

Dissecting the clonality of I1 plasmids using ORF-based binarized structure network

analysis of plasmids (OSNAp)

Running title: Dissecting the clonality of I1 plasmids

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31 Abstract

32 Phylogenetic relationship of 97 I1 plasmids harboring *bla*_{CTX-M} genes encoding
 33 extended-spectrum beta-lactamase (ESBL) was analyzed using the ORF-based binarized
 34 structure network analysis of plasmids (OSNAp). The majority of plasmids carrying
 35 *bla*_{CTX-M-1} or *bla*_{CTX-M-8}, ESBL genes primarily associated with domestic animals, were
 36 clonal. On the other hand, plasmids carrying *bla*_{CTX-M-14} or *bla*_{CTX-M-15}, identified from
 37 both humans and domestic animals, were diverse in their contents. The findings suggest
 38 that circulation of I1 plasmids among humans and animals may contribute to their
 39 diversity.

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Introduction

Extended-spectrum β -lactamase (ESBL) genes, especially *bla*_{CTX-M}, are widely disseminated among *Enterobacteriaceae* (1, 2). The *bla*_{CTX-M} genes are found on plasmids of various incompatibility groups, such as F, I1, and others (3-9). Among them, *bla*_{CTX-M-15} and *bla*_{CTX-M-14} are the major ESBL genes present in *Enterobacteriaceae* from various hosts including humans and domestic animals (10-12). On the other hand, *bla*_{CTX-M-1} and *bla*_{CTX-M-8} are mainly found in *Enterobacteriaceae* from domestic animals (13-16). Phylogenetics of plasmids provide useful information on the dissemination pattern of resistance genes across various hosts. However, practical methods to compare lineages of multiple plasmids are yet to be established and validated.

We recently developed the ORF-based binarized structure network analysis of plasmids (OSNAp), a novel, digitalized approach in analyzing phylogeny of plasmids (17). With OSNAp, plasmids are converted to binary sequences based on their gene contents, and the relationship among plasmids is visualized by the neighbor-net network. The network is drawn using splits that separate plasmids into two groups based on distances between the binary sequences (Fig. 1).

In this study, we analyzed *bla*_{CTX-M}-carrying I1 plasmids identified in *Enterobacteriaceae* collected from multiple hosts including humans and animals. I1

plasmids were selected in this proof of concept study since their structures are relatively conserved making them suitable for OSNAp (18).

Materials and Methods

A total of 190 I1 plasmid sequences consisting of publicly available I1 plasmid sequences (17) and I1 plasmid sequences newly obtained for this study were included. As publicly available I1 plasmid sequences, 140 I1 plasmid sequence data registered in the NCBI genome database as of 4-Aug-2018 were used (Table 1) (17). Among these publicly available plasmid sequences, 47 had *bla*_{CTX-M} genes (Table 3).

In addition, a total of 50 I1 plasmids containing *bla*_{CTX-M} were identified in *Escherichia coli* strains isolated from humans, chicken meat, dog feces, and pig feces between 2010 to 2015. Their whole genomes were sequenced by MiSeq using QIAseq FX DNA Library Kit (Qiagen, Hulsterweg, Netherlands) and MiSeq Reagent Kit v3 600 cycles (Illumina, San Diego, CA). To enrich for sequence reads originating from plasmids, purified I1 plasmids were used as the templates for sequencing according to our previous study (8). MinION long-read sequencing was also performed according to our previous study (19). MiSeq and MinION reads were hybrid-assembled using Unicycler v. 0.4.9b, or Flye v. 2.7 and Pilon v. 1.23. Accession numbers of plasmids were listed in Table 2.

Plasmid sequence types (pSTs) of the plasmids were identified using the plasmid multilocus sequence typing (pMLST) web site (<https://pubmlst.org/plasmid/>). Subtypes of *bla*_{CTX-M} genes were determined by ABRicate (<https://github.com/tseemann/abricate>) using the ResFinder database. Sequence types (STs) of the host cells were also identified using mlst command (<https://github.com/tseemann/mlst>). A total of 190 I1 plasmid sequences were analyzed by OSNAp under the relaxed condition (17). For the purpose of this analysis, ORFs having $\geq 80\%$ sequence identity and sequence coverage were considered identical. Dice coefficient values between plasmids were also calculated. Plasmid regions (core, shell and cloud regions) were defined according to our previous study (17).

Clusters constituting the *bla*_{CTX-M} plasmids were defined by splits which maximized the number of plasmids harboring the same *bla*_{CTX-M} gene and minimized others. While clusters are identical to splits in the OSNAp analysis, the term cluster is used in this study for ease of reading. The diversity of plasmids within the same cluster was defined by the average of Dice indices.

I1 plasmid sequences were annotated using DFAST-core (<https://dfast.ddbj.nig.ac.jp/dfc/distribution/>) supplemented with allele-level annotation of *bla*_{CTX-M} and IS genes using ResFinder

(https://bitbucket.org/genomicepidemiology/resfinder_db/src/master/) and ISfinder (<https://www-is.biotoul.fr/index.php>). Structures of modules carrying *bla*_{CTX-M} genes and their locations were determined from annotation data of plasmid sequences by checking the regions surrounding *bla*_{CTX-M}.

