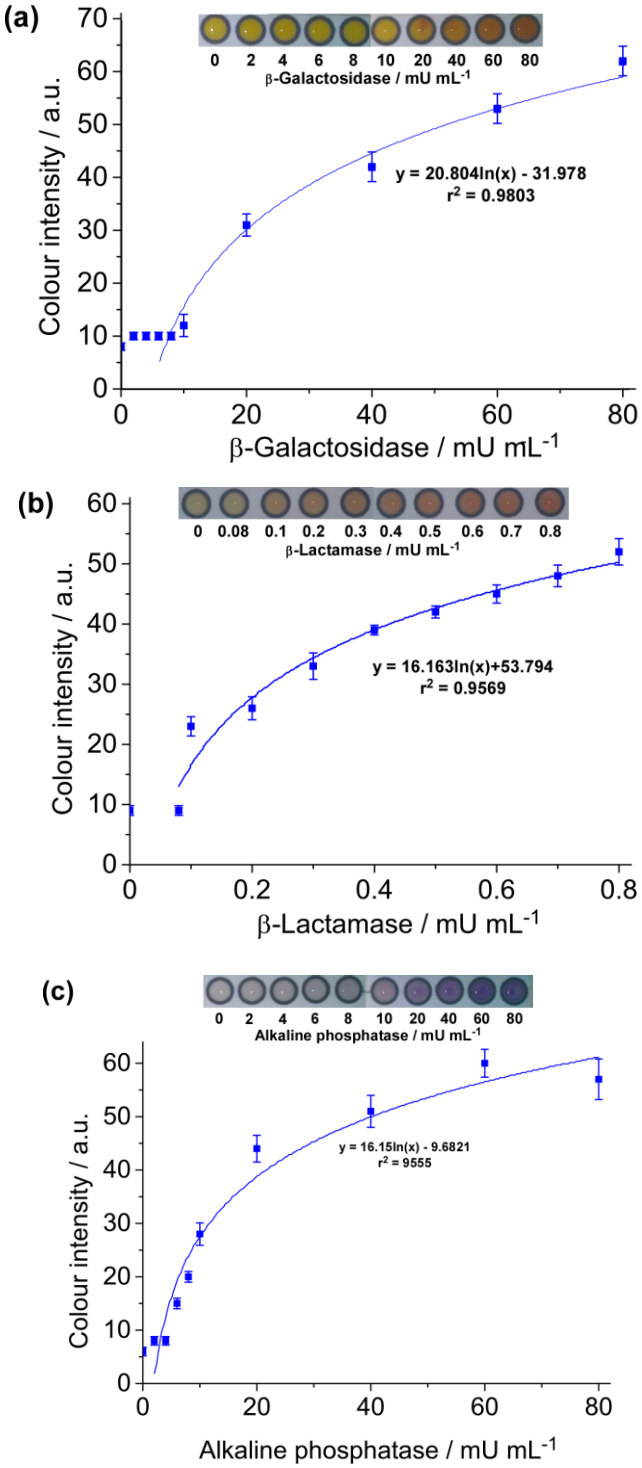


Figure S1 (a) Reaction on µPAD between CRPG substrate (3 mM) and β-galactosidase enzyme solutions. Concentrations down to 20 mU mL^{-1} could be detected. (b) Reaction on µPAD between nitrocefin (1 mM) and β-lactamase enzyme solutions. Concentrations down to 0.1 mU mL^{-1} of β-lactamase could be detected. (c) Reaction on µPAD between BCIP substrate (5.7 mM) and alkaline phosphatase enzyme solutions. Concentrations down to 6 mU mL^{-1} could be detected readily by naked eye. The colour reactions were carried out by adding different concentrations of β-galactosidase, alkaline phosphatase and β-lactamase onto the µPADs, followed by 3-4 h incubation at 37 °C. Data obtained from three separate experiments (n=3).

(C) Colourimetric reactions from pathogen cells in culture media

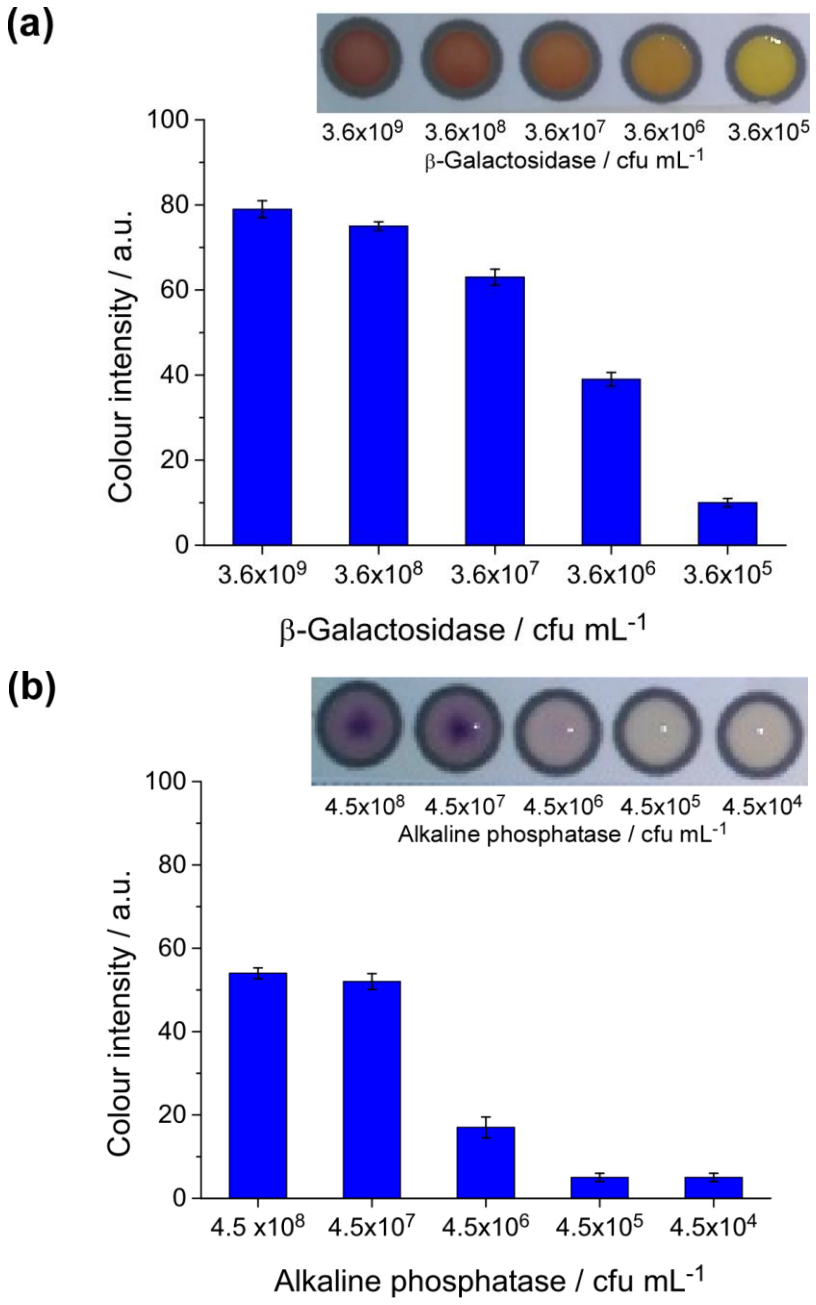


Figure S2 (a) Reaction on µPAD between the CRPG substrate (3 mM) and β-galactosidase enzyme from lysed *E. coli* cells. A colour change was detectable at 3.6 x 10^6 cfu mL^{-1}. (b) Reaction on µPAD between the BCIP substrate (5.7 mM) and alkaline phosphatase excreted from *S. aureus*. Colour change was detected from concentrations ≥ 4.5x10^6 cfu $mL.^{-1}$ Solutions of chromogenic substrates were pre-deposited on µPADs. Suspensions of live bacteria were added. For the detection of β-galactosidase from *E. coli*, the cells were lysed beforehand. The µPADs were incubated at 37 °C for 3-4 h before colour readout.

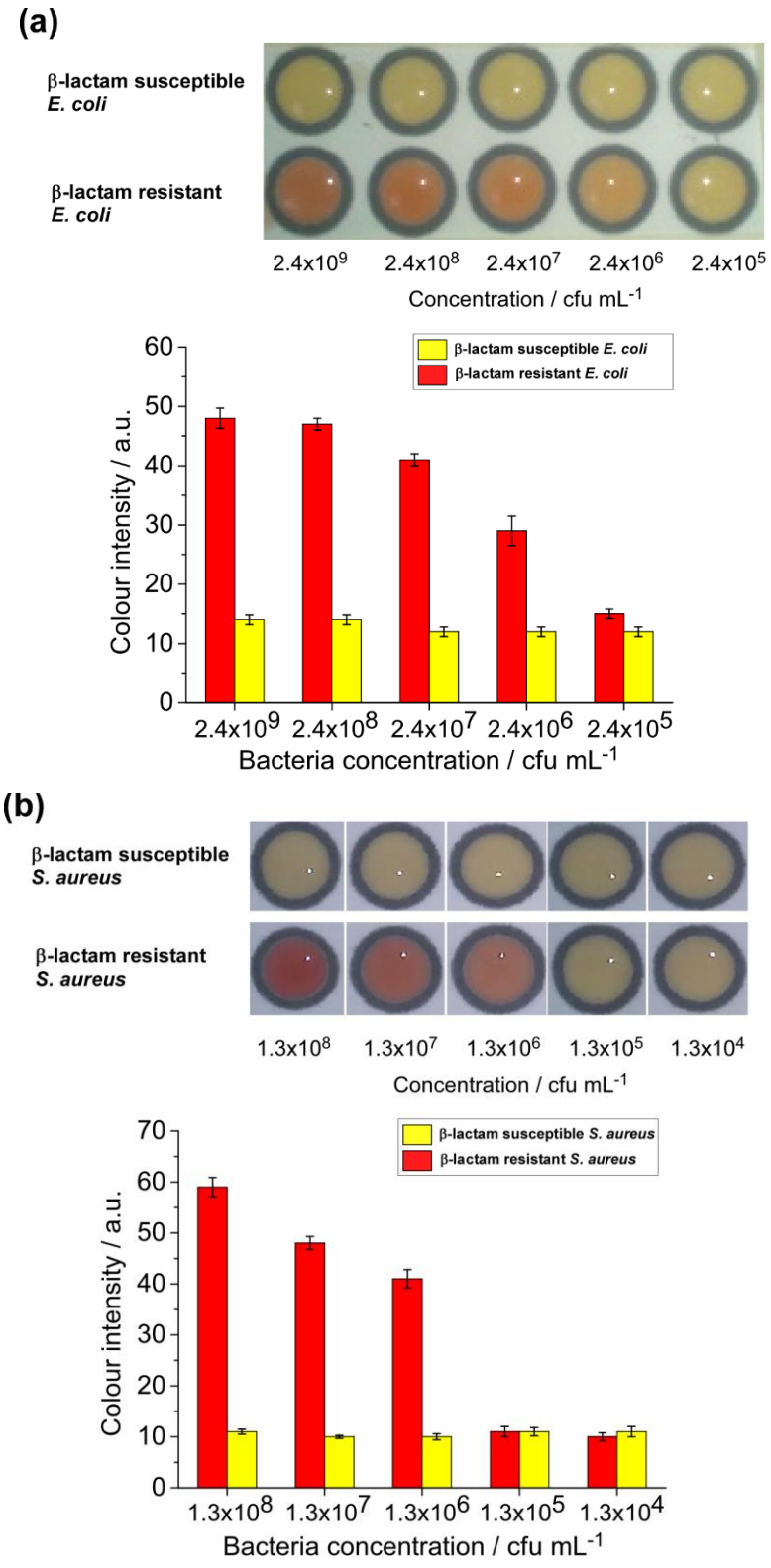


Figure S3 (a) Reaction between nitrocefin substrate and live bacteria of β-lactam resistant and β-lactam susceptible strains of *E. coli*. (b) Reaction between nitrocefin substrate and live bacteria of β-lactam resistant and β-lactam susceptible strains of *S. aureus.* (n=3)

