

Clinically isolated β -lactam-resistant Gram-negative bacilli in a Philippine tertiary care hospital harbor multi-class β -lactamase genes

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Supplementary Data

Table S1. Primers, PCR conditions, positive controls, and reference literature used for the amplification of the 16S rRNA and target β -lactamase genes.

Gene Targets	Primer Sequence (5'–3') F– Forward, R– Reverse	Amplicon Size (bp)	Annealing Temperature (°C)	Positive Control	Primer Reference
16S rRNA	F– AGAGTTTGATCCTGGCTCAG R– GGTTACCTTGTTACGACTT	1350	58	<i>S. aureus</i> ATCC 29213	[29]
<i>bla</i> _{TEM}	F– CATTTCGTCGCGCCCTTATTC R– CGTTCATCCATAGTTGCCTGAC	800	61	<i>E. coli</i> ATCC 35218	[26]
<i>bla</i> _{SHV}	F– TTATCTCCCTGTTAGCCACC R– GATTGCTGATTTGCTCGG	797	51	<i>K. pneumoniae</i> ATCC 700603	[30]
<i>bla</i> _{CTX–M}	F– TTAGGAAGTGTGCCGCTGTA R– CGATATCGTTGGTGGTGCCAT	688	60	<i>E. coli</i> NCTC 13353	[26]
<i>bla</i> _{FOX}	F– AACATGGGGTATCAGGGAGATG R– CAAAGCGCGTAACCGGATTGG	190	60	<i>K. pneumoniae</i> ATCC 2146	[31]
<i>bla</i> _{DHA}	F– TGATGGCACAGCAGGATATTC R– GCTTTGACTCTTTGCGTATTCCG	997	60	<i>E. coli</i> TMC EF1	[26]
<i>bla</i> _{IMP}	F– GGAATAGAGTGGCTTAATTCTC R– GGTTTAACAAAACAACCACC	233	52	<i>E. coli</i> NCTC 13476	[27]
<i>bla</i> _{VIM}	F– ATGTTAAAAAGTTATTAGTAGTTTATTG R– CTACTCGGCGACTGAGC	801	57	<i>K. pneumoniae</i> NCTC 13440	[32]
<i>bla</i> _{KPC}	F– ATGTCACTGTATCGCCGTCT R– TTAGTCCCGTTGACGCCC	882	63	<i>K. pneumoniae</i> ATCC BAA 1705	[33]
<i>bla</i> _{NDM}	F– GGTTTGCGGATCTGGTTTTTC R– CGGAATGGCTCATCACGATC	621	60	<i>K. pneumoniae</i> ATCC BAA 2146	[32]
<i>bla</i> _{OXA–1}	F– TCTGTTGTTTGGGTTTCGC R– TCTATGGTGTCTTCTATGGCTG	245	52	<i>E. coli</i> ATCC BAA 2469	[35]
<i>bla</i> _{OXA–2}	F– ATTTCAAGCCAAAGGCACGA R– GCCACTCAACCCATCCTACC	569	52	<i>K. pneumoniae</i> ATCC 700603	[34]
<i>bla</i> _{OXA–23-like}	F– CTATTTTTGTCGTGTACAGAGC R– GGATCACAACAATAAAAGCACTG	1008	62	<i>A. baumannii</i> ATCC BAA 1605	[35]
<i>bla</i> _{OXA–24-like}	F– GGTTAGTTGGCC CCCTTAAA R– AGTTGAGCGAAA AGGGGATT	249	53	<i>A. baumannii</i> ATCC BAA 1605	[28]
<i>bla</i> _{OXA–48-like}	F– TTGGTGGCATCGATTATCGG R– GAGCACTTCTTTTGTGATGGC	744	52	<i>K. pneumoniae</i> NCTC 13442	[36]
<i>bla</i> _{OXA–51-like}	F– CCATGGCAATGAACATTAAAGCACTC TTAC R– CTATAAAATACCTAATTGTTCT	825	51	<i>A. baumannii</i> ATCC BAA 1605	[37]

Gene Targets	Primer Sequence (5'–3') F– Forward, R– Reverse	Amplicon Size (bp)	Annealing Temperature (°C)	Positive Control	Primer Reference
<i>bla</i> _{OXA-58-like}	F– AAGTATTGGGGCTTGTGCTG R– CCCCTCTGCGCTCTACATAC	599	58	<i>A. baumannii</i> TMC 32	[38]

Table S2. GenBank® Accession Numbers of the sequenced sample isolate genes. The genes presented include 16S rRNA– 16S ribosomal ribonucleic acid, CTX–M– Cefotaximase–Munich, TEM– Temoneira β –lactamase, SHV– Sulfhydryl reagent variable β –lactamase, NDM– New Delhi metallo– β –lactamase, IMP– Imipenemase, DHA– Dharhan β –lactamase, OXA– Oxacillinase.

Organism/ Detected Gene	GenBank Accession Number
<i>Klebsiella pneumoniae</i> KF1 16S rRNA	OQ117104
<i>Klebsiella pneumoniae</i> KF2 16S rRNA	OQ117105
<i>Klebsiella pneumoniae</i> KF3 16S rRNA	OQ117106
<i>Klebsiella pneumoniae</i> KF4 16S rRNA	OQ117107
<i>Klebsiella pneumoniae</i> KM1 16S rRNA	OQ117108
<i>Klebsiella pneumoniae</i> KM2 16S rRNA	OQ117109
<i>Klebsiella pneumoniae</i> KM3 16S rRNA	OQ117110
<i>Klebsiella pneumoniae</i> KM4 16S rRNA	OQ117111
<i>Escherichia coli</i> EF1 16S rRNA	OQ117096
<i>Escherichia coli</i> EF2 16S rRNA	OQ117097
<i>Escherichia coli</i> EF3 16S rRNA	OQ117098
<i>Escherichia coli</i> EF4 16S rRNA	OQ117099
<i>Escherichia coli</i> EM1 16S rRNA	OQ117100
<i>Escherichia coli</i> EM2 16S rRNA	OQ117101
<i>Escherichia coli</i> EM3 16S rRNA	OQ117102
<i>Escherichia coli</i> EM4 16S rRNA	OQ117103
<i>Acinetobacter baumannii</i> AF1 16S rRNA	OQ117089
<i>Acinetobacter baumannii</i> AF2 16S rRNA	OQ117090
<i>Acinetobacter baumannii</i> AF3 16S rRNA	OQ117091
<i>Acinetobacter baumannii</i> AF4 16S rRNA	OQ117092
<i>Acinetobacter baumannii</i> AM1 16S rRNA	OQ117093
<i>Acinetobacter baumannii</i> AM2 16S rRNA	OQ117094
<i>Acinetobacter baumannii</i> AM3 16S rRNA	OQ117095
<i>Pseudomonas aeruginosa</i> AF2 16S rRNA	OQ117112
<i>Pseudomonas aeruginosa</i> AF3 16S rRNA	OQ117113
<i>Pseudomonas aeruginosa</i> AF4 16S rRNA	OQ117114
<i>Pseudomonas aeruginosa</i> AM2 16S rRNA	OQ117115
<i>Pseudomonas aeruginosa</i> AM3 16S rRNA	OQ117116
<i>Pseudomonas aeruginosa</i> AM4 16S rRNA	OQ117117
<i>Escherichia coli</i> EF1 <i>bla</i> _{CTX–M}	OQ096344
<i>Escherichia coli</i> EF2 <i>bla</i> _{CTX–M}	OQ096345
<i>Escherichia coli</i> EF3 <i>bla</i> _{CTX–M}	OQ096346
<i>Escherichia coli</i> EF4 <i>bla</i> _{CTX–M}	OQ096347
<i>Escherichia coli</i> EM1 <i>bla</i> _{CTX–M}	OQ096340
<i>Escherichia coli</i> EM2 <i>bla</i> _{CTX–M}	OQ096341

Organism/ Detected Gene	GenBank Accession Number
<i>Escherichia coli</i> EM3 <i>bla</i> _{CTX-M}	OQ096342
<i>Escherichia coli</i> EM4 <i>bla</i> _{CTX-M}	OQ096343
<i>Klebsiella pneumoniae</i> KF1 <i>bla</i> _{CTX-M}	OQ096352
<i>Klebsiella pneumoniae</i> KF2 <i>bla</i> _{CTX-M}	OQ096353
<i>Klebsiella pneumoniae</i> KF3 <i>bla</i> _{CTX-M}	OQ096354
<i>Klebsiella pneumoniae</i> KF4 <i>bla</i> _{CTX-M}	OQ096355
<i>Klebsiella pneumoniae</i> KM1 <i>bla</i> _{CTX-M}	OQ096348
<i>Klebsiella pneumoniae</i> KM2 <i>bla</i> _{CTX-M}	OQ096349
<i>Klebsiella pneumoniae</i> KM3 <i>bla</i> _{CTX-M}	OQ096350
<i>Klebsiella pneumoniae</i> KM4 <i>bla</i> _{CTX-M}	OQ096351
<i>Pseudomonas aeruginosa</i> PM3 <i>bla</i> _{CTX-M}	OQ096356
<i>Acinetobacter baumannii</i> AM1 <i>bla</i> _{CTX-M}	OQ096357
<i>Acinetobacter baumannii</i> AM3 <i>bla</i> _{CTX-M}	OQ096358
<i>Escherichia coli</i> EF1 <i>bla</i> _{DHA}	OQ096359
<i>Escherichia coli</i> EF2 <i>bla</i> _{DHA}	OQ096360
<i>Escherichia coli</i> EF1 <i>bla</i> _{IMP}	OQ096361
<i>Escherichia coli</i> EF4 <i>bla</i> _{IMP}	OQ096362
<i>Klebsiella pneumoniae</i> KF4 <i>bla</i> _{IMP}	OQ096364
<i>Klebsiella pneumoniae</i> KM3 <i>bla</i> _{IMP}	OQ096363
<i>Pseudomonas aeruginosa</i> PF2 <i>bla</i> _{IMP}	OQ096365
<i>Pseudomonas aeruginosa</i> PF3 <i>bla</i> _{IMP}	OQ096366
<i>Pseudomonas aeruginosa</i> PF4 <i>bla</i> _{IMP}	OQ270746
<i>Pseudomonas aeruginosa</i> PM2 <i>bla</i> _{IMP}	OQ270743
<i>Pseudomonas aeruginosa</i> PM3 <i>bla</i> _{IMP}	OQ270744
<i>Pseudomonas aeruginosa</i> PM4 <i>bla</i> _{IMP}	OQ270745
<i>Acinetobacter baumannii</i> AF1 <i>bla</i> _{IMP}	OQ096368
<i>Acinetobacter baumannii</i> AF2 <i>bla</i> _{IMP}	OQ270742
<i>Acinetobacter baumannii</i> AF4 <i>bla</i> _{IMP}	OQ096369
<i>Acinetobacter baumannii</i> AM3 <i>bla</i> _{IMP}	OQ096367
<i>Escherichia coli</i> EF2 <i>bla</i> _{NDM}	OQ096374
<i>Escherichia coli</i> EF3 <i>bla</i> _{NDM}	OQ096375
<i>Escherichia coli</i> EF4 <i>bla</i> _{NDM}	OQ096376
<i>Escherichia coli</i> EM1 <i>bla</i> _{NDM}	OQ096370
<i>Escherichia coli</i> EM2 <i>bla</i> _{NDM}	OQ096371
<i>Escherichia coli</i> EM3 <i>bla</i> _{NDM}	OQ096372
<i>Escherichia coli</i> EM4 <i>bla</i> _{NDM}	OQ096373
<i>Klebsiella pneumoniae</i> KF2 <i>bla</i> _{NDM}	OQ096380
<i>Klebsiella pneumoniae</i> KF4 <i>bla</i> _{NDM}	OQ270747
<i>Klebsiella pneumoniae</i> KM2 <i>bla</i> _{NDM}	OQ096377
<i>Klebsiella pneumoniae</i> KM3 <i>bla</i> _{NDM}	OQ096378
<i>Klebsiella pneumoniae</i> KM4 <i>bla</i> _{NDM}	OQ096379