Results

OSNAp analysis.

Plasmids harboring the same *bla*_{CTX-M} gene belonged to one or two clusters, and each plasmid cluster included the same *bla*_{CTX-M}, except for one cluster which contained *bla*_{CTX-M-3}, *bla*_{CTX-M-15}, and *bla*_{CTX-M-55}-harboring plasmids (Fig. 2).

Most (27/30) plasmids harboring *bla*_{CTX-M-1} were classified into the same OSNAp cluster. The average Dice index of the *bla*_{CTX-M-1} cluster was 0.942 ± 0.036 . Most plasmids in the cluster belonged to pST3 except for 3 plasmids which were of pST108, pST7, and a pST7 variant lacking *sogS*.

All 15 plasmids harboring *bla*_{CTX-M-8} were classified into the same cluster. Among them, 12 plasmids were similar with each other with an average Dice index of 0.979 ± 0.015 and belonged to pST113. However, 3 plasmids belonging to pST114, pST132 and pST235 showed lower levels of similarity compared with the other 12 plasmids. The average Dice

index between the former 12 and the latter 3 plasmids was 0.868 ± 0.038 .

Plasmids harboring *bla*_{CTX-M-15} were classified into 2 major and 1 minor clusters, and one singleton. The cluster including 11 *bla*_{CTX-M-15} plasmids showed an average Dice index of 0.970 ± 0.013 and represented pST31. Another major cluster also included plasmids harboring *bla*_{CTX-M-3} and *bla*_{CTX-M-55} as well as *bla*_{CTX-M-15}. The *bla*_{CTX-M-3} and *bla*_{CTX-M-55} modules showed structures specific to each *bla*_{CTX-M} gene. The average Dice index of the cluster was 0.967 ± 0.016 . All plasmids in this cluster belonged to pST16. The minor cluster was placed near the two major clusters in the OSNAp. Plasmids of the minor cluster belonged to pST37.

Unlike the other *bla*_{CTX-M} genes, plasmids harboring *bla*_{CTX-M-14} showed a polyclonal distribution. They were classified into 2 major and 1 minor clusters and a singleton. The largest cluster contained 10 plasmids with an average Dice index of 0.950 ± 0.049 . Most plasmids in the largest cluster belonged to pST275.

Structure of modules carrying *bla*_{CTX-M}.

*bla*_{CTX-M-1} was always carried on a module consisting of *ISEcp1*, *bla*_{CTX-M-1}, and an ORF for a hypothetical protein. In 25 of the 27 plasmids, the *bla*_{CTX-M-1} module was inserted between *rci* (encoding shufflon-specific DNA recombinase) and *pilVA* genes. In the remaining two plasmids, the *bla*_{CTX-M-1} module was inserted into a cloud region. The

*bla*_{CTX-M-1} module was inserted into *pilJ* in the three non-pST3 plasmids.

All *bla*_{CTX-M-8} modules consisted of IS26, Δ IS10, *bla*_{CTX-M-8}, and IS26. The modules were inserted between *impB* and *yfbB* genes.

*bla*_{CTX-M-15} plasmids of the major clusters had the same modules consisting of Tn2, *bla*_{CTX-M-15}, *ISEcp1* and Δ Tn2. Plasmids of the minor cluster belonged to pST37 and had another module consisting of *bla*_{CTX-M-15} surrounded by IS26. The *bla*_{CTX-M-15} modules were located in a cloud region of I1 plasmids.

*bla*_{CTX-M-14} plasmids in the largest cluster had a closely related plasmid structure and their *bla*_{CTX-M-14} modules consisted of *ISEcp1*, an ORF encoding a hypothetical protein, *bla*_{CTX-M-14} and IS903. In 8 of 10 plasmids, the *bla*_{CTX-M-14} module was integrated between *traH* and *traG* genes. The remaining 2 plasmids had the same *bla*_{CTX-M-14} module, which was integrated in a cloud region. However, the *bla*_{CTX-M-14} modules outside the largest cluster, including another major cluster consisting of pST80 plasmids, consisted of *ISEcp1*, *bla*_{CTX-M-14}, and IS903. Some of the modules were interrupted by other IS elements or partial deletion. They were integrated into the cloud region.

MLST of *E. coli* strains. Most of the host *E. coli* strains belonged to diverse STs even when they harbored the same *bla*_{CTX-M} genes (Table 2). Among them, 11 strains carrying *bla*_{CTX-M-1} were classified into 7 STs and no strains carrying *bla*_{CTX-M-15} or *bla*_{CTX-M-8}

showed the same ST. However, strains with I1 plasmids carrying *bla*_{CTX-M-14} gene were less diverse. In particular, 5 of 6 strains carrying pST275 and *bla*_{CTX-M-14} gene belonged to ST131.