(D) Differentiation of β-lactam resistant and ESBL-resistant bacteria

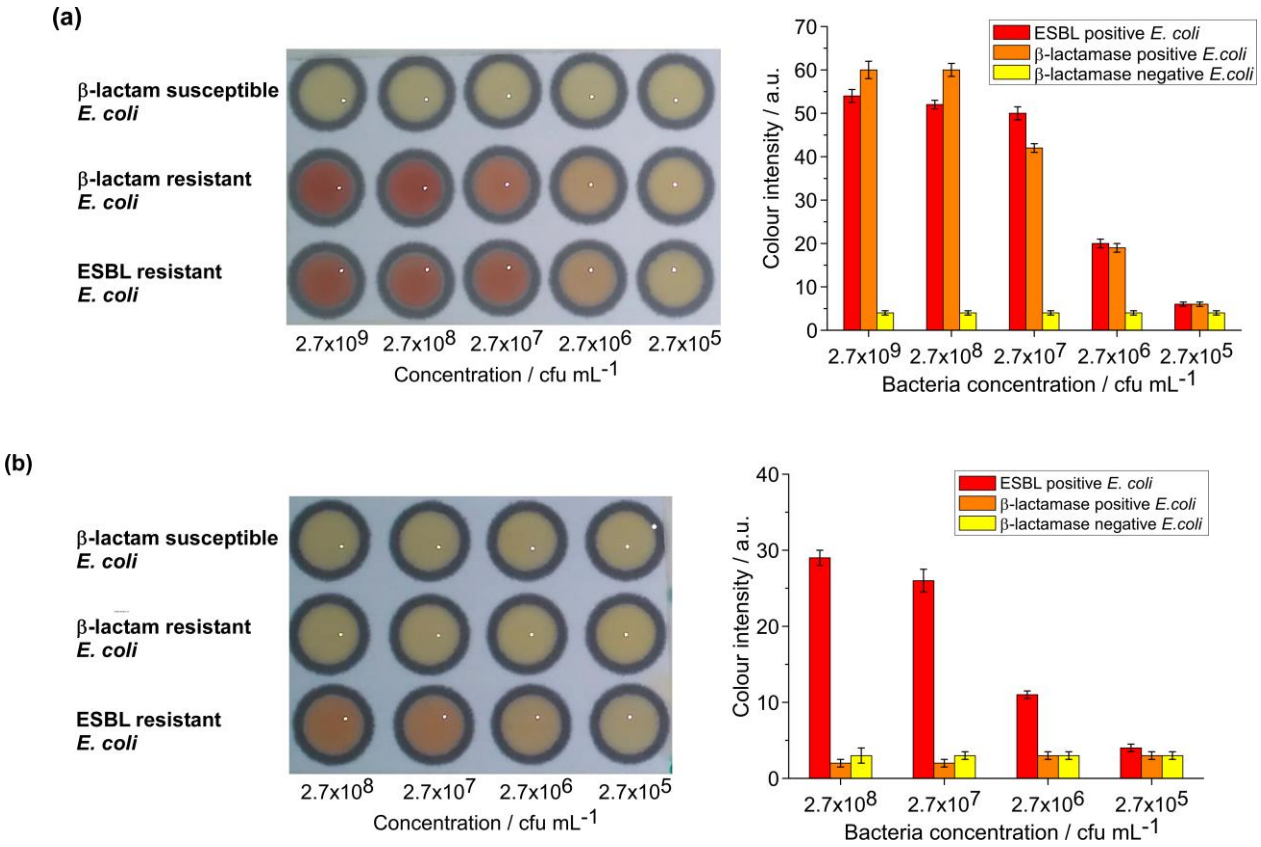


Figure S4 (a) Colourimetric reaction on nitrocefin-embedded µPADs with β-lactam susceptible *E. coli*, β-lactam resistant *E. coli* and ESBL-resistant *E. coli*. A red colour is observed both for β-lactam resistant *E. coli* and ESBL resistant *E. coli*. (b) Colourimetric reaction on HMRZ-86-embedded µ-PADs with β-lactam susceptible *E. coli*, β-lactam resistant *E. coli* and ESBL resistant *E. coli*. A red colour is observed only for ESBL resistant *E. coli*. (n=3)

(E) UV/vis spectrophotometric analysis

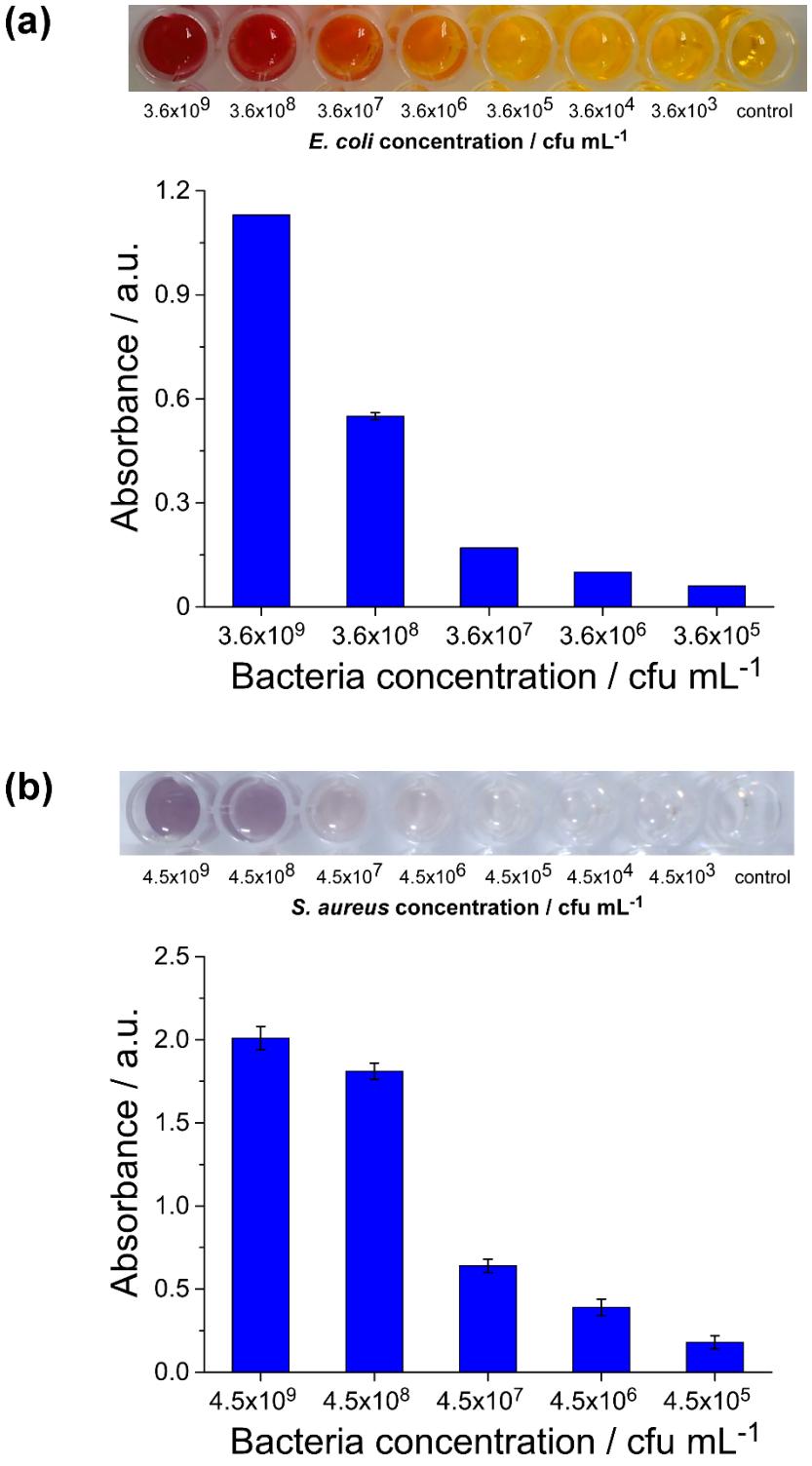


Figure S5 Reactions between the substrates and pathogen derived enzymes tested in conventional microtitre plates and read out through UV-visible spectrometry. (a) Detection of *E. coli* with CPRG substrate via UV-visible spectrophotometry at λ = 594 nm. (b) Detection of *S. aureus* with BCIP via UV-vis spectrophotometry at λ = 405 nm. (n=3)

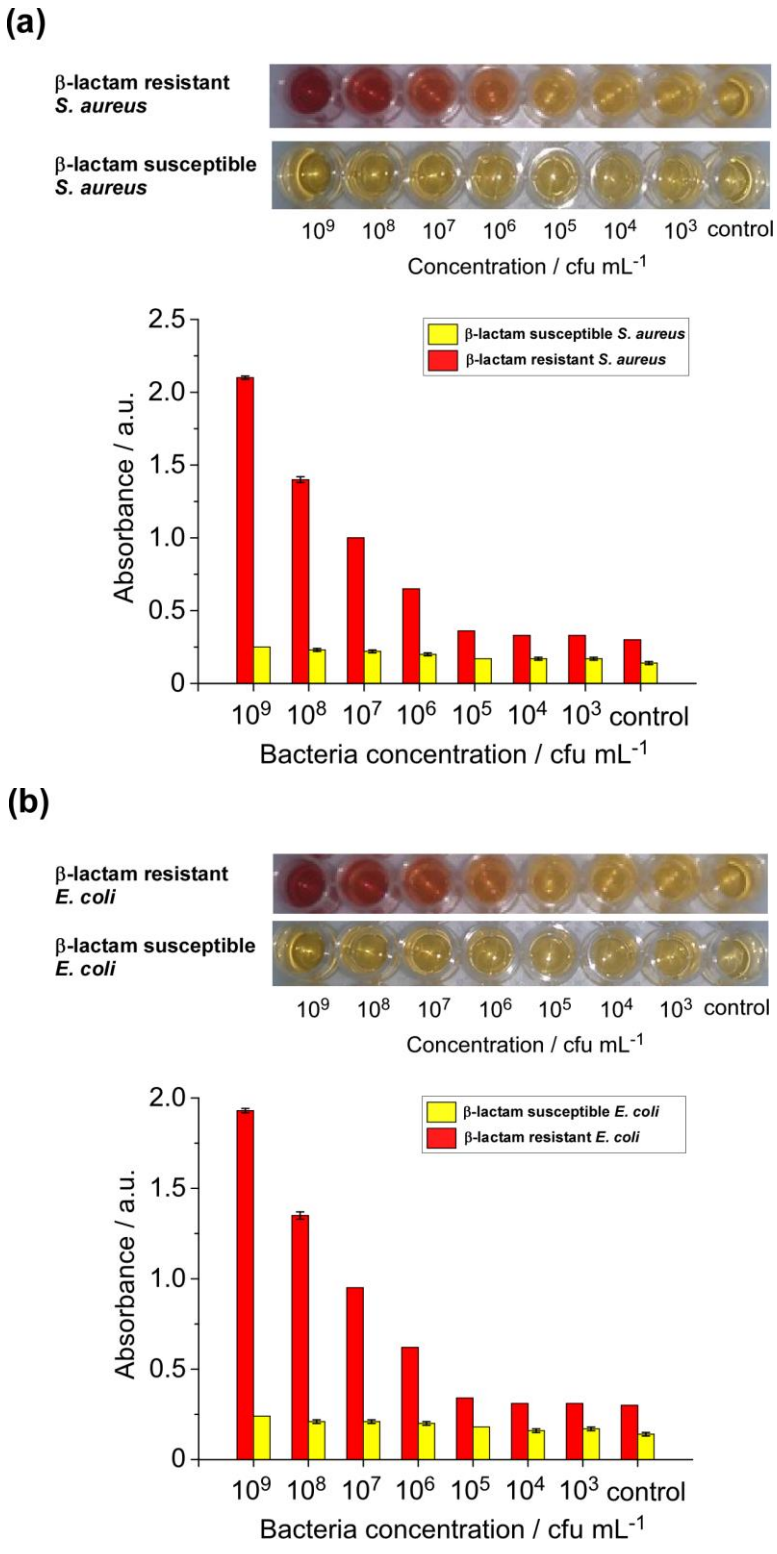


Figure S6 Reactions between the substrates and pathogen derived enzymes tested in conventional microtitre plates and read out through UV-visible spectrometry. (a) Detection of β-lactam resistant *S. aureus* with nitrocefin via UV-vis spectrophotometry at λ = 490 nm. (b) Detection of β-lactam resistant *E. coli* with nitrocefin via UV-vis spectrophotometry at λ = 490 nm. (n=3)