Organism/ Detected Gene	GenBank Accession Number
<i>Pseudomonas aeruginosa</i> PF2 <i>bla</i> _{NDM}	OQ096382
<i>Pseudomonas aeruginosa</i> PM3 <i>bla</i> _{NDM}	OQ096381
<i>Acinetobacter baumannii</i> AF1 <i>bla</i> _{NDM}	OQ096385
<i>Acinetobacter baumannii</i> AF2 <i>bla</i> _{NDM}	OQ096386
<i>Acinetobacter baumannii</i> AM1 <i>bla</i> _{NDM}	OQ096383
<i>Acinetobacter baumannii</i> AM3 <i>bla</i> _{NDM}	OQ096384
<i>Escherichia coli</i> EF1 <i>bla</i> _{OXA-1}	OQ096391
<i>Escherichia coli</i> EF2 <i>bla</i> _{OXA-1}	OQ096392
<i>Escherichia coli</i> EF3 <i>bla</i> _{OXA-1}	OQ096393
<i>Escherichia coli</i> EF4 <i>bla</i> _{OXA-1}	OQ096394
<i>Escherichia coli</i> EM1 <i>bla</i> _{OXA-1}	OQ096387
<i>Escherichia coli</i> EM2 <i>bla</i> _{OXA-1}	OQ096388
<i>Escherichia coli</i> EM3 <i>bla</i> _{OXA-1}	OQ096389
<i>Escherichia coli</i> EM4 <i>bla</i> _{OXA-1}	OQ096390
<i>Klebsiella pneumoniae</i> KF2 <i>bla</i> _{OXA-1}	OQ270748
<i>Klebsiella pneumoniae</i> KF3 <i>bla</i> _{OXA-1}	OQ270749
<i>Klebsiella pneumoniae</i> KF4 <i>bla</i> _{OXA-1}	OQ270750
<i>Klebsiella pneumoniae</i> KM1 <i>bla</i> _{OXA-1}	OQ096395
<i>Klebsiella pneumoniae</i> KM2 <i>bla</i> _{OXA-1}	OQ096396
<i>Klebsiella pneumoniae</i> KM3 <i>bla</i> _{OXA-1}	OQ096397
<i>Klebsiella pneumoniae</i> KM4 <i>bla</i> _{OXA-1}	OQ096398
<i>P. aeruginosa</i> PF2 <i>bla</i> _{OXA-1}	OQ270753
<i>P. aeruginosa</i> PF3 <i>bla</i> _{OXA-1}	OQ270754
<i>P. aeruginosa</i> PM3 <i>bla</i> _{OXA-1}	OQ270751
<i>P. aeruginosa</i> PM4 <i>bla</i> _{OXA-1}	OQ270752
<i>Escherichia coli</i> EF1 <i>bla</i> _{OXA-24-like}	OQ096438
<i>Escherichia coli</i> EF4 <i>bla</i> _{OXA-24-like}	OQ096439
<i>Escherichia coli</i> EM4 <i>bla</i> _{OXA-24-like}	OQ096437
<i>Klebsiella pneumoniae</i> KF4 <i>bla</i> _{OXA-24-like}	OQ096443
<i>Klebsiella pneumoniae</i> KM1 <i>bla</i> _{OXA-24-like}	OQ096440
<i>Klebsiella pneumoniae</i> KM3 <i>bla</i> _{OXA-24-like}	OQ096441
<i>Klebsiella pneumoniae</i> KM4 <i>bla</i> _{OXA-24-like}	OQ096442
<i>Pseudomonas aeruginosa</i> PF2 <i>bla</i> _{OXA-24-like}	OQ096447
<i>Pseudomonas aeruginosa</i> PM2 <i>bla</i> _{OXA-24-like}	OQ096444
<i>Pseudomonas aeruginosa</i> PM3 <i>bla</i> _{OXA-24-like}	OQ096445
<i>Pseudomonas aeruginosa</i> PM4 <i>bla</i> _{OXA-24-like}	OQ096446
<i>Acinetobacter baumannii</i> PF1 <i>bla</i> _{OXA-24-like}	OQ096451
<i>Acinetobacter baumannii</i> PF2 <i>bla</i> _{OXA-24-like}	OQ096452
<i>Acinetobacter baumannii</i> AF3 <i>bla</i> _{OXA-24-like}	OQ096453
<i>Acinetobacter baumannii</i> AF4 <i>bla</i> _{OXA-24-like}	OQ096454
<i>Acinetobacter baumannii</i> AM1 <i>bla</i> _{OXA-24-like}	OQ096448

Organism/ Detected Gene	GenBank Accession Number
<i>Acinetobacter baumannii</i> AM2 <i>bla</i> _{OXA-24-like}	OQ096449
<i>Acinetobacter baumannii</i> AM3 <i>bla</i> _{OXA-24-like}	OQ096450
<i>Acinetobacter baumannii</i> AF1 <i>bla</i> _{OXA-51-like}	OQ096402
<i>Acinetobacter baumannii</i> AF2 <i>bla</i> _{OXA-51-like}	OQ096403
<i>Acinetobacter baumannii</i> AF3 <i>bla</i> _{OXA-51-like}	OQ096404
<i>Acinetobacter baumannii</i> AF4 <i>bla</i> _{OXA-51-like}	OQ096405
<i>Acinetobacter baumannii</i> AM1 <i>bla</i> _{OXA-51-like}	OQ096399
<i>Acinetobacter baumannii</i> AM2 <i>bla</i> _{OXA-51-like}	OQ096400
<i>Acinetobacter baumannii</i> AM3 <i>bla</i> _{OXA-51-like}	OQ096401
<i>Klebsiella pneumoniae</i> KF1 <i>bla</i> _{SHV}	OQ096410
<i>Klebsiella pneumoniae</i> KF2 <i>bla</i> _{SHV}	OQ096411
<i>Klebsiella pneumoniae</i> KF4 <i>bla</i> _{SHV}	OQ096412
<i>Klebsiella pneumoniae</i> KM1 <i>bla</i> _{SHV}	OQ096406
<i>Klebsiella pneumoniae</i> KM2 <i>bla</i> _{SHV}	OQ096407
<i>Klebsiella pneumoniae</i> KM3 <i>bla</i> _{SHV}	OQ096408
<i>Klebsiella pneumoniae</i> KM4 <i>bla</i> _{SHV}	OQ096409
<i>Pseudomonas aeruginosa</i> PF3 <i>bla</i> _{SHV}	OQ096413
<i>Acinetobacter baumannii</i> AF2 <i>bla</i> _{SHV}	OQ096414
<i>Acinetobacter baumannii</i> AF3 <i>bla</i> _{SHV}	OQ096417
<i>Acinetobacter baumannii</i> AF4 <i>bla</i> _{SHV}	OQ096418
<i>Acinetobacter baumannii</i> AM1 <i>bla</i> _{SHV}	OQ096415
<i>Acinetobacter baumannii</i> AM3 <i>bla</i> _{SHV}	OQ096416
<i>Escherichia coli</i> EF1 <i>bla</i> _{TEM}	OQ096423
<i>Escherichia coli</i> EF2 <i>bla</i> _{TEM}	OQ096424
<i>Escherichia coli</i> EF3 <i>bla</i> _{TEM}	OQ096425
<i>Escherichia coli</i> EF4 <i>bla</i> _{TEM}	OQ096426
<i>Escherichia coli</i> EM1 <i>bla</i> _{TEM}	OQ096419
<i>Escherichia coli</i> EM2 <i>bla</i> _{TEM}	OQ096420
<i>Escherichia coli</i> EM3 <i>bla</i> _{TEM}	OQ096421
<i>Escherichia coli</i> EM4 <i>bla</i> _{TEM}	OQ096422
<i>Klebsiella pneumoniae</i> KF2 <i>bla</i> _{TEM}	OQ096431
<i>Klebsiella pneumoniae</i> KF3 <i>bla</i> _{TEM}	OQ096432
<i>Klebsiella pneumoniae</i> KF4 <i>bla</i> _{TEM}	OQ096433
<i>Klebsiella pneumoniae</i> KM1 <i>bla</i> _{TEM}	OQ096427
<i>Klebsiella pneumoniae</i> KM2 <i>bla</i> _{TEM}	OQ096428
<i>Klebsiella pneumoniae</i> KM3 <i>bla</i> _{TEM}	OQ096429
<i>Klebsiella pneumoniae</i> KM4 <i>bla</i> _{TEM}	OQ096430
<i>Pseudomonas aeruginosa</i> PF3 <i>bla</i> _{TEM}	OQ096436
<i>Pseudomonas aeruginosa</i> PM3 <i>bla</i> _{TEM}	OQ096434
<i>Pseudomonas aeruginosa</i> PM4 <i>bla</i> _{TEM}	OQ096435

Table S3. GenBank® Accession Numbers of the reference sequences and their corresponding phylogenetic tree construction parameters. The chosen reference sequences were based on the initial Basic Local Alignment Search Tool (BLAST) analysis of the study sequences. Tree construction parameters were based on the best nucleotide substitution model identified using Akaike Information Criterion (AIC) analysis in the jModelTest version 20160303. The genes presented include 16S rRNA– 16S ribosomal ribonucleic acid, CTX–M– Cefotaximase–Munich, TEM– Temoneira β –lactamase, SHV– Sulfhydryl reagent variable β –lactamase, NDM– New Delhi metallo– β –lactamase, IMP– Imipenemase, DHA– Dharhan β –lactamase, OXA– Oxacillinase. The chosen models include GTR+G– General Time Reversible model with gamma shape, TrN– Tamura–Nei model, TrN+I– Tamura–Nei with proportion of invariant sites, HKY+G– Hasegawa–Kishino–Yano model with gamma shape, TPM1uf– Three–parameter model 1 with unequal frequencies, TPM2uf– Three–parameter model 2 with unequal frequencies, TPM2uf+I– Three–parameter model 2 with unequal frequencies and proportion of invariant sites, K80– Kimura model.

Target Gene	Reference Sequences (GenBank Accession Numbers)	Tree Construction Parameters
16S rRNA	<i>Klebsiella pneumoniae</i> (LC557135) <i>Klebsiella aerogenes</i> (NR_102493) <i>Salmonella typhimurium</i> (DQ153191) <i>Citrobacter freundii</i> (MF953251) <i>Escherichia coli</i> (OP986844) <i>Escherichia coli</i> (NR_024570) <i>Yersinia pestis</i> (NR_025160) <i>Serratia liquefaciens</i> (NR_122057) <i>Proteus vulgaris</i> (NR_115878) <i>Pseudomonas aeruginosa</i> (NR_026078) <i>Pseudomonas denitrificans</i> (MK085084) <i>Pseudomonas fluorescens</i> (ON908465) <i>Pseudomonas syringae</i> (NR_043716) <i>Acinetobacter johnsonii</i> (NR_164627) <i>Acinetobacter calcoaceticus</i> (NR_042387) <i>Acinetobacter baumannii</i> (NR_117931) <i>Acinetobacter baumannii</i> (NR_026206)	Model = GTR+G Substitution rates: [AC] = 0.7468 [AG] = 2.4750 [AT] = 1.8033 [CG] = 0.8992 [CT] = 4.5721 [GT] = 1.0000 gamma shape = 0.3290
<i>bla</i> CTX–M	<i>bla</i> CTX–M–1 (DQ915955) <i>bla</i> CTX–M–10 (AF255298) <i>bla</i> CTX–M–15 (JN788266) <i>bla</i> CTX–M–42 (DQ061159) <i>bla</i> CTX–M–64 (GQ300937) <i>bla</i> CTX–M–117 (JN227085) <i>bla</i> CTX–M–123 (JN790864) <i>bla</i> CTX–M–167 (KR537428) <i>bla</i> CTX–M–179 (KU705393)	Model = TrN Substitution Rates: [AC] = 1.0000 [AG] = 1.4479 [AT] = 1.0000 [CG] = 1.0000 [CT] = 2.6278 [GT] = 1.0000
<i>bla</i> DHA	<i>bla</i> DHA–1 (JN638038) <i>bla</i> DHA–2 (AF259520) <i>bla</i> DHA–12 (HG798963) <i>bla</i> DHA–14 (DQ478687) <i>bla</i> DHA–20 (KM087848) <i>bla</i> DHA–22 (KM08756) <i>bla</i> DHA–23 (KX068223)	Model = TrN+I Substitution Rates [AC] = 1.0000 [AG] = 3.3526 [AT] = 1.0000 [CG] = 1.0000 [CT] = 7.8918 [GT] = 1.0000