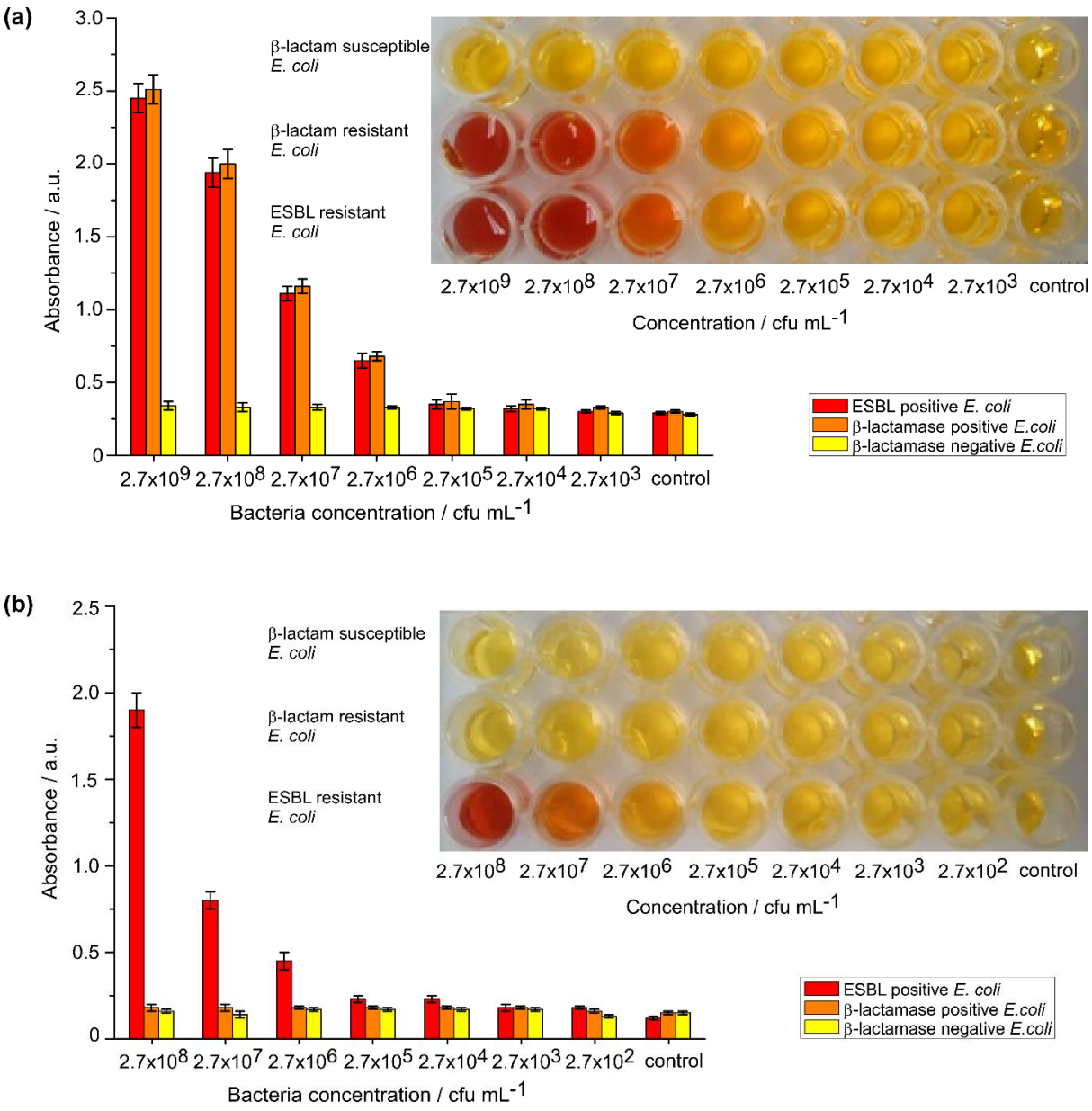


Figure S7 Reactions between the substrates and pathogen derived enzymes tested in conventional microtitre plates and read out through UV-visible spectrometry. (a) Analysis of β-lactam resistant and ESBL-resistant *E. coli* with nitrocefin via UV-vis spectrophotometry at λ = 490 nm. (b) Analysis of β-lactam resistant and ESBL resistant *E. coli* with HMRZ-86 via UV-vis spectrophotometry at λ = 490 nm. (n=3)

(F) Spiked milk samples

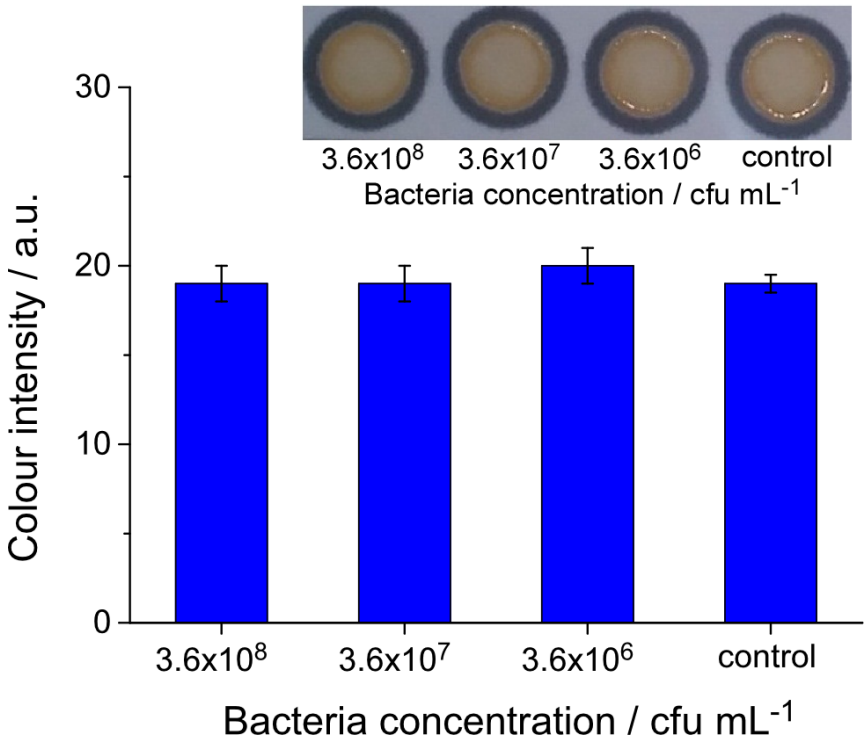


Figure S8 No colour change observed from reaction between CPRG and β-galactosidase enzyme from *E. coli* spiked milk. *E. coli* could not be detected in milk on the basis of β-galactosidase enzyme, probably as the released β-galactosidase reacts with lactose in the milk, thus no enzyme was available for interaction with the CPRG substrate. (n=3)

(G) Results from real milk samples

(G.1) µPADs

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
1.	YES 	YES 	YES 	YES 
2.	NO 	-	NO 	-
3.	NO 	-	NO 	-
4.	YES 	YES 	YES 	YES 
5.	NO 	-	YES 	YES 
6.	NO 	-	NO 	-
7.	NO 	-	NO 	-
8.	NO 	-	NO 	-
9.	YES 	YES 	NO 	-
10.	NO 	-	YES 	YES
11.	NO 	-	NO 	-
12.	YES 	YES	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
13.	NO 	-	YES	YES 
14.	NO 	-	NO	-
15.	YES 	YES 	YES	YES 
16.	NO 	-	YES 	YES 
17.	NO 	-	NO 	-
18.	NO 	-	NO	-
19.	YES 	YES 	NO	-
20.	NO 	-	NO 	-
21.	NO 	-	YES 	NO 
22.	NO 	-	NO 	-
23.	NO 	-	NO 	-
24.	YES 	YES 	YES	YES 
25.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
26.	NO 	-	NO	-
27.	NO 	-	NO	-
28.	NO 	-	NO 	-
29.	NO 	-	NO 	-
30.	YES 	YES 	YES	YES
31.	NO 	-	NO 	-
32.	NO 	-	NO 	-
33.	NO 	-	NO 	-
34.	NO 	-	NO 	-
35.	NO 	-	NO 	-
36.	NO 	-	NO 	-
37.	NO 	-	YES 	YES
38.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
39.	NO 	-	NO 	-
40.	NO 	-	NO 	-
41.	NO 	-	NO 	-
42.	NO 	-	YES 	YES 
43.	NO 	-	NO 	-
44.	NO 	-	NO 	-
45.	NO 	-	NO 	-
46.	NO 	-	NO 	-
47.	NO 	-	YES 	YES 
48.	NO 	-	NO 	-
49.	NO 	-	NO 	-
50.	NO 	-	NO 	-
51.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
52.	NO 	-	NO 	-
53.	NO 	-	NO 	-
54.	NO 	-	NO 	-
55.	NO 	-	YES 	YES 
56.	NO 	-	NO 	-
57.	YES 	YES 	NO 	-
58.	NO 	-	YES 	YES 
59.	NO 	-	NO 	-
60.	NO 	-	NO 	-
61.	NO 	-	NO 	-
62.	YES 	YES 	NO 	-
63.	NO 	-	YES 	YES 
64.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
65.	NO 	-	NO 	-
66.	NO 	-	NO 	-
67.	NO 	-	NO 	-
68.	YES 	YES 	NO 	-
69.	NO 	-	YES 	YES 
70.	NO 	-	NO 	-
71.	NO 	-	NO 	-
72.	NO 	-	NO 	-
73.	NO 	-	NO 	-
74.	NO 	-	YES 	YES 
75.	YES 	YES 	NO 	-
76.	NO 	-	NO 	-
77.	YES 	YES 	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
78.	NO 	-	NO 	-
79.	YES 	YES 	YES 	YES 
80.	NO 	-	NO 	-
81.	NO 	-	NO 	-
82.	YES 	YES 	YES 	YES 
83.	NO 	-	NO 	-
84.	NO 	-	NO 	-
85.	NO 	-	NO 	-
86.	NO 	-	NO 	-
87.	NO 	-	YES 	YES 
88.	YES 	YES 	NO 	-
89.	NO 	-	NO 	-
90.	NO 	-	YES 	YES 

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
91.	NO 	-	NO 	-
92.	NO 	-	NO 	-
93.	NO 	-	NO 	-
94.	NO 	-	NO 	-
95.	NO 	-	YES 	YES 
96.	NO 	-	NO 	-
97.	NO 	-	NO 	-
98.	NO 	-	YES 	YES 
99.	YES 	YES 	YES 	YES 
100.	NO 	-	NO 	-
101.	NO 	-	YES 	YES 
102.	YES 	YES 	YES 	YES 
103.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
104.	NO 	-	NO 	-
105.	NO 	-	YES 	YES 
106.	NO 	-	YES 	YES 
107.	YES 	NO 	NO 	-
108.	NO 	-	NO 	-
109.	NO 	-	NO 	-
110.	NO 	-	YES 	YES 
111.	NO 	-	YES 	NO 
112.	NO 	-	YES 	YES 
113.	NO 	-	NO 	-
114.	NO 	-	NO 	-
115.	NO 	-	NO 	-
116.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
117.	NO 	"	NO 	"
118.	NO 	"	NO 	"
119.	NO 	"	YES 	YES 
120.	NO 	"	YES 	YES 
121.	NO 	"	NO 	"
122.	NO 	"	NO 	"
123.	NO 	"	YES 	NO 
124.	NO 	"	NO 	"
125.	NO 	"	NO 	"
126.	NO 	"	NO 	"
127.	NO 	"	NO 	"
128.	NO 	"	NO 	"
129.	NO 	"	NO 	"

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
130.	NO 	-	NO 	-
131.	NO 	-	YES 	YES
132.	NO 	-	NO 	-
133.	NO 	-	NO 	-
134.	YES 	YES 	NO 	-
135.	NO 	-	NO 	-
136.	NO 	-	NO 	-
137.	NO 	-	NO 	-
138.	NO 	-	NO 	-
139.	NO 	-	NO 	-
140.	NO 	-	NO 	-
141.	NO 	-	NO 	-
142.	NO 	-	YES 	YES

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
143.	NO 	"	NO 	"
144.	NO 	"	NO 	"
145.	NO 	"	NO 	"
146.	NO 	"	NO 	"
147.	NO 	"	NO 	"
148.	NO 	"	YES 	YES 
149.	NO 	"	NO 	"
150.	NO 	"	NO 	"
151.	NO 	"	NO 	"
152.	NO 	"	NO 	"
153.	NO 	"	NO 	"
154.	YES 	NO 	NO 	"
155.	NO 	"	NO 	"

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
156.	NO 	-	NO 	-
157.	NO 	-	NO 	-
158.	NO 	-	YES 	YES 
159.	YES 	YES 	NO 	-
160.	NO 	-	NO 	-
161.	NO 	-	YES 	YES 
162.	NO 	-	NO 	-
163.	NO 	-	YES 	YES 
164.	NO 	-	NO 	-
165.	NO 	-	NO 	-
166.	NO 	-	YES 	YES 
167.	NO 	-	NO 	-
168.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
169.	NO 	-	YES 	YES 
170.	NO 	-	NO 	-
171.	YES 	YES 	NO 	-
172.	NO 	-	YES 	NO 
173.	NO 	-	YES 	YES 
174.	YES 	YES 	NO 	-
175.	NO 	-	YES 	YES 
176.	NO 	-	NO 	-
177.	NO 	-	NO 	-
178.	NO 	-	YES 	YES 
179.	NO 	-	NO 	-
180.	NO 	-	NO 	-
181.	NO 	-	YES 	YES 

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
182.	NO 	-	NO 	-
183.	NO 	-	NO 	-
184.	NO 	-	NO 	-
185.	NO 	-	YES 	YES 
186.	NO 	-	NO 	-
187.	NO 	-	NO 	-
188.	NO 	-	NO 	-
189.	NO 	-	YES 	YES 
190.	NO 	-	YES 	YES 
191.	NO 	-	YES 	YES 
192.	NO 	-	NO 	-
193.	NO 	-	NO 	-
194.	NO 	-	YES 	YES 

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
195.	NO 	-	NO 	-
196.	NO 	-	YES 	YES 
197.	NO 	-	YES 	YES 
198.	NO 	-	NO 	-
199.	NO 	-	NO 	-
200.	NO 	-	NO 	-
201.	NO 	-	NO 	-
202.	NO 	-	NO 	-
203.	NO 	-	NO 	-
204.	NO 	-	NO 	-
205.	NO 	-	NO 	-
206.	YES 	YES 	NO 	-
207.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
208.	NO 	-	NO 	-
209.	NO 	-	YES 	NO 
210.	NO 	-	NO 	-
211.	NO 	-	NO 	-
212.	NO 	-	NO 	-
213.	NO 	-	NO 	-
214.	NO 	-	NO 	-
215.	NO 	-	NO 	-
216.	NO 	-	NO 	-
217.	NO 	-	NO 	-
218.	NO 	-	NO 	-
219.	NO 	-	NO 	-
220.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
221.	NO 	-	NO 	-
222.	NO 	-	NO 	-
223.	NO 	-	NO 	-
224.	NO 	-	NO 	-
225.	NO 	-	NO 	-
226.	YES 	YES 	NO 	-
227.	NO 	-	NO 	-
228.	NO 	-	NO 	-
229.	NO 	-	NO 	-
230.	NO 	-	NO 	-
231.	NO 	-	NO 	-
232.	NO 	-	NO 	-
233.	YES 	NO 	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
234.	NO 	-	YES 	YES 
235.	NO 	-	NO 	-
236.	NO 	-	NO 	-
237.	NO 	-	NO 	-
238.	NO 	-	NO 	-
239.	NO 	-	NO 	-
240.	NO 	-	YES 	YES 
241.	NO 	-	NO 	-
242.	NO 	-	NO 	-
243.	YES 	YES 	NO 	-
244.	NO 	-	NO 	-
245.	NO 	-	YES 	YES 
246.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
247.	NO 	-	NO 	-
248.	NO 	-	NO 	-
249.	NO 	-	NO 	-
250.	NO 	-	NO 	-
251.	NO 	-	NO 	-
252.	NO 	-	NO 	-
253.	NO 	-	NO 	-
254.	NO 	-	NO 	-
255.	NO 	-	NO 	-
256.	NO 	-	YES 	YES 
257.	NO 	-	NO 	-
258.	NO 	-	NO 	-
259.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
260.	NO 	-	NO 	-
261.	NO 	-	NO 	-
262.	NO 	-	NO 	-
263.	NO 	-	NO 	-
264.	NO 	-	NO 	-
265.	NO 	-	NO 	-
266.	NO 	-	NO 	-
267.	NO 	-	NO 	-
268.	NO 	-	NO 	-
269.	NO 	-	NO 	-
270.	NO 	-	NO 	-
271.	NO 	-	NO 	-
272.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
273.	NO 	-	NO 	-
274.	YES 	YES 	NO 	-
275.	NO 	-	NO 	-
276.	NO 	-	NO 	-
277.	NO 	-	NO 	-
278.	NO 	-	YES 	NO 
279.	NO 	-	NO 	-
280.	NO 	-	YES 	YES 
281.	NO 	-	NO 	-
282.	NO 	-	NO 	-
283.	NO 	-	NO 	-
284.	NO 	-	NO 	-
285.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
286.	NO 	-	NO 	-
287.	NO 	-	YES 	YES 
288.	NO 	-	YES 	YES 
289.	NO 	-	NO 	-
290.	NO 	-	NO 	-
291.	NO 	-	NO 	-
292.	NO 	-	NO 	-
293.	NO 	-	NO 	-
294.	NO 	-	NO 	-
295.	NO 	-	NO 	-
296.	NO 	-	NO 	-
297.	NO 	-	NO 	-
298.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
299.	NO 	-	YES 	YES 
300.	NO 	-	NO 	-
301.	NO 	-	NO 	-
302.	NO 	-	YES 	YES 
303.	NO 	-	NO 	-
304.	NO 	-	NO 	-
305.	NO 	-	NO 	-
306.	NO 	-	NO 	-
307.	NO 	-	NO 	-
308.	NO 	-	YES 	YES 
309.	NO 	-	NO 	-
310.	NO 	-	NO 	-
311.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
312.	NO 	-	NO 	-
313.	NO 	-	NO 	-
314.	NO 	-	NO 	-
315.	NO 	-	NO 	-
316.	NO 	-	NO 	-
317.	NO 	-	YES 	YES 
318.	NO 	-	NO 	-
319.	NO 	-	NO 	-
320.	NO 	-	NO 	-
321.	YES 	NO 	NO 	-
322.	NO 	-	NO 	-
323.	NO 	-	NO 	-
324.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
325.	NO 	-	YES 	NO 
326.	NO 	-	NO 	-
327.	NO 	-	NO 	-
328.	NO 	-	NO 	-
329.	NO 	-	NO 	-
330.	NO 	-	NO 	-
331.	NO 	-	YES 	YES 
332.	NO 	-	NO 	-
333.	NO 	-	NO 	-
334.	NO 	-	NO 	-
335.	NO 	-	NO 	-
336.	NO 	-	NO 	-
337.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
338.	NO 	-	YES 	NO 
339.	NO 	-	YES 	NO 
340.	NO 	-	NO 	-
341.	NO 	-	NO 	-
342.	NO 	-	NO 	-
343.	NO 	-	NO 	-
344.	NO 	-	YES 	YES 
345.	NO 	-	NO 	-
346.	NO 	-	NO 	-
347.	NO 	-	NO 	-
348.	NO 	-	NO 	-
349.	NO 	-	NO 	-
350.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
351.	NO 	-	YES 	YES 
352.	NO 	-	YES 	NO 
353.	NO 	-	YES 	NO 
354.	NO 	-	NO 	-
355.	NO 	-	YES 	NO 
356.	NO 	-	NO 	-
357.	YES 	NO 	NO 	-
358.	NO 	-	NO 	-
359.	NO 	-	NO 	-
360.	NO 	-	NO 	-
361.	NO 	-	NO 	-
362.	NO 	-	YES 	YES 
363.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
364.	NO 	-	NO 	-
365.	NO 	-	YES 	YES 
366.	NO 	-	NO 	-
367.	NO 	-	NO 	-
368.	NO 	-	NO 	-
369.	NO 	-	NO 	-
370.	NO 	-	YES 	NO 
371.	NO 	-	NO 	-
372.	NO 	-	NO 	-
373.	NO 	-	NO 	-
374.	NO 	-	NO 	-
375.	NO 	-	NO 	-
376.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
377.	NO 	-	NO 	-
378.	NO 	-	NO 	-
379.	NO 	-	NO 	-
380.	NO 	-	NO 	-
381.	YES 	YES 	YES 	NO 
382.	NO 	-	NO 	-
383.	NO 	-	NO 	-
384.	NO 	-	NO 	-
385.	NO 	-	YES 	N 
386.	NO 	-	YES 	N 
387.	NO 	-	NO 	-
388.	NO 	-	NO 	-
389.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
390.	NO 	-	NO 	-
391.	NO 	-	YES 	NO 
392.	NO 	-	NO 	-
393.	NO 	-	NO 	-
394.	NO 	-	NO 	-
395.	NO 	-	NO 	-
396.	NO 	-	NO 	-
397.	NO 	-	NO 	-
398.	NO 	-	NO 	-
399.	NO 	-	NO 	-
400.	NO 	-	YES 	NO 
401.	NO 	-	NO 	
402.	N 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
403.	NO 	-	NO 	-
404.	NO 	-	NO 	-
405.	NO 	-	NO 	-
406.	NO 	-	NO 	-
407.	NO 	-	YES 	NO 
408.	NO 	-	NO 	-
409.	NO 	-	NO 	-
410.	NO 	-	NO 	-
411.	NO 	-	NO 	-
412.	NO 	-	YES 	NO 
413.	NO 	-	NO 	-
414.	NO 	-	NO 	-
415.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
416.	NO 	"	NO 	"
417.	NO 	"	NO 	"
418.	NO 	"	NO 	"
419.	NO 	"	YES 	NO 
420.	NO 	"	NO 	"
421.	NO 	"	NO 	"
422.	NO 	"	NO 	"
423.	NO 	"	NO 	"
424.	YES 	NO 	NO 	"
425.	NO 	"	NO 	"
426.	NO 	"	NO 	"
427.	N 	"	NO 	"
428.	NO 	"	NO 	"

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
429.	NO 	-	NO 	-
430.	NO 	-	NO 	-
431.	N 	-	NO 	-
432.	NO 	-	YES 	NO 
433.	NO 	-	NO 	-
434.	NO 	-	NO 	-
435.	NO 	-	NO 	-
436.	YES 	YES 	YES 	YES 
437.	NO 	-	NO 	-
438.	NO 	-	NO 	-
439.	NO 	-	NO 	-
440.	NO 	-	NO 	-
441.	YES 	YES 	YES 	NO 

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
442.	NO 	-	NO 	-
443.	NO 	-	NO 	-
444.	NO 	-	NO 	-
445.	YES 	NO 	NO 	-
446.	NO 	-	NO 	-
447.	NO 	-	NO 	-
448.	NO 	-	NO 	-
449.	NO 	-	NO 	-
450.	NO 	-	YES 	NO 
451.	YES 	YES 	NO 	-
452.	YES 	YES 	YES 	NO 
453.	NO 	-	NO 	-
454.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
455.	NO 	-	NO 	-
456.	YES 	NO 	YES 	YES 
457.	NO 	-	NO 	-
458.	NO 	-	NO 	-
459.	NO 	-	NO 	-
460.	YES 	YES 	NO 	-
461.	NO 	-	NO 	-
462.	NO 	-	NO 	-
463.	NO 	-	YES 	YES 
464.	NO 	-	NO 	-
465.	YES 	NO	NO 	-
466.	NO 	-	NO 	-
467.	NO 	-	YES 	NO 

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
468.	NO 	-	NO 	-
469.	YES 	NO 	NO 	-
470.	NO 	-	NO 	-
471.	NO 	-	YES 	NO 
472.	NO 	-	YES 	YES 
473.	YES 	YES 	YES 	NO 
474.	NO 	-	NO 	-
475.	YES 	YES 	NO 	-
476.	NO 	-	YES 	NO 
477.	NO 	-	NO 	-
478.	NO 	-	NO 	-
479.	NO 	-	NO 	-
480.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
481.	YES 	YES 	NO 	-
482.	NO 	-	NO 	-
483.	NO 	-	NO 	-
484.	YES 	YES 	NO 	-
485.	NO 	-	YES 	YES 
486.	NO 	-	NO 	-
487.	NO 	-	NO 	-
488.	NO 	-	NO 	-
489.	NO 	-	NO 	-
490.	YES 	YES 	NO 	-
491.	NO 	-	YES 	NO 
492.	NO 	-	NO 	-
493.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
494.	NO 	-	NO 	-
495.	NO 	-	NO 	-
496.	NO 	-	NO 	-
497.	NO 	-	NO 	-
498.	NO 	-	NO 	-
499.	NO 	-	NO 	-
500.	NO 	-	NO 	-
501.	NO 	-	NO 	-
502.	YES 	NO 	YES 	YES 
503.	NO 	-	NO 	-
504.	NO 	-	NO 	-
505.	NO 	-	NO 	-
506.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
507.	NO 	-	NO 	-
508.	NO 	-	NO 	-
509.	NO 	-	NO 	-
510.	NO 	-	NO 	-
511.	YES 	YES 	NO 	-
512.	NO 	-	NO 	-
513.	NO 	-	NO 	-
514.	NO 	-	NO 	-
515.	YES 	NO 	NO 	-
516.	NO 	-	NO 	-
517.	YES 	NO 	YES 	NO 
518.	YES 	YES 	YES 	NO 
519.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
520.	NO 	-	YES 	NO 
521.	NO 	-	NO 	-
522.	NO 	-	NO 	-
523.	NO 	-	YES 	NO 
524.	YES 	NO 	NO 	-
525.	NO 	-	NO 	-
526.	NO 	-	YES 	NO 
527.	NO 	-	YES 	NO 
528.	NO 	-	NO 	-
529.	NO 	-	NO 	-
530.	NO 	-	NO 	-
531.	YES 	YES 	NO 	-
532.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
533.	NO 	-	YES 	YES 
534.	NO 	-	NO 	-
535.	NO 	-	NO 	-
536.	YES 	YES 	YES 	NO 
537.	NO 	-	NO 	-
538.	NO 	-	YES 	YES 
539.	NO 	-	NO 	-
540.	NO 	-	YES 	YES 
541.	NO 	-	YES 	NO 
542.	NO 	-	NO 	-
543.	NO 	-	NO 	-
544.	NO 	-	YES 	YES 
545.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
546.	NO 	-	NO 	-
547.	NO 	-	YES 	YES 
548.	NO 	-	YES 	NO 
549.	NO 	-	NO 	-
550.	NO 	-	NO 	-
551.	NO 	-	NO 	-
552.	NO 	-	YES 	NO 
553.	NO 	-	NO 	-
554.	YES 	YES 	NO 	-
555.	YES 	NO 	NO 	-
556.	NO 	-	YES 	YES 
557.	NO 	-	YES 	YES 
558.	YES 	YES 	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
559.	NO 	-	NO 	-
560.	NO 	-	NO 	-
561.	NO 	-	YES 	NO 
562.	YES 	YES 	NO 	-
563.	YES 	NO 	NO 	-
564.	NO 	-	YES 	NO 
565.	NO 	-	NO 	-
566.	NO 	-	NO 	-
567.	NO 	-	NO 	-
568.	NO 	-	NO 	-
569.	NO 	-	NO 	-
570.	NO 	-	NO 	-
571.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
572.	NO 	-	NO 	-
573.	YES 	NO 	YES 	NO 
574.	YES 	YES 	NO 	-
575.	YES 	NO 	NO 	-
576.	YES 	NO 	NO 	-
577.	NO 	-	YES 	NO 
578.	NO 	-	NO 	-
579.	NO 	-	YES 	NO 
580.	YES 	YES 	NO 	-
581.	NO 	-	NO 	-
582.	NO 	-	NO 	-
583.	NO 	-	NO 	-
584.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
585.	YES 	YES 	NO 	-
586.	NO 	-	NO 	-
587.	NO 	-	NO 	-
588.	NO 	-	NO 	-
589.	NO 	-	NO 	-
590.	NO 	-	NO 	-
591.	NO 	-	NO 	-
592.	NO 	-	NO 	-
593.	NO 	-	NO 	-
594.	NO 	-	NO 	-
595.	YES 	YES 	NO 	-
596.	NO 	-	NO 	-
597.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
598.	YES 	YES 	YES 	YES 
599.	NO 	-	NO 	-
600.	NO 	-	NO 	-
601.	NO 	-	YES 	NO 
602.	NO 	-	NO 	
603.	NO 	-	NO 	-
604.	NO 	-	NO 	-
605.	NO 	-	NO 	-
606.	NO 	-	NO 	-
607.	YES 	NO 	NO 	-
608.	NO 	-	NO 	-
609.	NO 	-	NO 	-
610.	NO 	-	NO 	-

Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
611.	NO 	-	NO 	-
612.	YES 	YES 	NO	-
613.	NO 	-	YES 	NO 
614.	YES 	YES 	NO 	-
615.	NO 	-	NO 	-
616.	YES 	YES 	NO 	-
617.	NO 	-	NO 	-
618.	NO 	-	NO 	-
619.	NO 	-	NO 	-
620.	YES 	NO 	YES 	NO 
621.	NO 	-	NO 	-
622.	NO 	-	NO 	-
623.	NO 	-	NO 	-

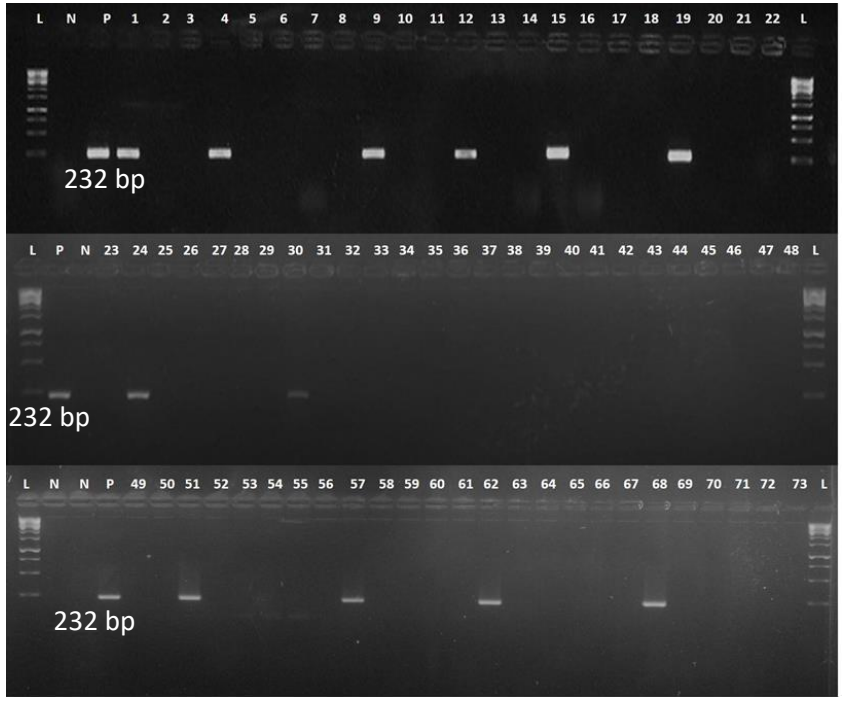
Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
624.	NO 	-	NO 	-
625.	NO 	-	NO 	-
626.	NO 	-	NO 	-
627.	NO 	-	NO 	-
628.	NO 	-	NO 	-
629.	YES 	NO 	NO 	-
630.	YES 	NO 	NO 	-
631.	NO 	-	NO 	-
632.	YES 	NO 	NO 	-
633.	NO 	-	NO 	-
634.	NO 	-	NO 	-
635.	NO 	-	NO 	-
636.	NO 	-	NO 	-

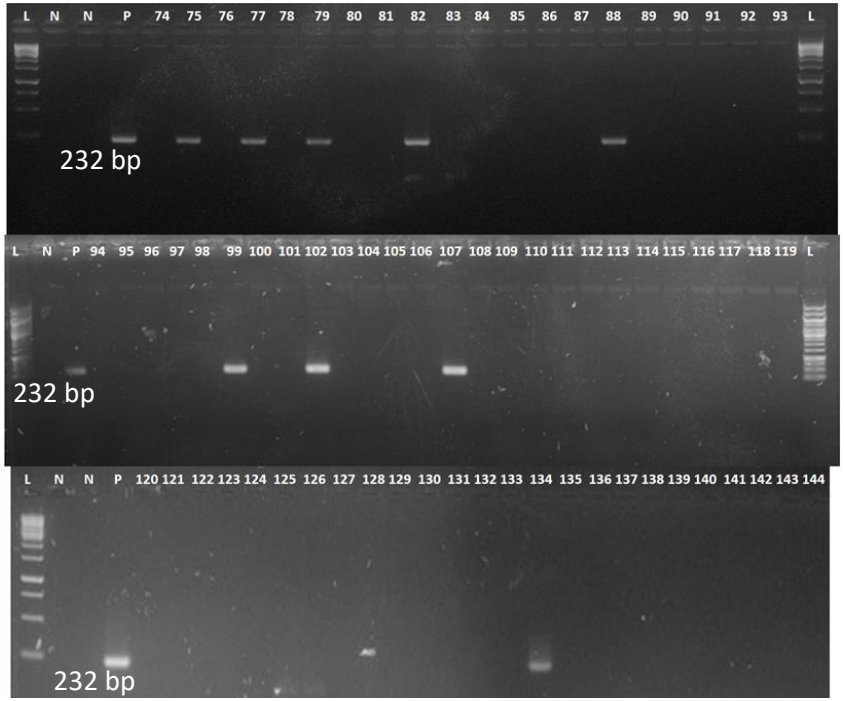
Sample No.	Positive for *E.coli*	Positive for Beta lactam resistant *E.coli*	Positive for *S. aureus*	Positive for Beta lactam resistant *S. aureus*
637.	NO 	-	NO 	-
638.	YES 	NO 	NO 	-
639.	NO 	-	NO 	-
640.	NO 	-	NO 	-
Total positives	**77**	**52**	**129**	**81**

(G.2) PCR

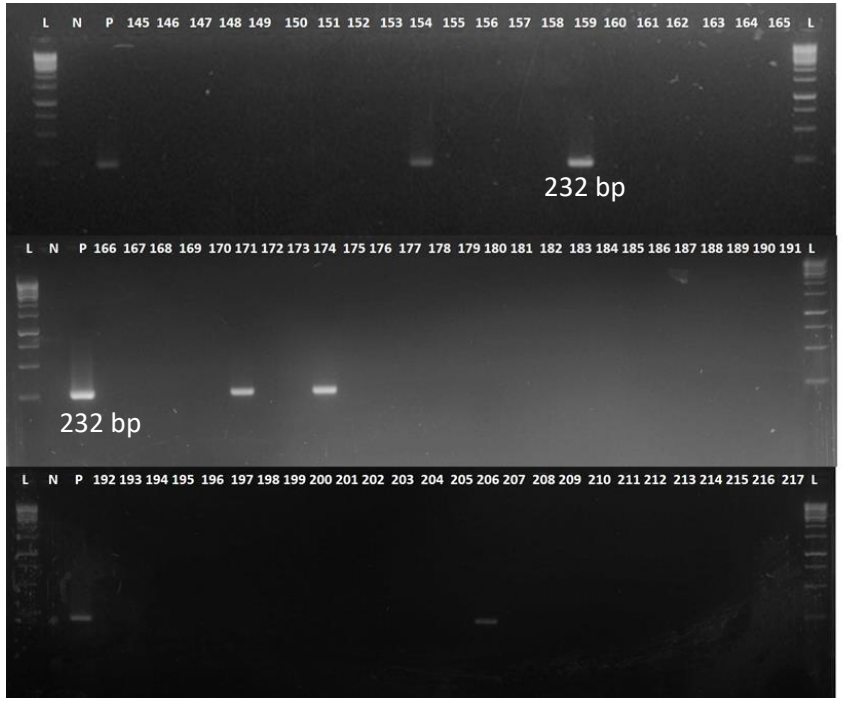
(G.2.1) *E. coli*

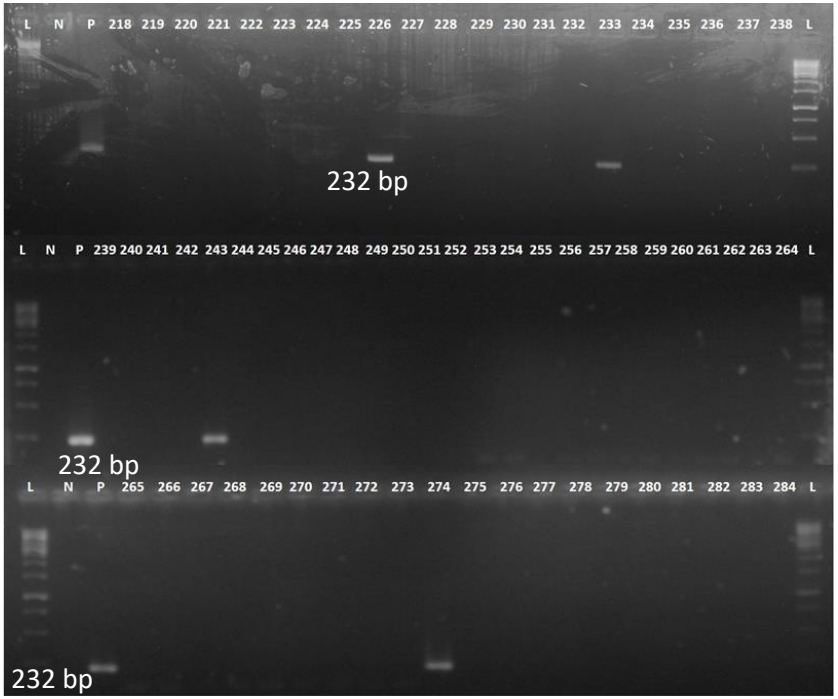
E. coli positive = presence of band at 232 bp