Target Gene	Reference Sequences (GenBank Accession Numbers)	Tree Construction Parameters
		proportion of invariant sites = 0.7340
<i>blaIMP</i>	<i>blaIMP-1</i> (EF027105) <i>blaIMP-2</i> (AJ243491) <i>blaIMP-4</i> (AF244145) <i>blaIMP-5</i> (AF290912) <i>blaIMP-7</i> (AF318077) <i>blaIMP-9</i> (FJ655384) <i>blaIMP-11</i> (AB074436) <i>blaIMP-12</i> (AJ420864) <i>blaIMP-14</i> (KJ406505) <i>blaIMP-18</i> (AY780674)	Model = HKY+I kappa = 4.3430 transition/ transversion= 2.0136 proportion of invariant sites = 0.4180
<i>blaNDM</i>	<i>blaNDM-1</i> (KJ018857) <i>blaNDM-7</i> (KP826705) <i>blaNDM-29</i> (MN624980)	Model = TPM1uf Substitution Rates: [AC] = 1.0000 [AG] = 1.0110 [AT] = 0.0100 [CG] = 0.0100 [CT] = 1.0110 [GT] = 1.0000
<i>blaOXA-1</i>	<i>blaOXA-1</i> (HQ170510) <i>blaOXA-47</i> (AY237830) <i>blaOXA-675</i> (MH780096)	Model = HKY kappa = 11.7342 transition/ transversion = 5.0996
<i>blaOXA-24-like</i>	<i>blaOXA-24</i> (AY082394) <i>blaOXA-25</i> (AF201826) <i>blaOXA-72</i> (AY739646) <i>blaOXA-160</i> (GU199038)	Model = TPM1uf Substitution Rates: [AC] = 1.0000 [AG] = 3.0912 [AT] = 2.2553 [CG] = 2.2553 [CT] = 3.0912 [GT] = 1.0000
<i>blaOXA-51-like</i>	<i>blaOXA-51</i> (DQ385606) <i>blaOXA-66</i> (AY750909) <i>blaOXA-68</i> (AY750910) <i>blaOXA-69</i> (AY859527) <i>blaOXA-70</i> (AY750912) <i>blaOXA-71</i> (AY859528) <i>blaOXA-90</i> (EU547443)	Model = HKY+G kappa = 8.1963 transition/ transversion = 3.9285 gamma shape = 0.0740
<i>blaSHV</i>	<i>blaSHV-1</i> (AY787643) <i>blaSHV-5</i> (EF653399) <i>blaSHV-27</i> (AF293345) <i>blaSHV-28</i> (AF299299)	Model = TPM2uf+I Substitution Rates: [AC] = 0.0100 [AG] = 6.7465 [AT] = 0.0100 [CG] = 1.0000 [CT] = 6.7465 [GT] = 1.0000 proportion of invariant sites = 0.9700
<i>blaTEM</i>	<i>blaTEM-1</i> (AY263331) <i>blaTEM-3</i> (FKZZ01000044) <i>blaTEM-5</i> (NG_068215) <i>blaTEM-20</i> (EU527189)	Model = K80 kappa = 2.2351 transition/ transversion = 1.1175

Table S4. Antibigram profiles of the study isolates showed category-specific resistance to different antibiotics. The profiles were generated from Vitek® 2 Compact (bioMérieux, France). Resistance Category 1 (RC1; strains F1, M1) refers to the isolates non-susceptible to third-generation cephalosporins, RC2 (strains F2, M2) refers to those non-susceptible to third-generation cephalosporins and penicillin-β-lactamase inhibitors, RC3 to those resistant to carbapenems and other β-lactams (strains F3, M3), and RC4 to those with clinically unusual resistance profiles (levofloxacin-resistant but ertapenem-susceptible *Enterobacteriaceae*; carbapenem-resistant but cephalosporin-susceptible *P. aeruginosa* and *A. baumannii*, strains F4, M4). The antibiotics presented include AMC– amoxicillin-clavulanic acid, AMP– ampicillin, AMK– amikacin, CAZ– ceftazidime, CIP– ciprofloxacin, CTR– ceftriaxone, CFX– cefuroxime, ERT– ertapenem, CEF– cefepime, NIT– nitrofurantoin, GEN– gentamicin, IMI– imipenem, LEV– levofloxacin, MEM– meropenem, SX – trimethoprim-sulfamethoxazole, TZP– piperacillin-tazobactam. The profiles presented are based on the latest Clinical and Laboratory Standards Institute breakpoints and recommendations: S– susceptible, I– intermediate, R– resistant, NT– not tested.

	AMC	AMP	AMK	CAZ	CIP	CTR	CFX	ERT	CEF	NIT	GEN	IMI	LEV	MEM	SXT	TZP
<i>Klebsiella pneumoniae</i>																
KF1	S	R	S	R	R	R	R	S	R	NT	S	S	S	S	S	S
KF2	R	R	S	R	R	R	R	S	R	NT	S	S	S	S	R	I
KF3	R	R	S	R	R	R	R	R	R	NT	R	R	R	R	R	R
KF4	S	R	S	R	R	R	R	S	R	NT	S	S	R	S	S	S
KM1	S	R	S	R	I	R	R	S	R	NT	S	S	S	S	S	S
KM2	S	R	S	R	S	R	R	S	R	NT	S	S	I	S	S	I
KM3	R	R	R	R	R	R	R	R	R	NT	R	R	R	R	R	R
KM4	S	R	S	R	R	R	R	S	R	NT	S	S	R	S	S	S
<i>Escherichia coli</i>																
EF1	S	R	S	R	S	R	R	S	R	NT	S	S	S	S	S	S
EF2	R	R	NT	R	S	R	R	S	R	NT	S	S	S	S	S	S
EF3	R	R	S	R	R	R	R	R	R	S	S	R	R	R	R	R
EF4	S	R	S	R	R	R	R	S	R	S	S	S	R	S	S	S
EM1	S	R	S	R	I	R	R	S	R	NT	S	S	NT	S	S	S
EM2	R	R	S	R	R	R	R	S	R	S	R	S	R	S	S	I
EM3	R	R	S	R	R	R	R	S	R	S	R	R	R	R	R	R
EM4	S	R	S	R	R	R	R	S	R	S	S	S	R	S	R	S
<i>Acinetobacter baumannii</i>																
AF1	NT	NT	NT	S	R	I	NT	NT	I	NT	S	S	NT	S	S	S
AF2	I	NT	S	I	S	I	NT	NT	I	NT	S	S	S	S	S	S
AF3	R	NT	NT	R	R	R	NT	NT	R	NT	R	R	NT	R	R	R
AF4	NT	NT	S	S	S	I	NT	NT	S	NT	S	R	S	R	S	S
AM1	NT	NT	NT	R	R	R	NT	NT	R	NT	R	S	NT	S	R	S
AM2	R	NT	S	S	S	I	NT	NT	S	NT	S	S	S	S	R	S
AM3	R	NT	S	R	R	R	NT	NT	R	NT	R	R	NT	R	R	R
<i>Pseudomonas aeruginosa</i>																
PF2	NT	NT	S	R	S	NT	NT	NT	I	NT	S	S	S	S	NT	R
PF3	NT	NT	S	R	R	NT	NT	NT	R	NT	R	R	R	R	NT	R
PF4	NT	NT	S	S	S	NT	NT	NT	S	NT	I	R	S	R	NT	S
PM2	NT	NT	S	R	S	NT	NT	NT	R	NT	S	S	S	S	NT	R
PM3	NT	NT	R	R	R	NT	NT	NT	R	NT	R	R	R	R	NT	R
PM4	NT	NT	S	S	S	NT	NT	NT	S	NT	S	R	S	R	NT	I

Table S5. Results of the disk diffusion–based phenotypic assays for detecting extended–spectrum β –lactamase (ESBL) and carbapenemase (CP) among the study isolates showed the production of ESBLs in the majority of the isolates, and CPs in most RC3 isolates (KF3, KM3, EF3, EM3, AF3, AM3, PF3, PM3). All tests are based on the latest Clinical and Laboratory Standards Institute protocols. For ESBL testing, the antibiotic disks used include CAC–ceftazidime–clavulanic acid (30 μ g/10 μ g), CAZ–ceftazidime (30 μ g), CEC–cefotaxime–clavulanic acid (30 μ g/10 μ g), CTX–cefotaxime (30 μ g). The values presented under this test are the average differences between the zones of inhibitions (ZOI in millimeters mm) of the combination disks and the standalone cephalosporin disks in triplicate setups. A difference of at least 5mm in ZOI in either CAC–CAZ or CEC–CTX indicated ESBL production (+), otherwise, the isolate was reported to be a non–ESBL producer (–). CP production was detected using modified carbapenem inactivation (mCIM) and ethylenediamine tetraacetic acid (EDTA)–modified carbapenem inactivation (eCIM) methods. The antibiotic disk used is 10 μ g meropenem. The values presented are the average ZOI of the disk (in mm) in triplicate setups. A 6–15 mm ZOI (or 16–18 mm ZOI with pinpoint colonies within the zones) in the mCIM setups is indicated putative serine CP production (+). For all mCIM positive isolates, at least 5 mm increase in the ZOI from the mCIM to the eCIM setups indicated that the CP produced is a metallo– β –lactamase (++). Isolates not confirming with mCIM criteria (regardless of eCIM results) are reported as non–CP producers (–).

Organism	ESBL Testing			mCIM and eCIM Testing		
	CAC–CAZ (mm)	CEC–CTX (mm)	ESBL	mCIM (mm)	eCIM (mm)	CP
<i>K. pneumoniae</i> KF1	9.3	16.33	+	0	0	–
<i>K. pneumoniae</i> KF2	2.2	24.67	+	21.67	24.67	–
<i>K. pneumoniae</i> KF3	0	8.5	+	6	23.83	++
<i>K. pneumoniae</i> KF4	21.33	14.17	+	22.67	23.67	–
<i>K. pneumoniae</i> KM1	10.67	9.67	+	22	22.33	–
<i>K. pneumoniae</i> KM2	26.17	16.33	+	21.67	21	–
<i>K. pneumoniae</i> KM3	0	9.67	+	6	20.67	++
<i>K. pneumoniae</i> KM4	12.5	16.33	+	24.33	21.67	–
<i>E. coli</i> EF1	6.33	15.61	+	20.67	20	–
<i>E. coli</i> EF2	–0.75	–0.5	–	18.67	21.33	–
<i>E. coli</i> EF3	0	10	+	10.67	20.33	++
<i>E. coli</i> EF4	4.66	13.83	+	20.67	20.67	–
<i>E. coli</i> EM1	8.5	16.17	+	21.67	23	–
<i>E. coli</i> EM2	28.67	32.17	+	20.67	25.33	–
<i>E. coli</i> EM3	0	18.33	+	11.33	17.33	++
<i>E. coli</i> EM4	6	–6.33	+	19.67	20	–
<i>A. baumannii</i> AF1	–1.5	3.33	–	22.33	23	–
<i>A. baumannii</i> AF2	0	4	–	23.33	23.67	–
<i>A. baumannii</i> AF3	0	4	–	20	20	–
<i>A. baumannii</i> AF4	–0.33	1.17	–	25	24	–
<i>A. baumannii</i> AM1	4.83	22.17	+	20.33	20.67	–
<i>A. baumannii</i> AM2	–1	2.67	–	19.33	20	–
<i>A. baumannii</i> AM3	–1.33	2.33	–	16.33	18	+
<i>P. aeruginosa</i> PF2	1	9	+	22.67	22	–
<i>P. aeruginosa</i> PF3	0	13.67	+	22	0	+
<i>P. aeruginosa</i> PF4	0.5	6.17	+	25	24.5	–
<i>P. aeruginosa</i> PM2	0.5	10.33	+	21.33	21.67	–
<i>P. aeruginosa</i> PM3	2.67	14.83	+	23	23	–
<i>P. aeruginosa</i> PM4	1.33	9.5	+	22.87	23	–

Table S6. Results of the Xpert® Carba–R PCR (Cepheid, California, USA) revealed the production of *bla*_{NDM} in some test isolates. The genes tested using the kit include IMP– imipenemase, VIM– Verona integron–encoded metallo– β –lactamase, NDM– New Delhi metallo– β –lactamase, KPC– *K. pneumoniae* carbapenemase, and OXA–48– oxacillinase–48. The presence (+) or absence (–) of the genes was reported qualitatively by GeneXpert® IV System (Cepheid, California, USA). *K. pneumoniae* F1 was excluded due to invalid results.

Organism	Target Genes				
	<i>bla</i> _{IMP}	<i>bla</i> _{VIM}	<i>bla</i> _{NDM}	<i>bla</i> _{KPC}	<i>bla</i> _{OXA–48}
<i>K. pneumoniae</i> KF2	–	–	–	–	–
<i>K. pneumoniae</i> KF3	–	–	+	–	–
<i>K. pneumoniae</i> KF4	–	–	–	–	–
<i>K. pneumoniae</i> KM1	–	–	–	–	–
<i>K. pneumoniae</i> KM2	–	–	–	–	–
<i>K. pneumoniae</i> KM3	–	–	+	–	–
<i>K. pneumoniae</i> KM4	–	–	–	–	–
<i>E. coli</i> EF1	–	–	–	–	–
<i>E. coli</i> EF2	–	–	–	–	–
<i>E. coli</i> EF3	–	–	+	–	–
<i>E. coli</i> EF4	–	–	–	–	–
<i>E. coli</i> EM1	–	–	–	–	–
<i>E. coli</i> EM2	–	–	–	–	–
<i>E. coli</i> EM3	–	–	–	–	–
<i>E. coli</i> EM4	–	–	–	–	–
<i>A. baumannii</i> AF1	–	–	–	–	–
<i>A. baumannii</i> AF2	–	–	–	–	–
<i>A. baumannii</i> AF3	–	–	–	–	–
<i>A. baumannii</i> AF4	–	–	–	–	–
<i>A. baumannii</i> AM1	–	–	–	–	–
<i>A. baumannii</i> AM2	–	–	–	–	–
<i>A. baumannii</i> AM3	–	–	–	–	–
<i>P. aeruginosa</i> PF2	–	–	–	–	–
<i>P. aeruginosa</i> PF3	–	–	–	–	–
<i>P. aeruginosa</i> PF4	–	–	–	–	–
<i>P. aeruginosa</i> PM2	–	–	–	–	–
<i>P. aeruginosa</i> PM3	–	–	–	–	–
<i>P. aeruginosa</i> PM4	–	–	–	–	–

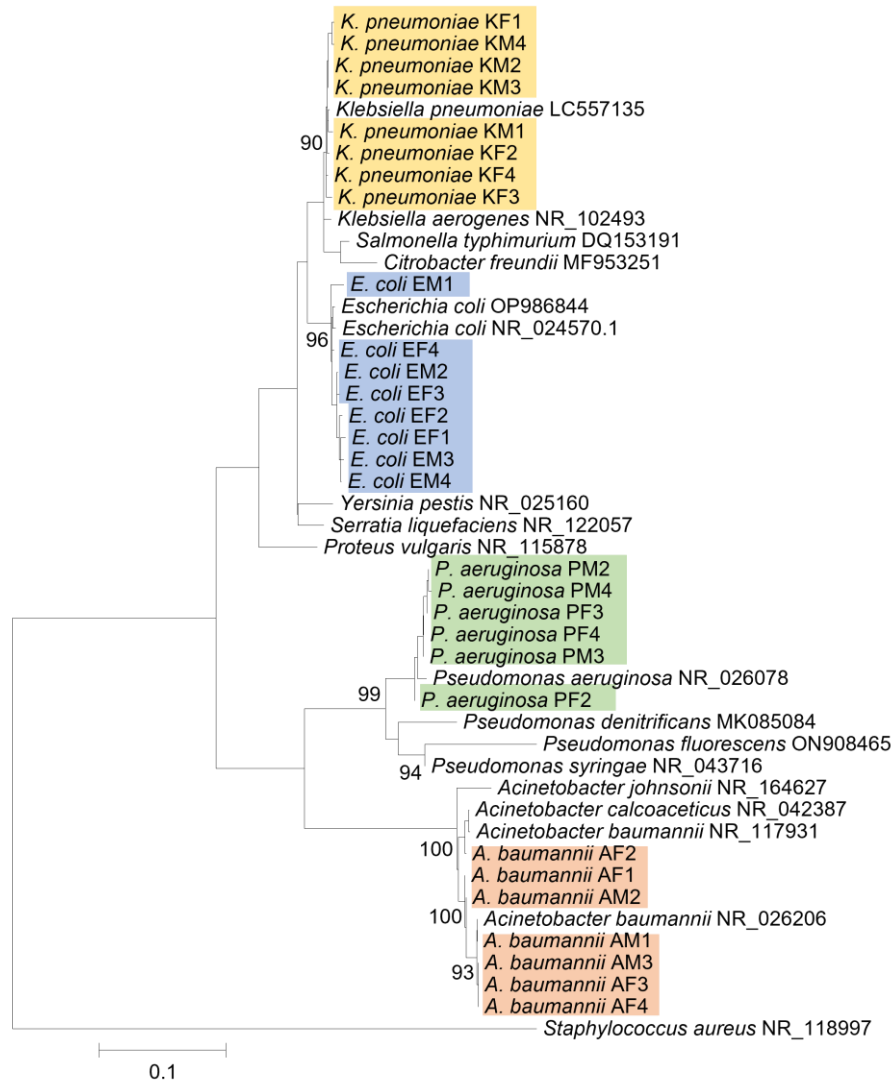
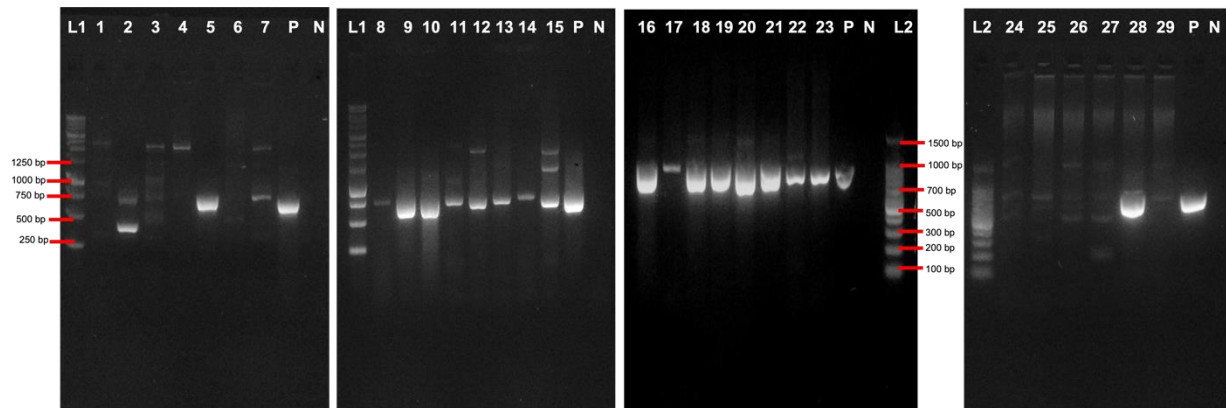
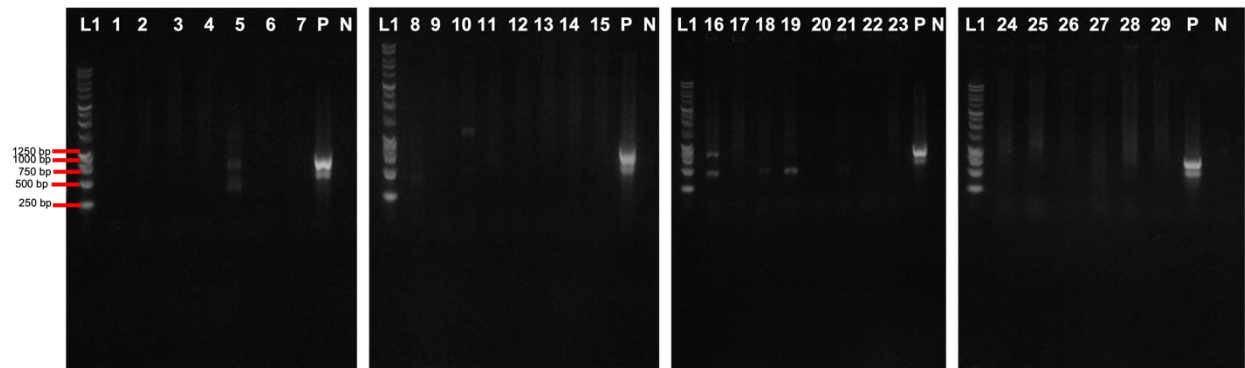


Figure S1. Neighbor-joining tree of the study isolates (colored entries) and reference species based on 694 nucleotides of 16S rRNA gene using the General Time Reversible with gamma distribution (GTR+G) model of DNA substitution. The tree is rooted in *Staphylococcus aureus*. Numbers on the nodes represent bootstrap values out of 1000 replicates. Values less than 90% are not shown. The scale bar represents 10 substitutions for every 100 nucleotides. General tree topology and bootstrap probability values confirm the identities of the study isolates as *Klebsiella pneumoniae* (90% bootstrap value in *K. pneumoniae* reference clade), *Escherichia coli* (96% bootstrap value in *E. coli* reference clade), *Pseudomonas aeruginosa* (99% bootstrap value in *P. aeruginosa* reference clade), and *Acinetobacter baumannii* (100% bootstrap value in *A. baumannii* reference clade).

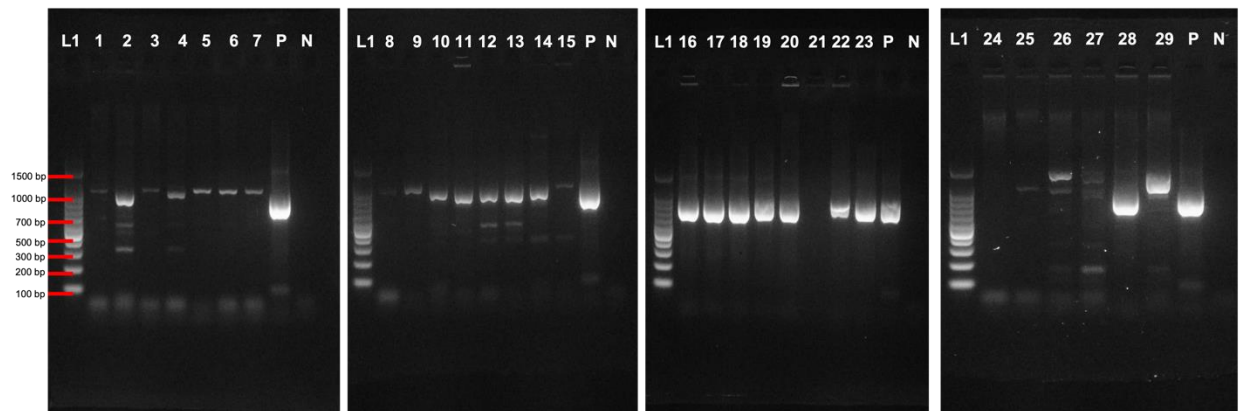
A.



B.



C.



D.