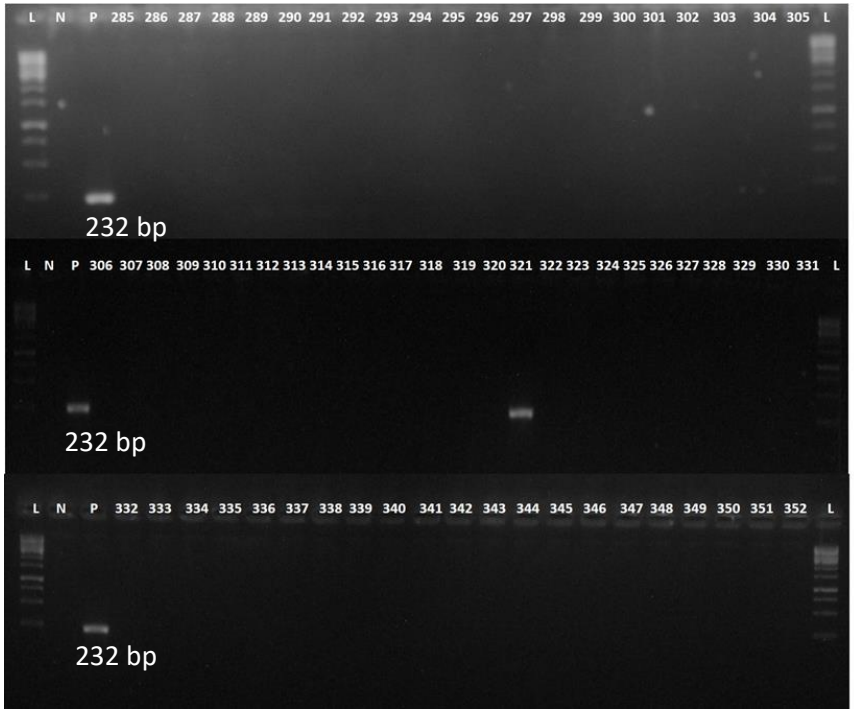


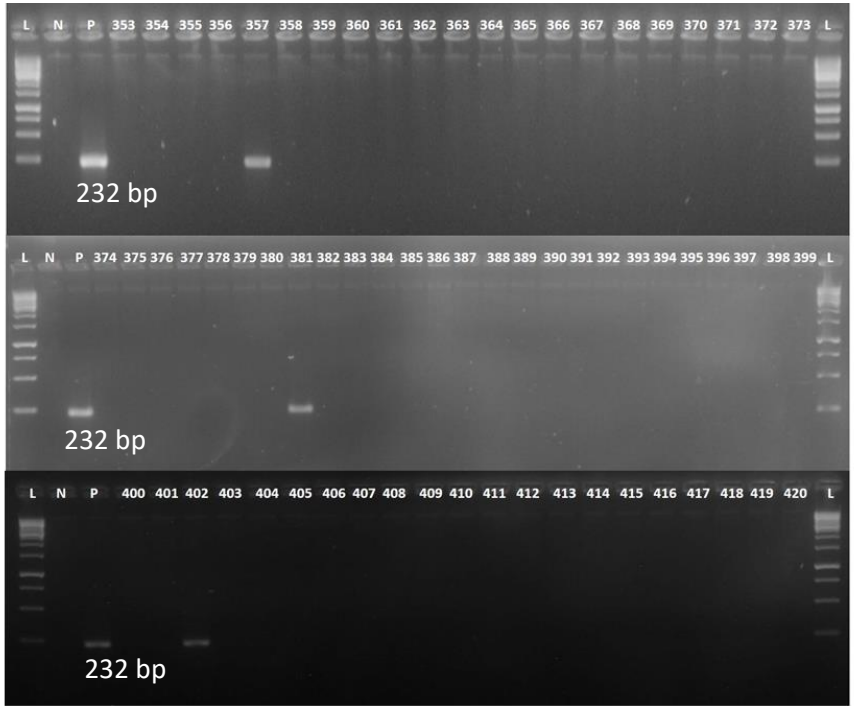
E. coli positive = presence of band at 232 bp



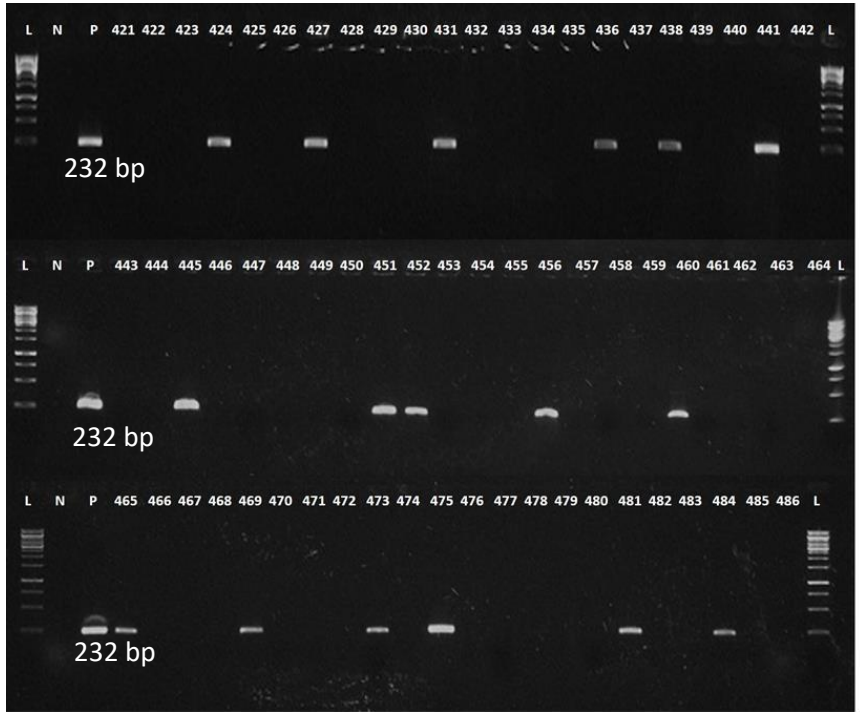


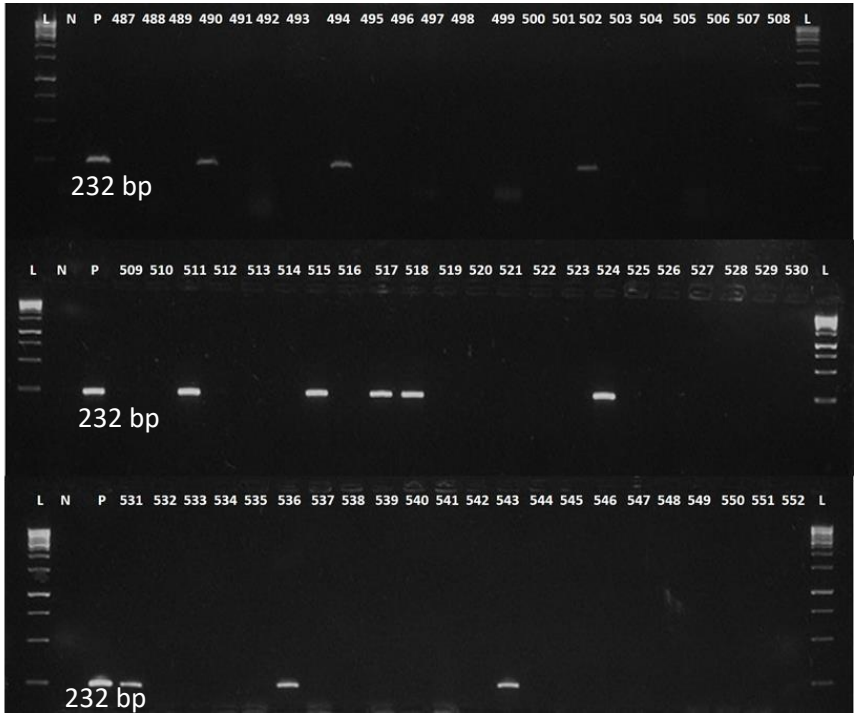
E. coli positive = presence of band at 232 bp