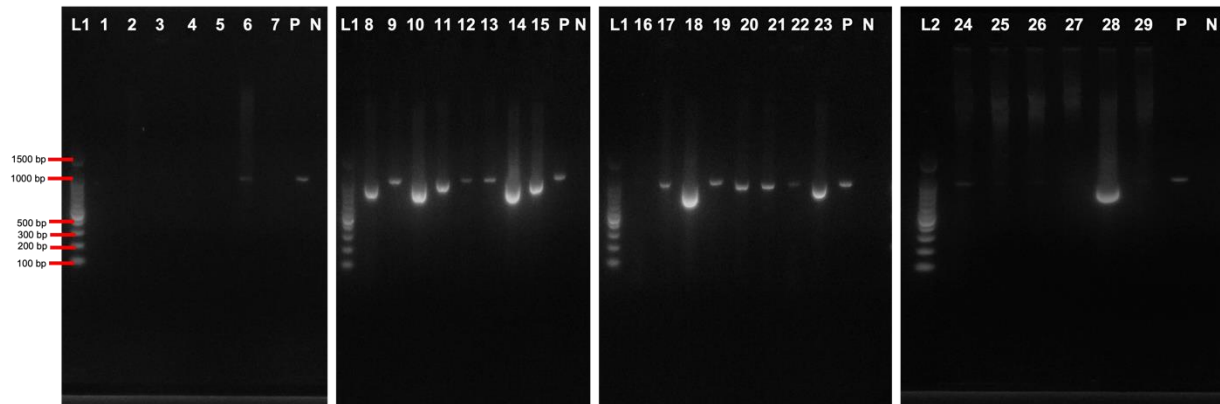
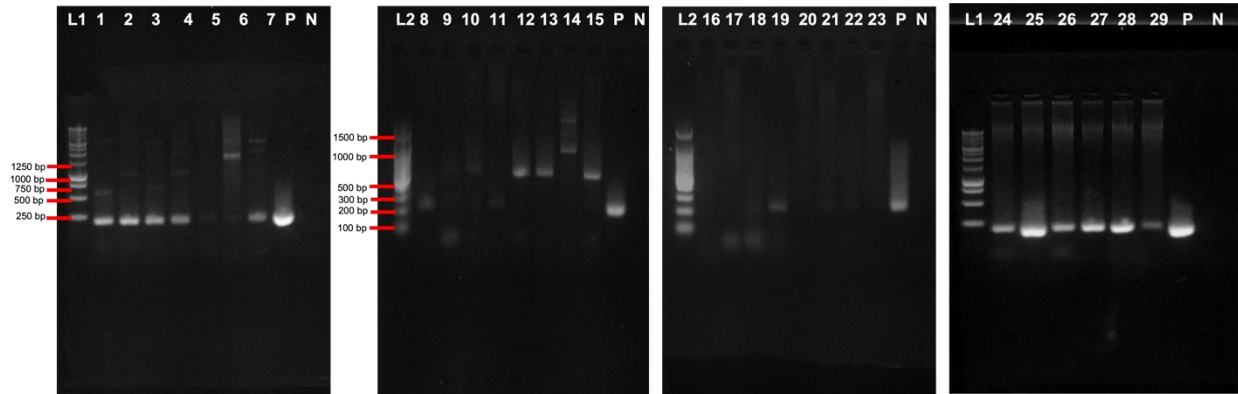
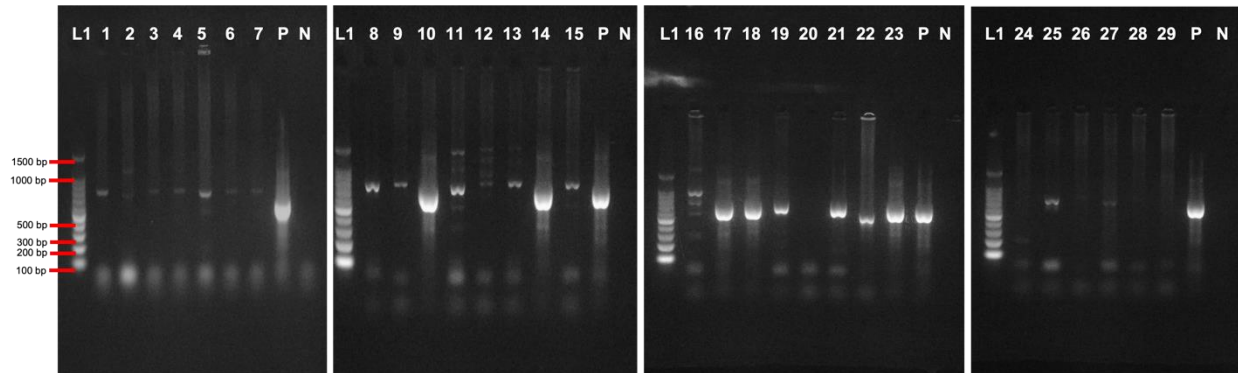


Figure S2. Agarose gel electrophoresis profiles of the PCR amplicons for the tested Ambler Class A β -lactamases. For convenience, the images of these four gels were assembled using Microsoft PowerPoint and the numbering of the lanes of the four gels was continuous (1 through 29). Gel exposure was optimized in E-Gel™ Power Snap imaging system (Thermo Fisher Scientific, USA). Gel contrast and brightness were adjusted using Microsoft Powerpoint to improve band clarity. **A.** Agarose gel electrophoresis profiles of the *bla*_{CTX-M} amplicons. The expected amplicon band size is 688 bp. Lanes 1–7 (Lanes 1–4 *A. baumannii* AF1–AF4, Lanes 5–7 – *A. baumannii* AM1–AM3), Lanes 8–15 (Lanes 8–11, *E. coli* EF1–EF4, Lanes 12–15 – *E. coli* EM1–EM4), Lanes 16–23 (Lanes 16–19 *K. pneumoniae* KF1–KF4, Lanes 20–23 *K. pneumoniae* KM1–KM4). Lanes 24–29 (Lanes 24–26 *P. aeruginosa* PF2–PF4, Lanes 27–29 *P. aeruginosa* PM2–PM4). L1 – 1 Kbp DNA Ladder. L2 – 100 bp DNA Ladder. P – Positive Control (*E. coli* NCTC 13353). N – Negative Control (*S. aureus* ATCC 29213). **B.** Agarose gel electrophoresis profiles of the *bla*_{KPC} amplicons. The expected amplicon band size is 882 bp. Lanes 1–7 (Lanes 1–3 *A. baumannii* AM1–AM3, Lanes 4–7 *A. baumannii* AF1–AF4). Lanes 8–15 (Lanes 8–11 *E. coli* EM1–EM4, Lanes 12–15 *E. coli* EF1–EF4). Lanes 16–23 (Lanes 16–19 *K. pneumoniae* KM1–KM4, Lanes 20–23 *K. pneumoniae* KF1–KF4). Lanes 24–29 (Lanes 24–26 *P. aeruginosa* PM2–PM4, Lanes 27–29 *P. aeruginosa* PF2–PF4). L1 – 1 Kbp DNA Ladder. P – Positive Control (*K. pneumoniae* ATCC BAA 1705). N – Negative Control (*S. aureus* ATCC 29213). **C.** Agarose gel electrophoresis profiles of the *bla*_{SHV} amplicons. The expected amplicon band size is 797 bp. Lanes 1–7 (Lanes 1–3 *A. baumannii* AM1–AM3, Lanes 4–7 *A. baumannii* AF1–AF4). Lanes 8–15 (Lanes 8–11 *E. coli* EM1–EM4, Lanes 12–15 *E. coli* EF1–EF4). Lanes 16–23 (Lanes 16–19 *K. pneumoniae* KM1–KM4, Lanes 20–23 *K. pneumoniae* KF1–KF4). Lanes 24–29 (Lanes 24–26 *P. aeruginosa* PM2–PM4, Lanes 27–29 *P. aeruginosa* PF2–PF4). L1 – 100 bp DNA Ladder. P – Positive Control (*K. pneumoniae* ATCC 700603). N – Negative Control (*S. aureus* ATCC 29213). Lane numbers 1 and 6 showed presence of putative target amplicons, but sequencing revealed mixed or not high-quality chromatogram signals. These sequences were not used in the analyses. Lane numbers 8 to 15 had low quality sequences with matching 24 to 30 bp only in references. **D.** Agarose gel electrophoresis profiles of the *bla*_{TEM} amplicons. The expected amplicon band size is 800 bp. Lanes 1–7 (Lanes 1–4 *A. baumannii* AF1–AF4, Lane 5–7 – *A. baumannii* AM1–AM3), Lanes 8–15 (Lanes 8–11, *E. coli* EF1–EF4, Lanes 12–15 – *E. coli* EM1–EM4), Lanes 16–23 (Lanes 16–19 *K. pneumoniae* KF1–KF4, Lanes 20–23 *K. pneumoniae* KM1–KM4). Lanes 24–29 (Lanes 24–26 *P. aeruginosa* PF2–PF4, Lanes 27–29 *P. aeruginosa* PM2–PM4). L1 – 100 bp DNA Ladder. P – Positive Control (*E. coli* NCTC 13353). N – Negative Control (*S. aureus* ATCC 29213). All putative amplicons of the gene targets were sequenced to confirm gene identities. Only the sequence-confirmed PCR amplicons were used in the analysis. The genes presented include CTX-M– Cefotaximase–Munich, KPC– *K. pneumoniae* carbapenemase, SHV– Sulfhydryl reagent variable β -lactamase, and TEM– Temoneira β -lactamase.

A.



B.



C.

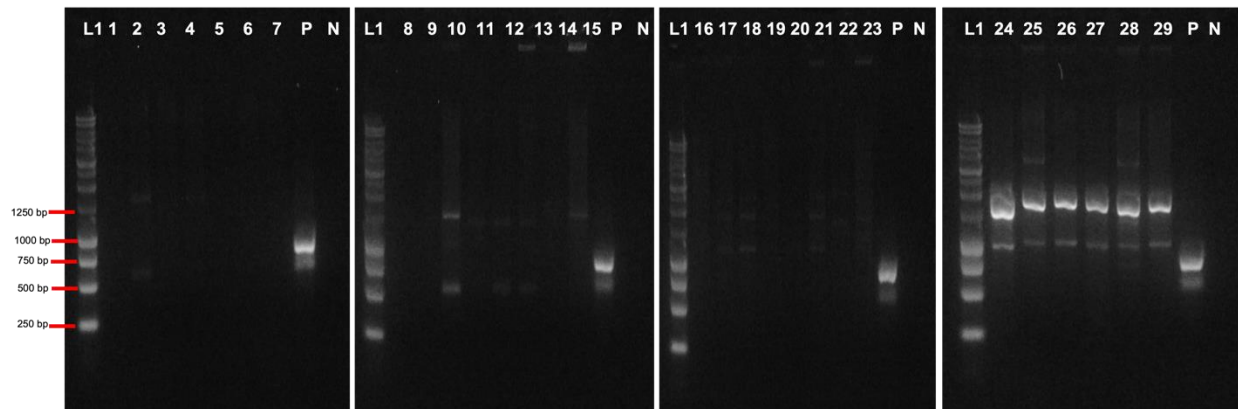
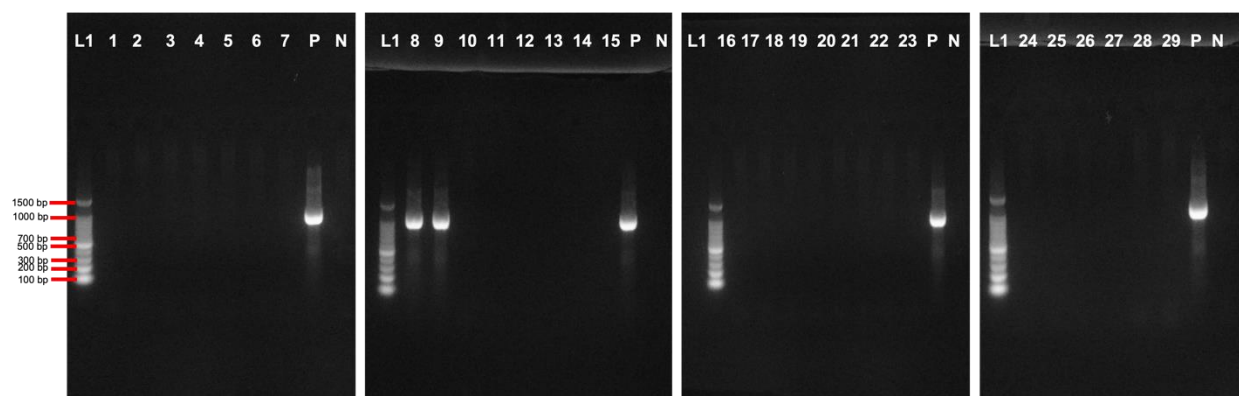


Figure S3. Agarose gel electrophoresis profiles of the PCR amplicons for the tested Ambler Class B β -lactamases. For convenience, the images of these four gels were assembled using Microsoft PowerPoint and the numbering of the lanes of the four gels was continuous (1 through 29). Gel exposure was optimized in E-Gel™ Power Snap imaging system (Thermo Fisher Scientific, USA). Gel contrast and brightness were adjusted using Microsoft PowerPoint to improve band clarity. **A.** Agarose gel electrophoresis profiles of the *bla*_{IMP} amplicons. The expected amplicon

band size is 233 bp. Lanes 1–7 (Lanes 1–4 *A. baumannii* AF1–AF4, Lanes 5–7 – *A. baumannii* AM1–AM3), Lanes 8–15 (Lanes 8–11, *E. coli* EF1–EF4, Lanes 12–15 – *E. coli* EM1–EM4), Lanes 16–23 (Lanes 16–19 *K. pneumoniae* KF1–KF4, Lanes 20–23 *K. pneumoniae* KM1–KM4). Lanes 24–29 (Lanes 24–26 *P. aeruginosa* PF2–PF4, Lanes 27–29 *P. aeruginosa* PM2–PM4). L1 – 1 Kbp DNA Ladder. L2 – 100 bp DNA Ladder. P – Positive Control (*E. coli* NCTC 13476). N – Negative Control (*S. aureus* ATCC 29213). Lane number 6 had low quality sequence with matching 20 bp only in reference. **B.** Agarose gel electrophoresis profiles of the *bla*_{NDM} amplicons. The expected amplicon band size is 621 bp. Lanes 1–7 (Lanes 1–3 *A. baumannii* AM1–AM3, Lanes 4–7 *A. baumannii* AF1–AF4). Lanes 8–15 (Lanes 8–11 *E. coli* M1–M4, Lanes 12–15 *E. coli* EF1–EF4). Lanes 16–23 (Lanes 16–19 *K. pneumoniae* KM1–KM4, Lanes 20–23 *K. pneumoniae* KF1–KF4). Lanes 24–29 (Lanes 24–26 *P. aeruginosa* PM2–PM4, Lanes 27–29 *P. aeruginosa* PF2–PF4). L1 – 100 bp DNA Ladder P – Positive Control (*K. pneumoniae* ATCC BAA 2146). N – Negative Control (*S. aureus* ATCC 29213). Lane number 22 had low quality sequence with matching 20 bp only in reference. **C.** Agarose gel electrophoresis profiles of the *bla*_{VIM} amplicons. The expected amplicon band size is 801 bp. Lanes 1–7 (Lanes 1–3 *A. baumannii* AM1–AM3, Lanes 4–7 *A. baumannii* AF1–AF4). Lanes 8–15 (Lanes 8–11 *E. coli* EM1–EM4, Lanes 12–15 *E. coli* EF1–EF4). Lanes 16–23 (Lanes 16–19 *K. pneumoniae* KM1–KM4, Lanes 20–23 *K. pneumoniae* KF1–KF4). Lanes 24–29 (Lanes 24–26 *P. aeruginosa* PM2–PM4, Lanes 27–29 *P. aeruginosa* PF2–PF4). L1 – 1 Kbp DNA Ladder. P – Positive Control (*K. pneumoniae* NCTC BAA 13439). N – Negative Control (*S. aureus* ATCC 29213). All putative amplicons of the gene targets were sequenced to confirm gene identities. Only the sequence-confirmed PCR amplicons were used in the analysis. The genes presented include IMP– Imipenemase, NDM– New Delhi metallo- β -lactamase, and VIM– Verona integron-encoded metallo- β -lactamase.

A.



B.

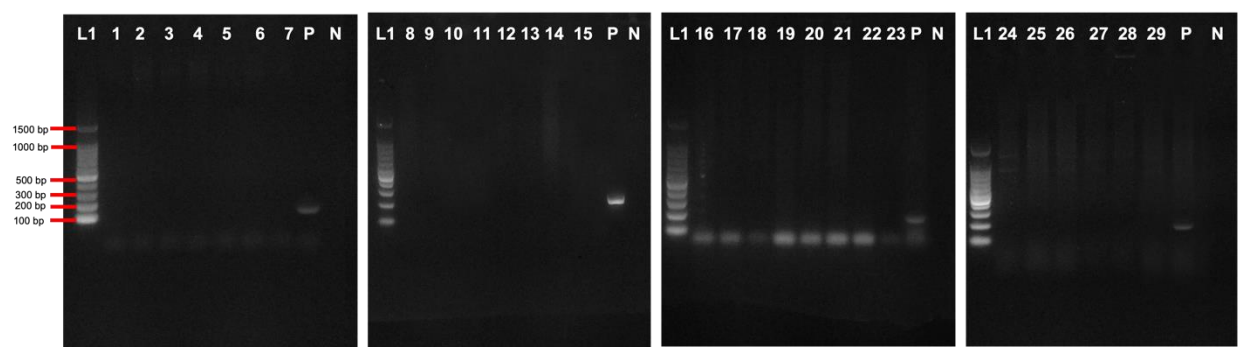
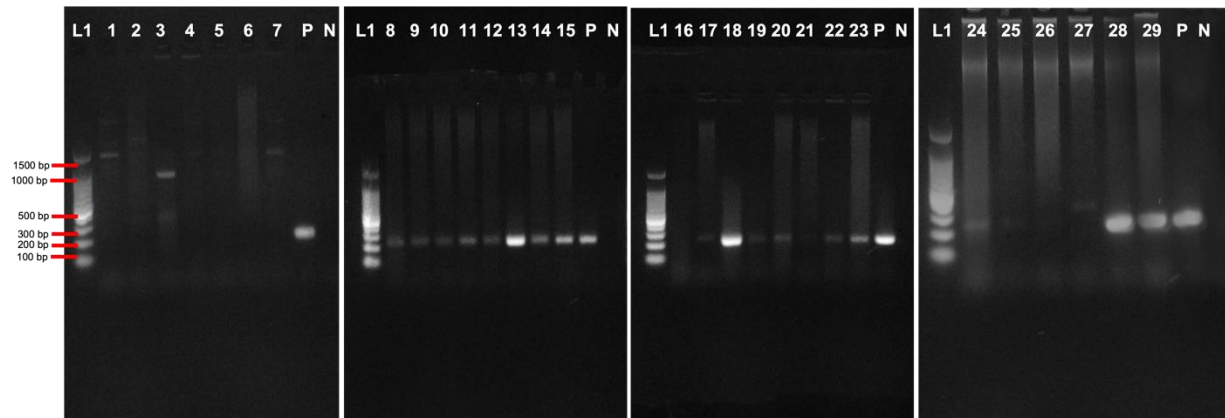
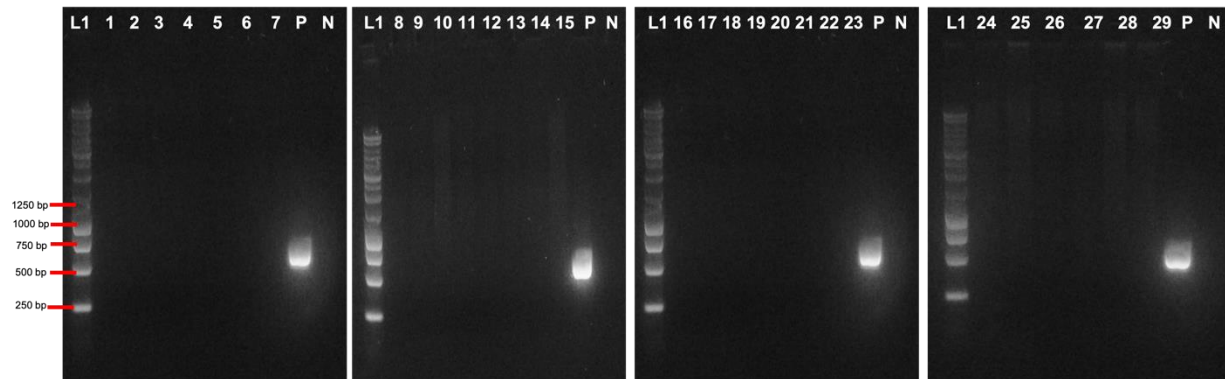


Figure S4. Agarose gel electrophoresis profiles of the PCR amplicons for the tested Ambler Class C β -lactamases. For convenience, the images of these four gels were assembled using Microsoft PowerPoint and the numbering of the lanes of the four gels was continuous (1 through 29). Gel exposure was optimized in E-Gel™ Power Snap imaging system (Thermo Fisher Scientific, USA). Gel contrast and brightness were adjusted using Microsoft PowerPoint to improve band clarity. **A.** Agarose gel electrophoresis profiles of the *bla*_{DHA} amplicons. The expected amplicon band size is 997 bp. Lanes 1–7 (Lanes 1–4 *A. baumannii* AF1–AF4, Lane 5–7 – *A. baumannii* AM1–AM3), Lanes 8–15 (Lanes 8–11, *E. coli* EF1–EF4, Lanes 12–15 – *E. coli* EM1–EM4), Lanes 16–23 (Lanes 16–19 *K. pneumoniae* KF1–KF4, Lanes 20–23 *K. pneumoniae* KM1–KM4). Lanes 24–29 (Lanes 24–26 *P. aeruginosa* PF2–PF4, Lanes 27–29 *P. aeruginosa* PM2–PM4). L1 – 100 bp DNA Ladder. P – Positive Control (*K. pneumoniae* ATCC 2146). N – Negative Control (*S. aureus* ATCC 29213). **B.** Agarose gel electrophoresis profiles of the *bla*_{FOX} amplicons. The expected amplicon band size is 190 bp. Lanes 1–7 (Lanes 1–4 *A. baumannii* AF1–AF4, Lane 5–7 – *A. baumannii* AM1–AM3), Lanes 8–15 (Lanes 8–11, *E. coli* EF1–EF4, Lanes 12–15 – *E. coli* EM1–EM4), Lanes 16–23 (Lanes 16–19 *K. pneumoniae* KF1–KF4, Lanes 20–23 *K. pneumoniae* KM1–KM4). Lanes 24–29 (Lanes 24–26 *P. aeruginosa* PF2–PF4, Lanes 27–29 *P. aeruginosa* PM2–PM4). L1 – 100 bp DNA Ladder. All putative amplicons of the gene targets were sequenced to confirm gene identities. Only the sequence-confirmed PCR amplicons were used in the analysis. The genes presented include DHA– Dharhan β -lactamase, and FOX– cefoxitin-active β -lactamase.

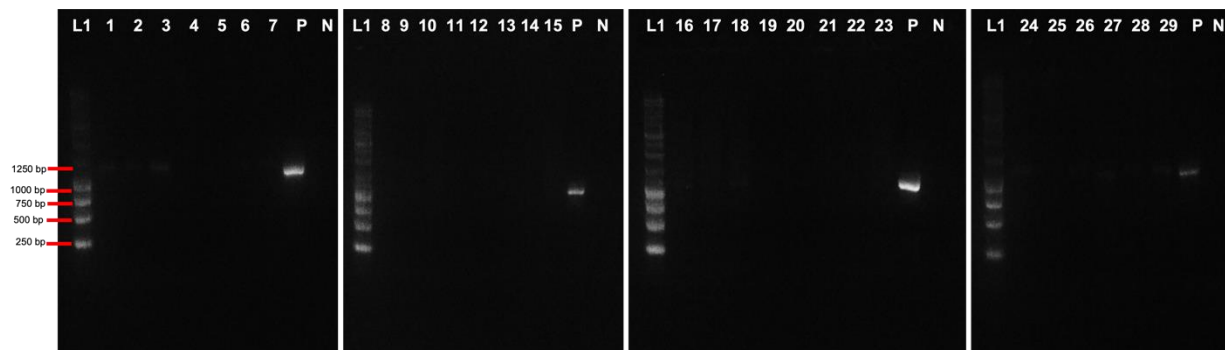
A.