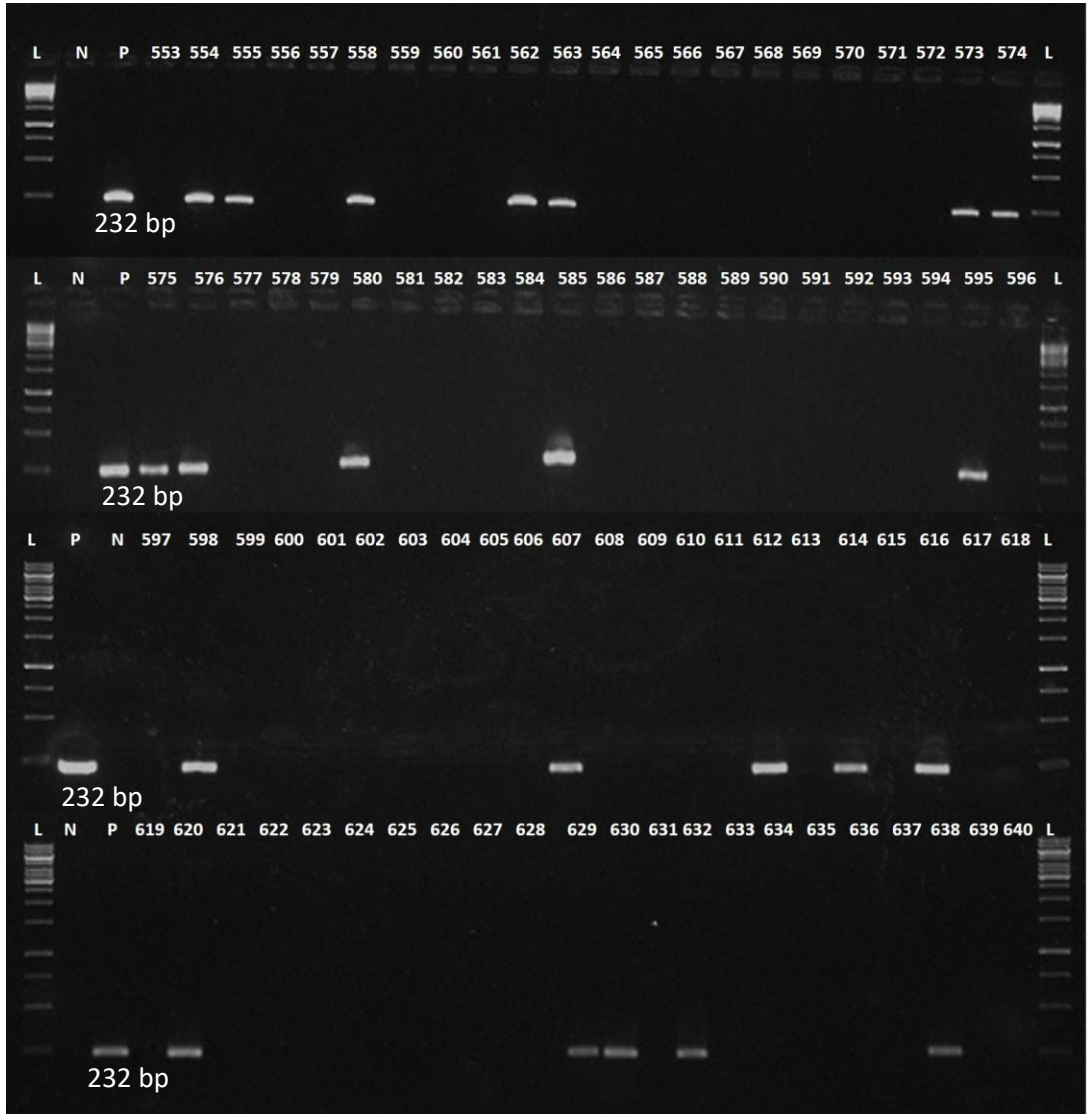


E. coli positive = presence of band at 232 bp



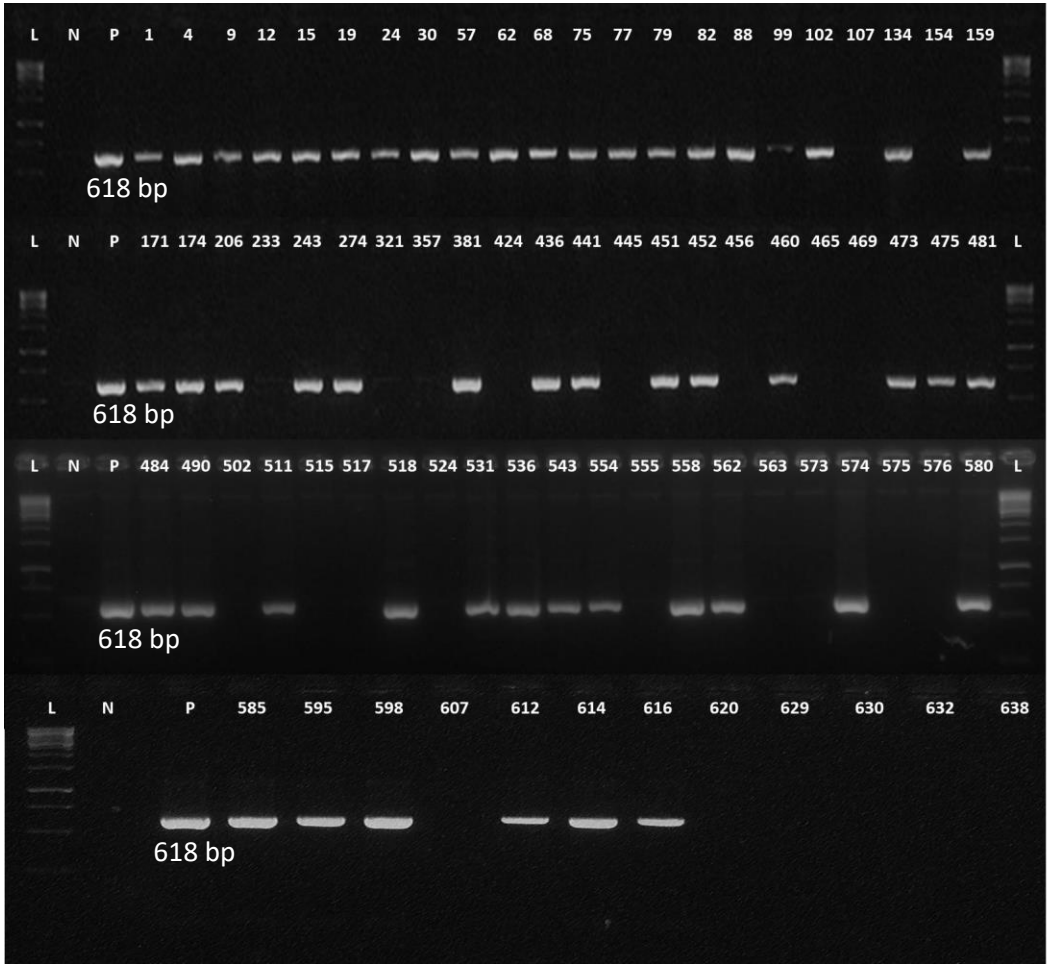


E. coli positive = presence of band at 232 bp



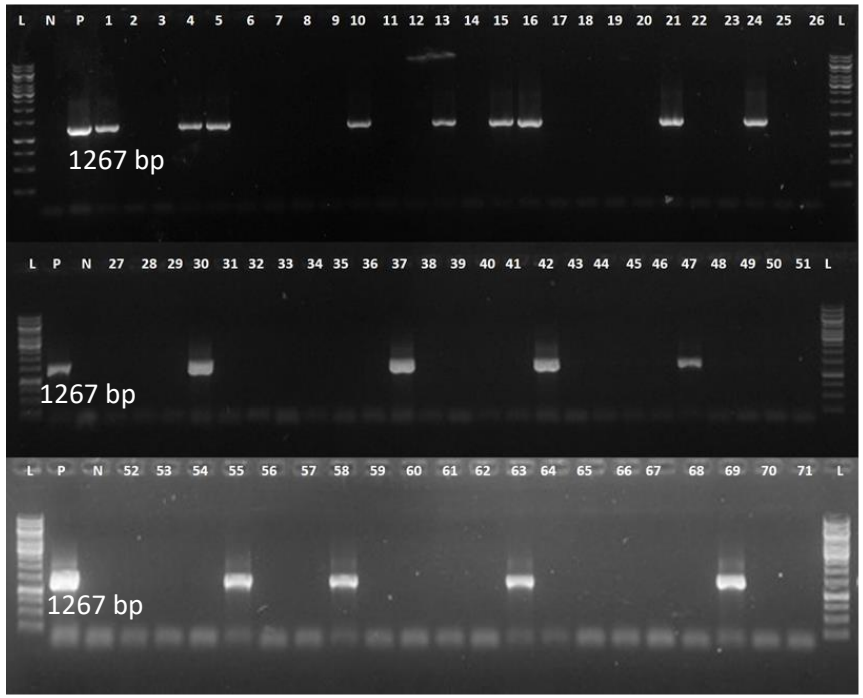
(G.2.2) Beta-lactam resistant *E. coli*

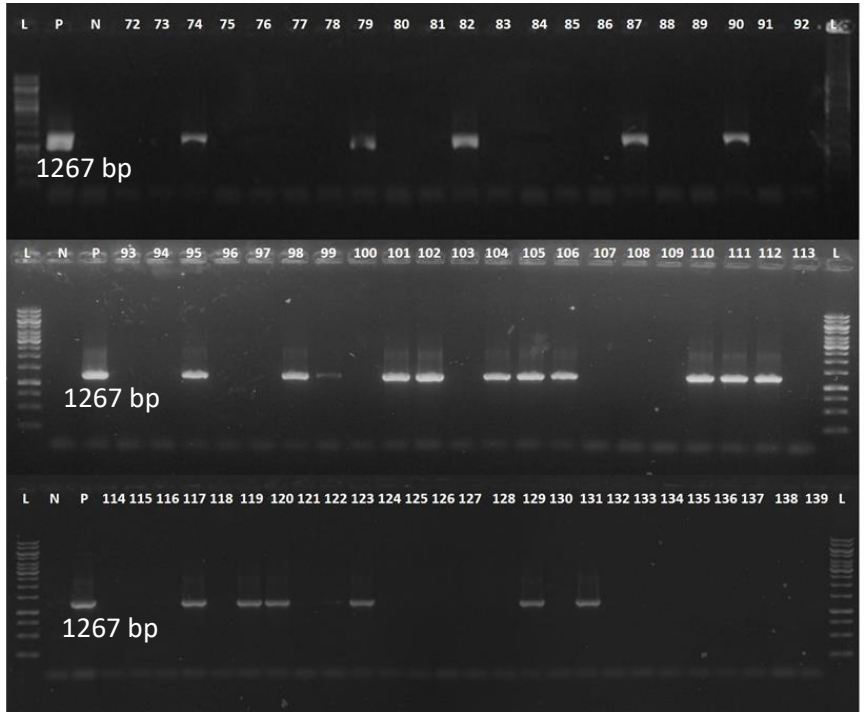
Beta-lactam resistant *E. coli* positive = presence of band at 618 bp



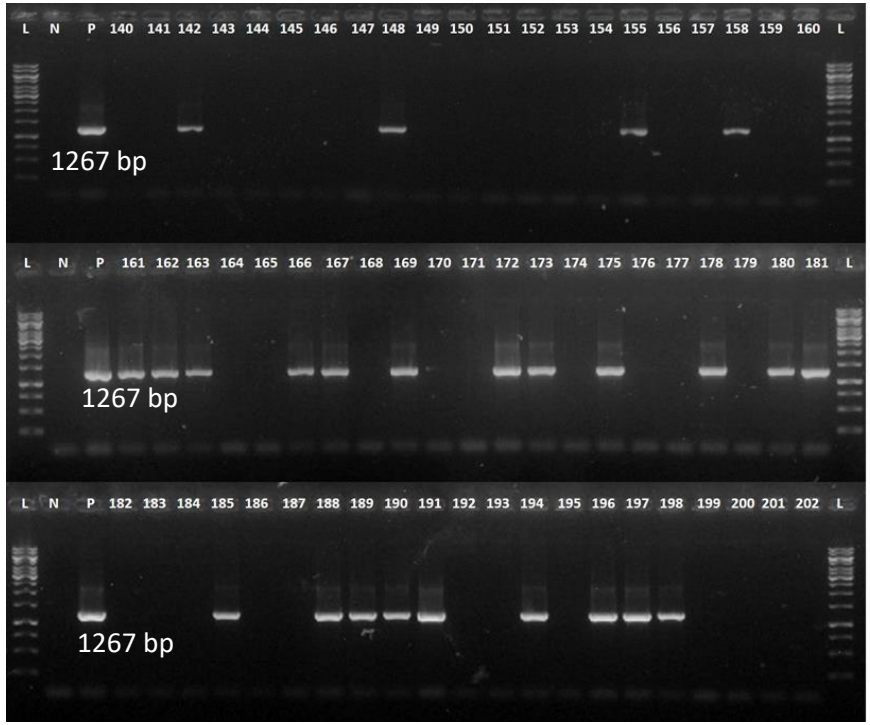
(G.2.3) *S. aureus*

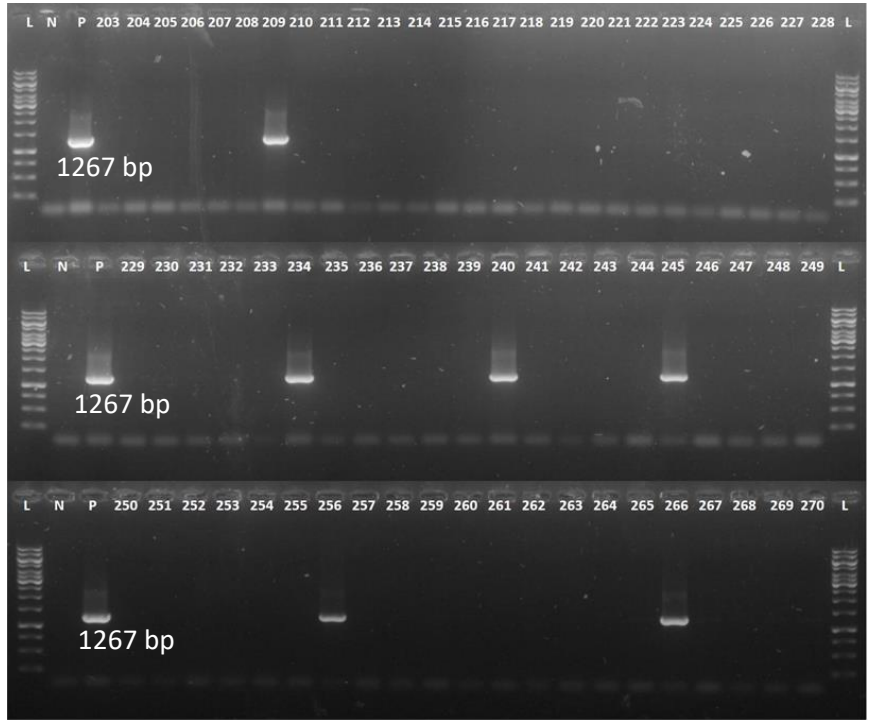
S. aureus positive = presence of band at 1267 bp



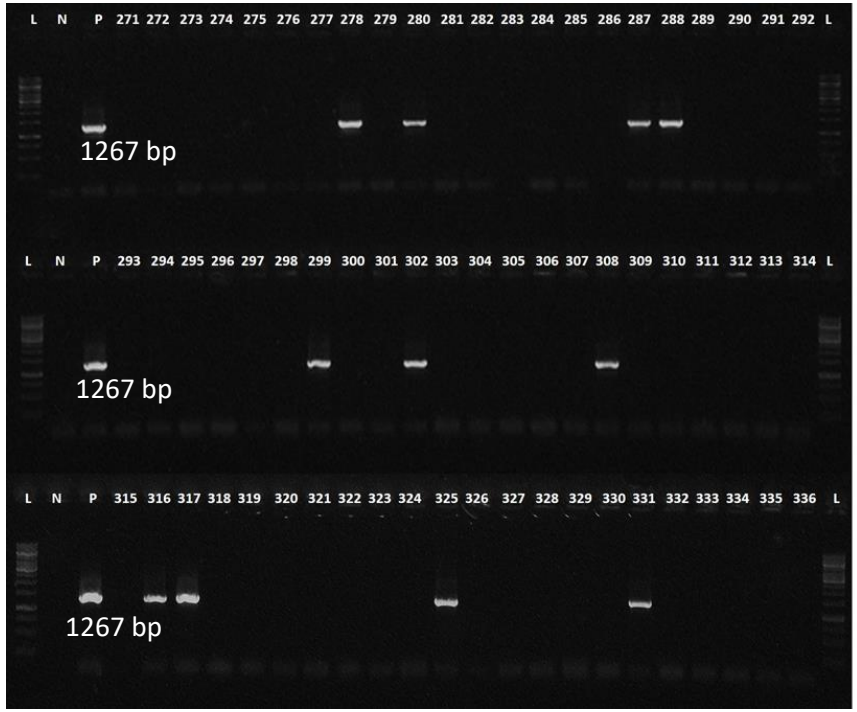


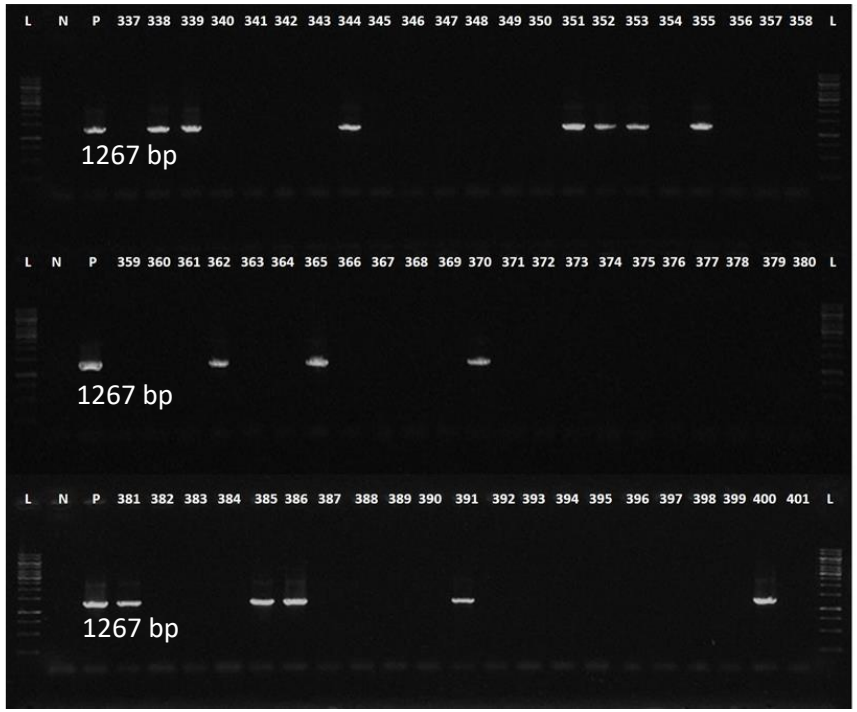
S. aureus positive = presence of band at 1267 bp