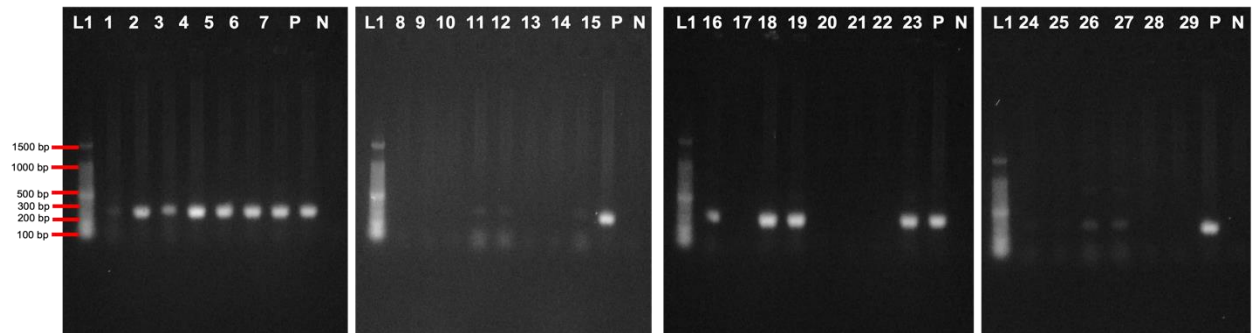
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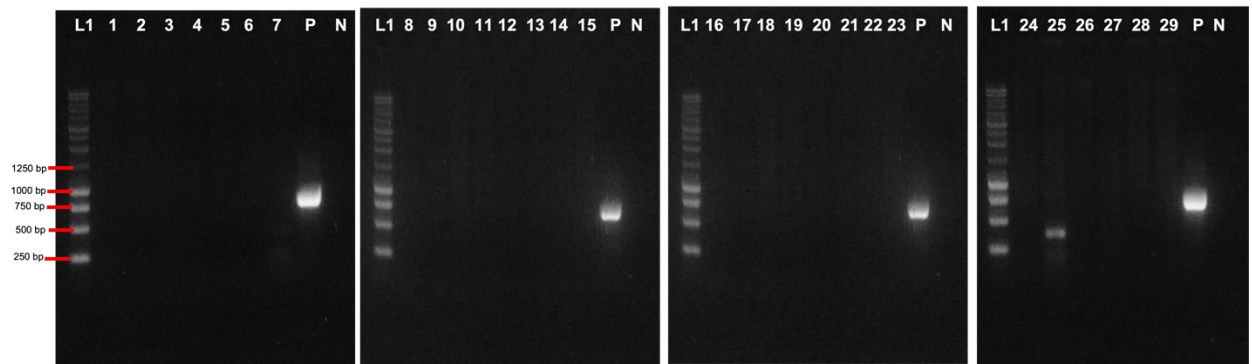
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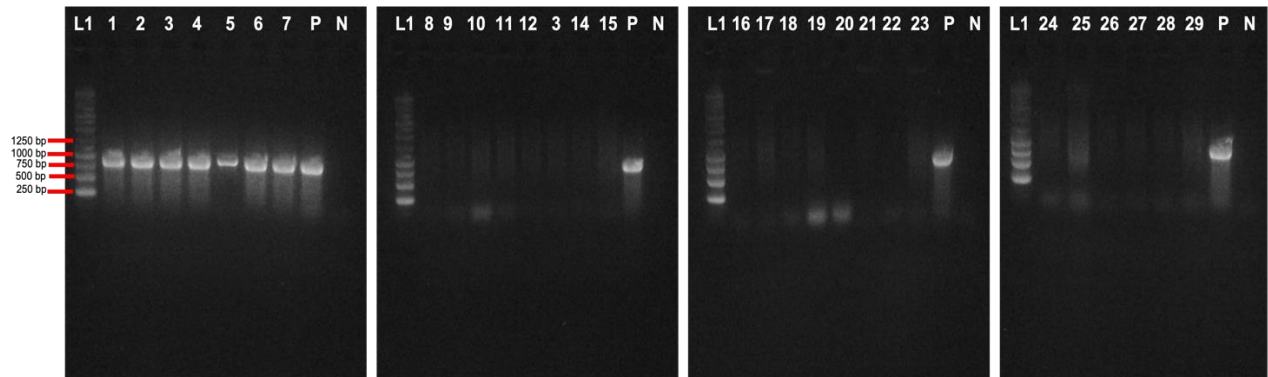
D.



E.



F.



G.

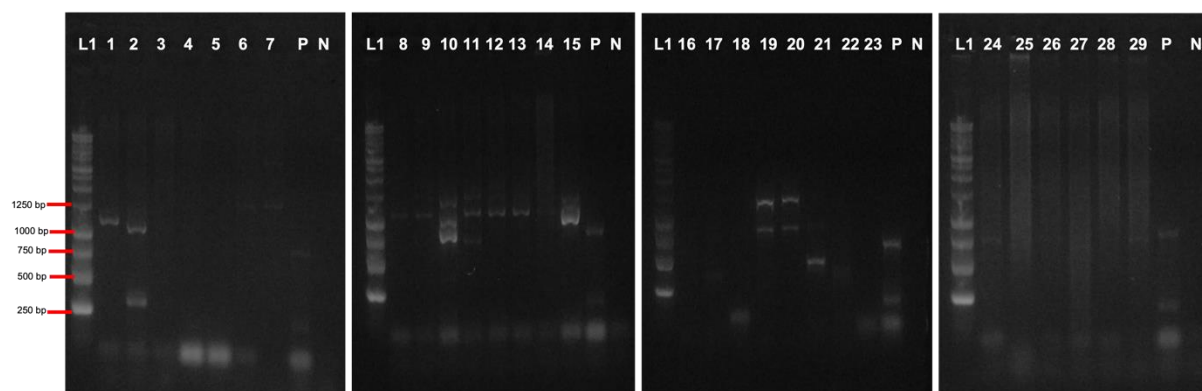


Figure S5. Agarose gel electrophoresis profiles of the PCR amplicons for the tested Ambler Class B β -lactamases. For convenience, the images of these four gels were assembled using Microsoft PowerPoint and the numbering of the lanes of the four gels was continuous (1 through 29). Gel exposure was optimized in E-Gel™ Power Snap imaging system (Thermo Fisher Scientific, USA). Gel contrast and brightness were adjusted using Microsoft PowerPoint to improve band clarity. **A.** Agarose Gel Electrophoresis of the *bla*_{OXA-1} amplicons. The expected amplicon band size is 245 bp. Lanes 1–7 (Lanes 1–3 *A. baumannii* AM1–AM3, Lanes 4–7 *A. baumannii* AF1–AF4). Lanes 8–15 (Lanes 8–11 *E. coli* EM1–EM4, Lanes 12–15 *E. coli* EF1–EF4). Lanes 16–23 (Lanes 16–19 *K. pneumoniae* KM1–KM4, Lanes 20–23 *K. pneumoniae* KF1–KF4). Lanes 24–29 (Lanes 24–26 *P. aeruginosa* PM2–PM4, Lanes 27–29 *P. aeruginosa* PF2–PF4). L1 – 1 Kbp DNA Ladder. P – Positive Control (*E. coli* ATCC BAA 2469). N – Negative Control (*S. aureus* ATCC 29213). **B.** Agarose gel electrophoresis profiles of the *bla*_{OXA-2} amplicons. 569 bp. Lanes 1–7 (Lanes 1–4 *A. baumannii* AF1–AF4, Lane 5–7 – *A. baumannii* AM1–AM3), Lanes 8–15 (Lanes 8–11, *E. coli* EF1–EF4, Lanes 12–15 – *E. coli* EM1–EM4), Lanes 16–23 (Lanes 16–19 *K. pneumoniae* KF1–KF4, Lanes 20–23 *K. pneumoniae* KM1–KM4). Lanes 24–29 (Lanes 24–26 *P. aeruginosa* PF2–PF4, Lanes 27–29 *P. aeruginosa* PM2–PM4). L1 – 1 Kbp DNA Ladder. P – Positive Control (*K. pneumoniae* ATCC 700603). N – Negative Control (*S. aureus* ATCC 29213). **C.** Agarose gel electrophoresis profiles of the *bla*_{OXA-23} amplicons. The expected amplicon band size is 1 Kbp. Lanes 1–7 (Lanes 1–3 *A. baumannii* AM1–AM3, Lanes 4–7 *A. baumannii* AF1–AF4). Lanes 8–15 (Lanes 8–11 *E. coli* AM1–AM4, Lanes 12–15 *E. coli* AF1–AF4). Lanes 16–23 (Lanes 16–19 *K. pneumoniae* KM1–KM4, Lanes 20–23 *K. pneumoniae* KF1–KF4). Lanes 24–29 (Lanes 24–26 *P. aeruginosa* PM2–PM4, Lanes 27–29 *P. aeruginosa* PF2–PF4). L1 – 100 bp DNA Ladder. P – Positive Control (*A. baumannii* ATCC BAA–1605). N – Negative Control (*S. aureus* ATCC 29213). **D.** Agarose gel electrophoresis profiles of the *bla*_{OXA-24-like} amplicons. The expected amplicon band size is 250 bp. Lanes 1–7 (Lanes 1–3 *A. baumannii* AM1–AM3, Lanes 4–7 *A. baumannii* AF1–AF4). Lanes 8–15 (Lanes 8–11 *E. coli* EM1–EM4, Lanes 12–15 *E. coli* EF1–EF4). Lanes 16–23 (Lanes 16–19 *K. pneumoniae* KM1–KM4, Lanes 20–23 *K. pneumoniae* KF1–KF4). Lanes 24–29 (Lanes 24–26 *P. aeruginosa* PM2–PM4, Lanes 27–29 *P. aeruginosa* PF2–PF4). L1 – 100 bp DNA Ladder. P – Positive Control (*A. baumannii* ATCC BAA–1605). N – Negative Control (*S. aureus* ATCC 29213). **E.** Agarose gel electrophoresis profiles of the *bla*_{OXA-48} amplicons. The expected amplicon band size is 744 bp. Lanes 1–7 (Lanes 1–3 *A. baumannii* AM1–AM3, Lanes 4–7 *A. baumannii* AF1–AF4). Lanes 8–15 (Lanes 8–11 *E. coli* EM1–EM4,

Lanes 12–15 *E. coli* EF1–EF4). Lanes 16–23 (Lanes 16–19 *K. pneumoniae* KM1–KM4, Lanes 20–23 *K. pneumoniae* KF1–KF4). Lanes 24–29 (Lanes 24–26 *P. aeruginosa* PM2–PM4, Lanes 27–29 *P. aeruginosa* PF2–PF4). L1 – 1 Kbp DNA Ladder. P – Positive Control (*K. pneumoniae* NCTC 13442). N – Negative Control (*S. aureus* ATCC 29213). **F.** Agarose gel electrophoresis profiles of the *bla*_{OXA-51-like} amplicons. The expected amplicon band size is 825 bp. Lanes 1–7 (Lanes 1–3 *A. baumannii* AM1–AM3, Lanes 4–7 *A. baumannii* AF1–AF4). Lanes 8–15 (Lanes 8–11 *E. coli* EM1–EM4, Lanes 12–15 *E. coli* EF1–EF4). Lanes 16–23 (Lanes 16–19 *K. pneumoniae* KM1–KM4, Lanes 20–23 *K. pneumoniae* KF1–KF4). Lanes 24–29 (Lanes 24–26 *P. aeruginosa* PM2–PM4, Lanes 27–29 *P. aeruginosa* PF2–PF4). L1 – 1 Kbp DNA Ladder. P – Positive Control (*A. baumannii* ATCC BAA-1605). N – Negative Control (*S. aureus* ATCC 29213). **G.** Agarose Gel Electrophoresis of the *bla*_{OXA-58} amplicons. The expected amplicon band size is 599 bp. Lanes 1–7 (Lanes 1–3 *A. baumannii* AM1–AM3, Lanes 4–7 *A. baumannii* AF1–AF4). Lanes 8–15 (Lanes 8–11 *E. coli* EM1–EM4, Lanes 12–15 *E. coli* EF1–EF4). Lanes 16–23 (Lanes 16–19 *K. pneumoniae* KM1–KM4, Lanes 20–23 *K. pneumoniae* KF1–KF4). Lanes 24–29 (Lanes 24–26 *P. aeruginosa* PM2–PM4, Lanes 27–29 *P. aeruginosa* PF2–PF4). L1 – 1 Kbp DNA Ladder. P – Positive Control (*A. baumannii* TMC 32). N – Negative Control (*S. aureus* ATCC 29213). All putative amplicons of the gene targets were sequenced to confirm gene identities. Only the sequence-confirmed PCR amplicons were used in the analysis. The genes presented include OXA– Oxacillinases.