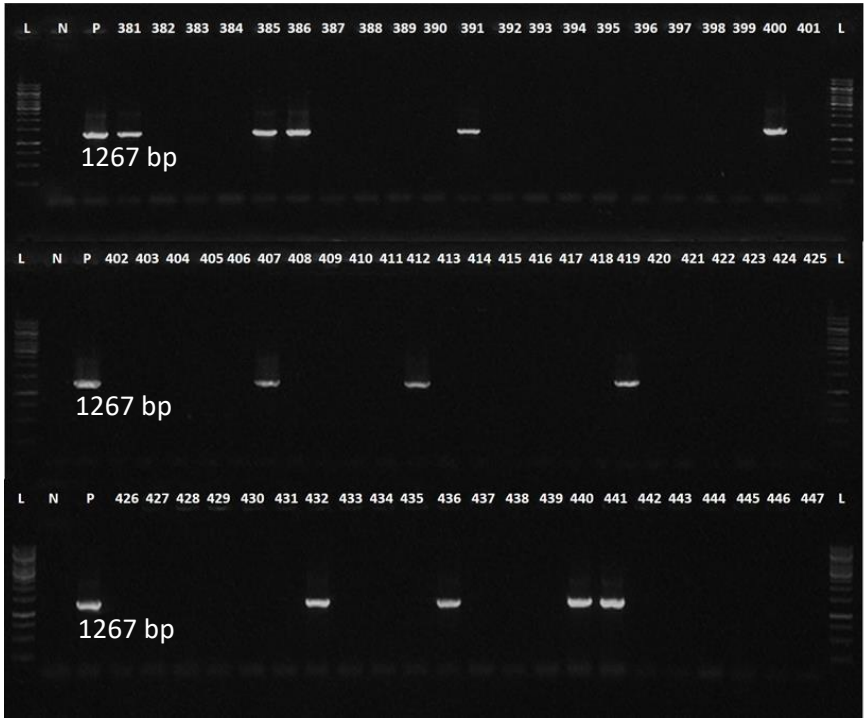


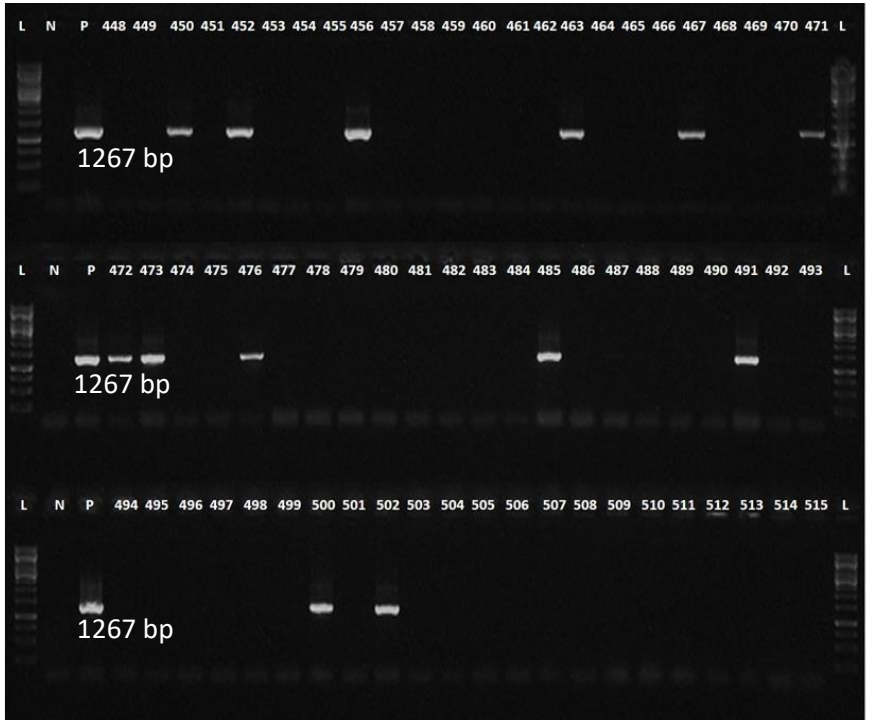
S. aureus positive = presence of band at 1267 bp



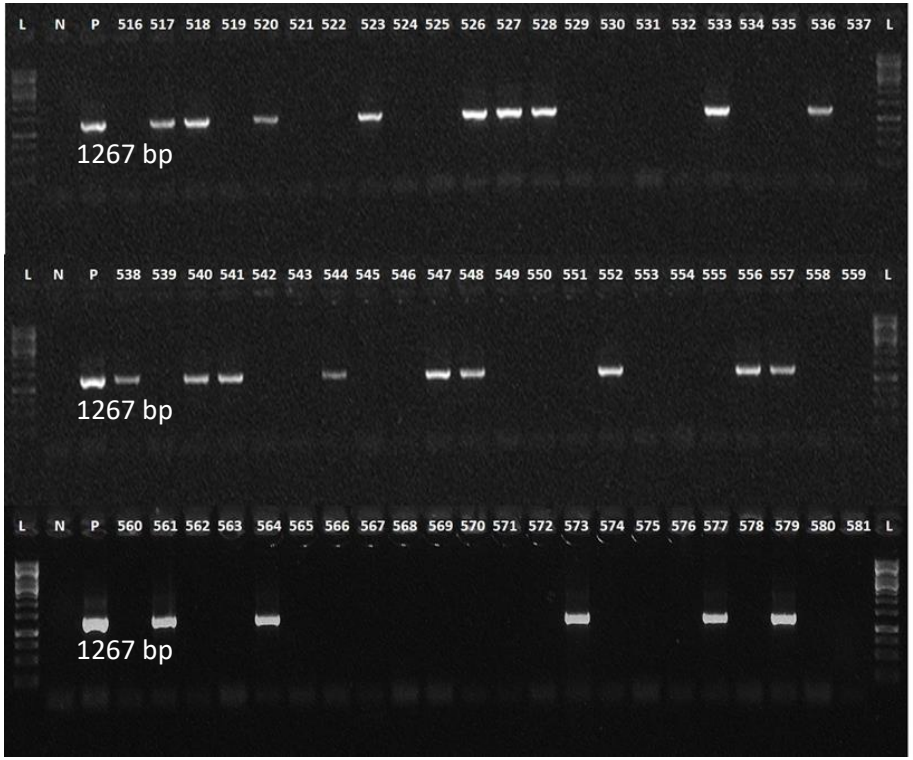


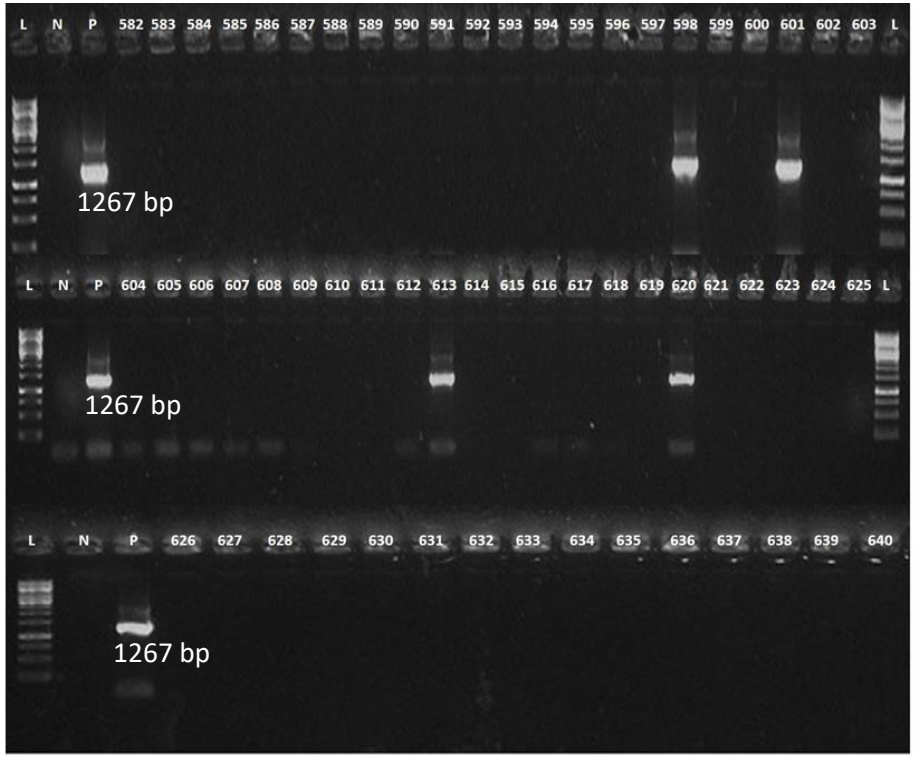
S. aureus positive = presence of band at 1267 bp





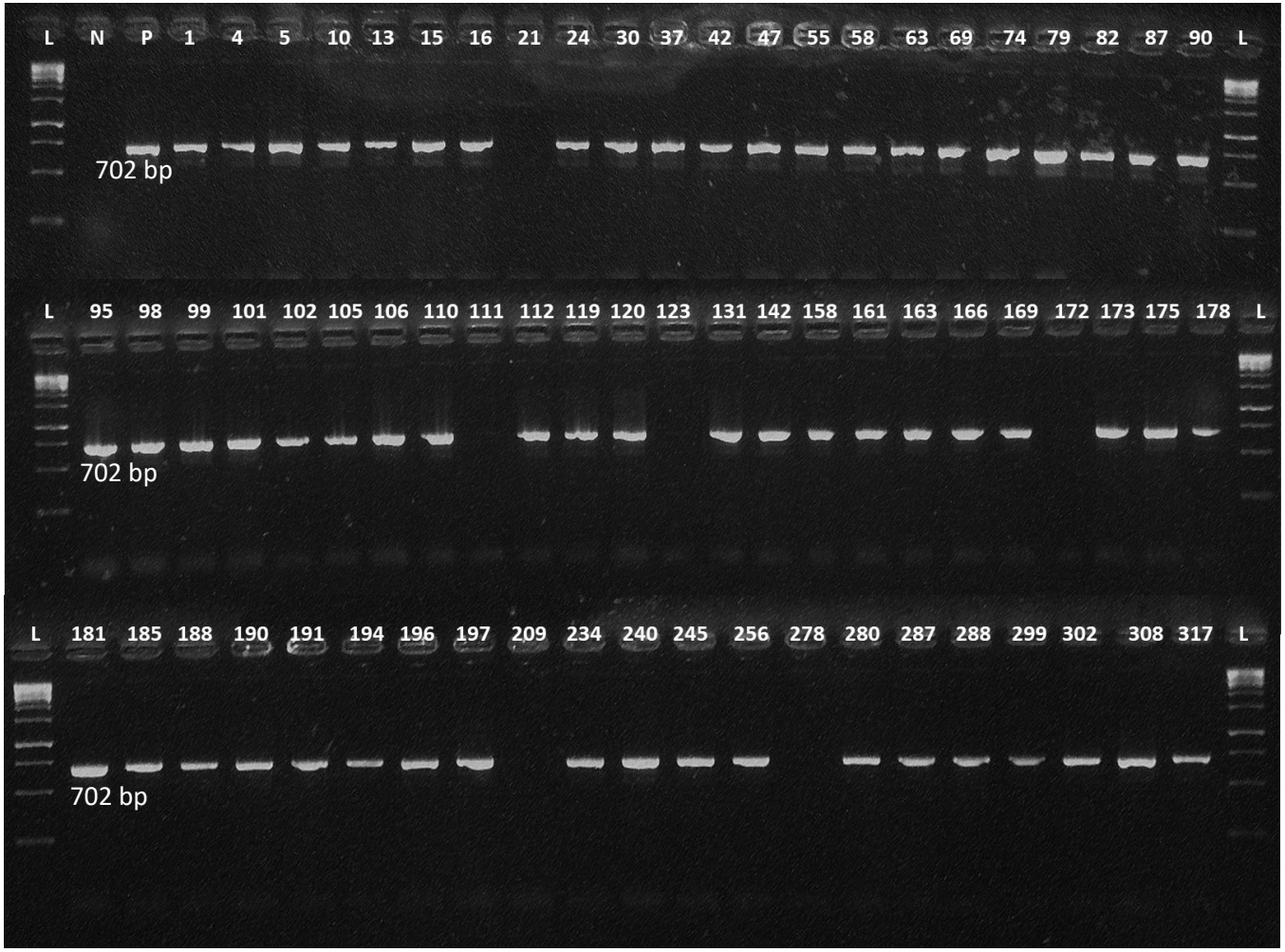
S. aureus positive = presence of band at 1267 bp





(G.2.4) Beta-lactam resistant *S. aureus*

Beta-lactam resistant *S. aureus* positive = presence of band at 702 bp



Beta-lactam resistant *S. aureus* positive = presence of band at 702 bp