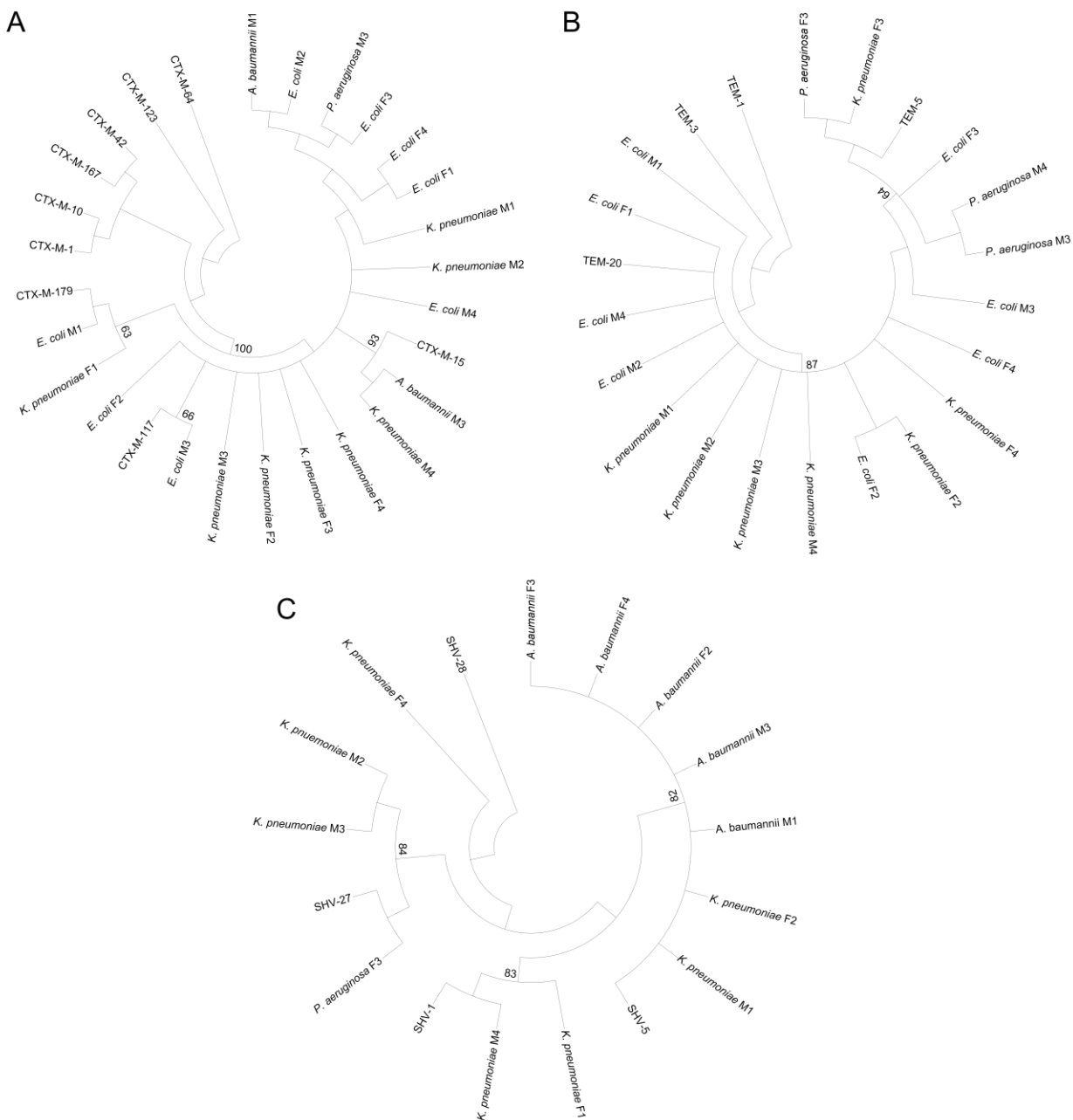


Figure S6. Unrooted maximum likelihood trees of the detected class A β -lactamase genes showed clustering of study isolates' genes in specific gene variants. Panel (A) refers to *bla*_{CTX-M}, (B) to *bla*_{TEM}, and (C) to *bla*_{SHV} trees. Numbers on the nodes represent bootstrap values out of 1000 replicates. Values less than 50% are not shown. All trees were generated in PhyML version 3.1 using the tree-construction parameters described in Supplementary Table 3. All trees were visualized in TreeExplorer version 2.12. General tree topology and high bootstrap probability values support the relatedness of the isolate's genes to specific reference genes. The genes presented include: CTX-M– Cefotaximase–Munich, TEM– Temoneira β -lactamase, and SHV– Sulfhydryl reagent variable β -lactamase.

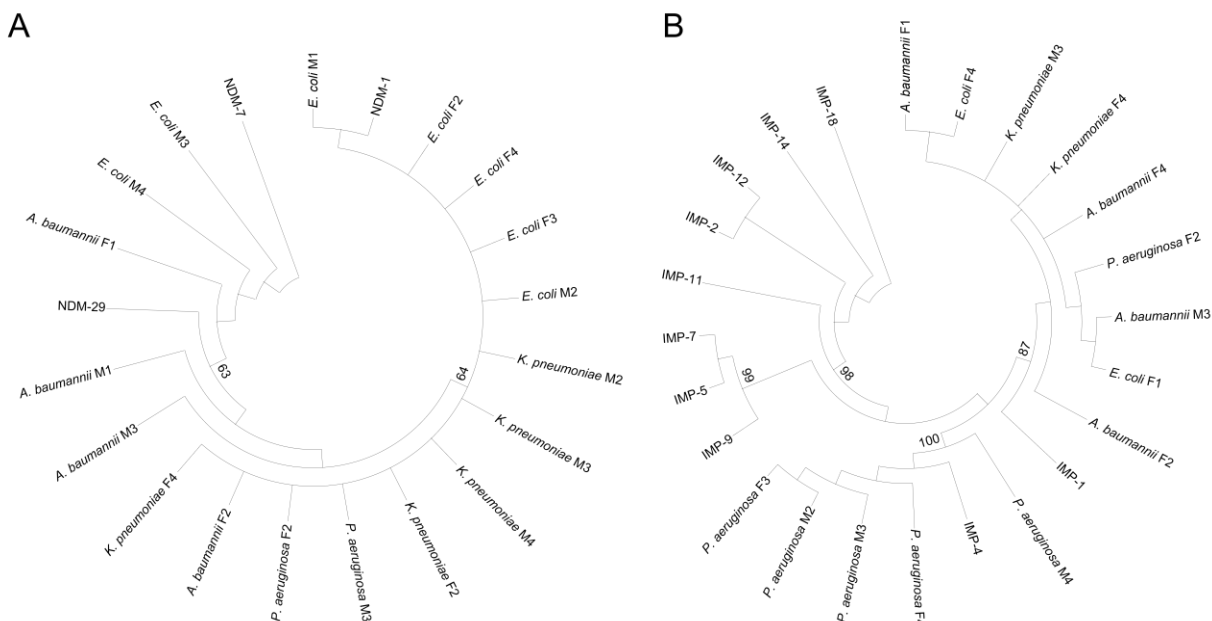


Figure S7. Unrooted maximum likelihood trees of the detected class B β -lactamase genes showed clustering of study isolates' genes in specific gene variants. Panel (A) refers to *bla*_{NDM1}, and (B) to *bla*_{IMP} trees. Numbers on the nodes represent bootstrap values out of 1000 replicates. Values less than 50% are not shown. All trees were generated in PhyML version 3.1 using the tree-construction parameters described in Supplementary Table 3. All trees were visualized in TreeExplorer version 2.12. General tree topology and high bootstrap probability values support the relatedness of the isolate's genes to specific reference genes. The genes presented include NDM– New Delhi metallo- β -lactamase, and IMP– Imipenemase.



Figure S8. The unrooted maximum likelihood tree of class C *bla*_{DHA} gene showed clustering of study isolates' genes in specific *bla*_{DHA} variants. Numbers on the nodes represent bootstrap values out of 1000 replicates. Values less than 50% are not shown. All trees were generated in PhyML version 3.1 using the tree-construction parameters described in Supplementary Table 3. The tree was visualized in TreeExplorer version 2.12. General tree topology and high bootstrap probability values support the relatedness of the isolate's genes to specific reference genes. The gene presented include DHA– Dharhan β–lactamase.

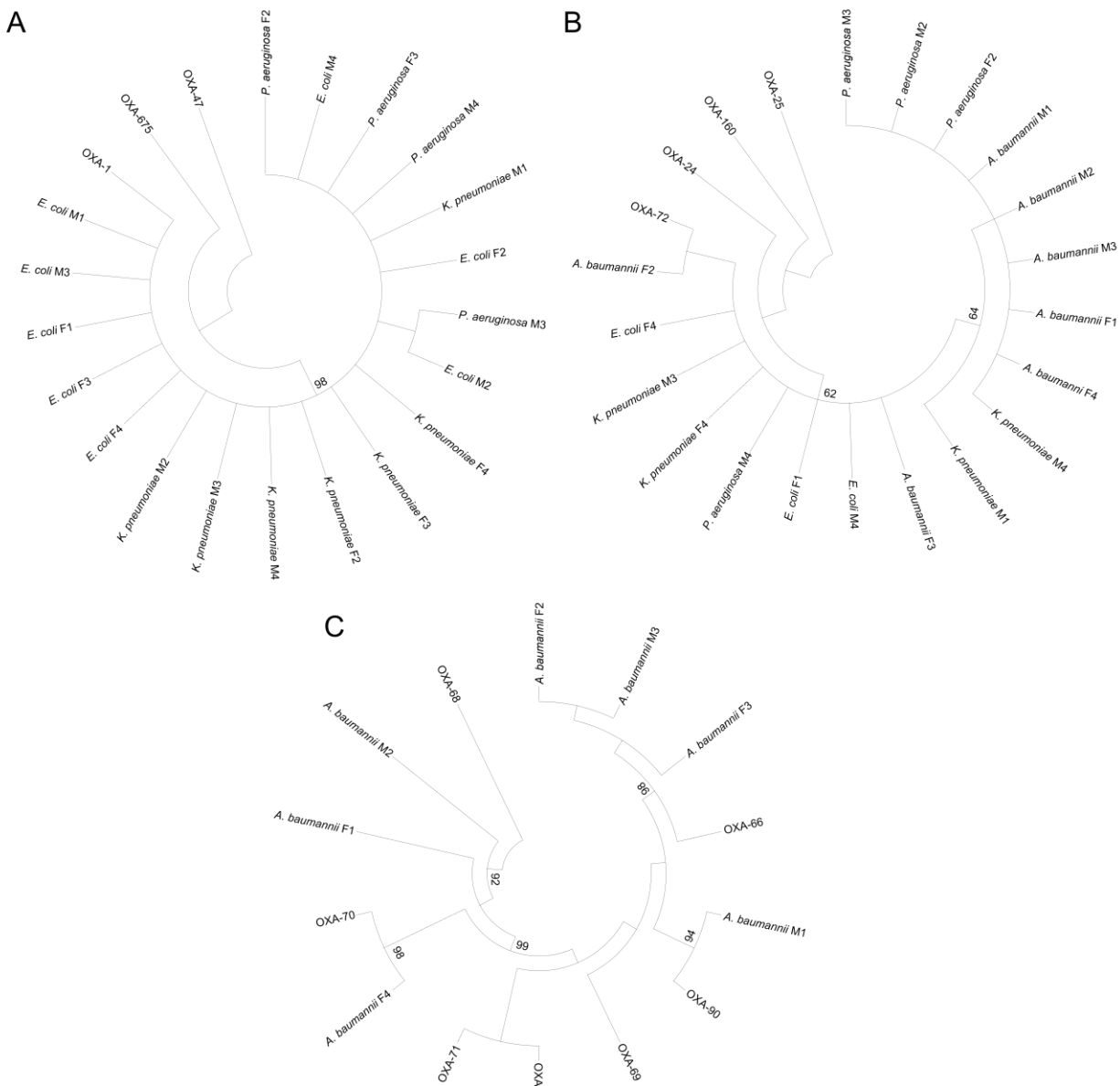


Figure S9. Unrooted maximum likelihood trees of the detected class D β -lactamase genes showed clustering of study isolates' genes in specific gene variants. Panel (A) refers to *bla*_{OXA-1}, (B) to *bla*_{OXA-24-like}, and (C) to *bla*_{OXA-51-like}. Numbers on the nodes represent bootstrap values out of 1000 replicates. Values less than 50% are not shown. All trees were generated in PhyML version 3.1 using the tree-construction parameters described in Supplementary Table 3. All trees were visualized in TreeExplorer version 2.12. General tree topology and high bootstrap probability values support the relatedness of the isolate's genes to specific reference genes. The genes presented include OXA- Oxacillinase.