Discussion

I1 plasmids are found in multiple host bacterial species but especially frequently in *Escherichia coli* and *Salmonella enterica*. Therefore, I1 plasmids may contribute to exchanging of antimicrobial resistant genes between these species. Moreover, I1 plasmids are capable of hosting multiple *bla*_{CTX-M} variants (20). Plasmid structures of the I1 lineage are relatively well conserved, making them good targets of OSNAp, a novel approach in analyzing phylogeny of plasmids we developed recently.

Our data indicate that I1 plasmids harboring the same *bla*_{CTX-M} genes generally spread clonally among different strains since they were classified into a limited number of OSNAp clusters whereas the host *E. coli* strains belonged to various STs. Our data support previous studies suggesting that I1 plasmids harboring the same *bla*_{CTX-M} gene shared similar plasmid structures (8, 9, 21-23) .

The I1 plasmids harboring *bla*_{CTX-M} could be divided into two major groups according to their similarity. One included plasmids harboring *bla*_{CTX-M-1} and *bla*_{CTX-M-8} which

showed clonal dissemination overall. The other included *bla*_{CTX-M-14} and *bla*_{CTX-M-15} plasmids which showed polyclonal dissemination consisting of two or more plasmid clones.

Interestingly, clonal plasmids carrying *bla*_{CTX-M} genes are largely restricted to animals (6, 8, 22, 24). Moreover, *bla*_{CTX-M} genes in this group are mainly associated with I1 plasmids (6, 8, 22, 25, 26), and not reported from F plasmids which are the dominant plasmid type overall in *E. coli*. Therefore, *bla*_{CTX-M} genes of this group may seldom exchange *bla*_{CTX-M} genes with other plasmids. Moreover, I1 plasmids harboring them appear to spread in *E. coli* in conjunction with international trade of meat products (27).

On the other hand, *bla*_{CTX-M} genes such as *bla*_{CTX-M-14} or *bla*_{CTX-M-15} were detected from various sources including humans and domestic animals. Moreover, *bla*_{CTX-M} genes in this group were frequently found on F plasmids in addition to I1 plasmids (3, 5, 7, 28, 29). This suggests that these *bla*_{CTX-M} genes may have transmitted between I1 and F plasmids. Adaptation to a variety of host animals and plasmids may contribute to the extensive spread of this group of *bla*_{CTX-M} genes.

In this study, we analyzed I1 plasmids as proof of concept because of their relative structural simplicity. However, F plasmids are the most common plasmid type especially carrying *bla*_{CTX-M-14} and *bla*_{CTX-M-15} genes, which are the dominant ESBL genes (28, 29).

The phylogeny of F plasmids is complex due to their extensive structural diversity (19, 28, 30). Phylogenetic analysis of F plasmids will therefore be challenging and may require adjustments to OSNAp such as analyzing subgroups of plasmids based on the similarity of gene contents.

In summary, I1 plasmids harboring *bla*_{CTX-M} genes have disseminated clonally, and the diversity of plasmid gene contents harboring the same *bla*_{CTX-M} subtypes may depend on their occurrence and history of their spread. I1 plasmids carrying *bla*_{CTX-M-1} or *bla*_{CTX-M-8}, which are known to spread by the global meat supply chain, showed clonal phylogeny. On the other hand, *bla*_{CTX-M-14} and *bla*_{CTX-M-15} showed polyclonal phylogeny. In particular, the latter *bla*_{CTX-M} genes are frequently reported on F plasmids (3, 5, 7, 28, 29), and it can be hypothesized that their ability to integrate into various plasmids may have contributed to their success in becoming predominant *bla*_{CTX-M} genes. The ability to integrate into various plasmids is also considered to be an important factor underpinning successful spread of certain drug resistance genes. Circulation among human and animals may help diversification of plasmids carrying *bla*_{CTX-M} genes.

Acknowledgements

This study was supported by grants from the Food Safety Commission, Cabinet Office,

Government of Japan (Research Program for Risk Assessment Study on Food Safety, no. 1504), JSPS KAKENHI Grant Number JP20K10457, and AMED under Grant Number JP20fk0108061.

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Figure legend

Figure 1

Outline of OSNAp analysis. Plasmid sequences (p1 – p3) are broken down into ORFs (1 – 8) and binarized based on the presence of ORFs. Phylogenetic relationships among plasmids are depicted as neighbor-net network based on the distance matrix of the binary sequences. ORF contents of plasmid 4 to 8 (p4 – p8) are omitted.

Figure 2

OSNAp analysis of 11 plasmids. Plasmids harboring *bla*_{CTX-M-8} were classified into single cluster and most *bla*_{CTX-M-1} harboring plasmids also belonged to a cluster. On the other hand, plasmids harboring *bla*_{CTX-M-14} and *bla*_{CTX-M-15} were classified into multiple clusters. Representative pST is shown for each cluster.

Table 1. 11 plasmids obtained from NCBI database

No.	Accession No.	Species	Plasmid
1	AP005147.1	<i>Salmonella enterica</i>	R64
2	AP011954.1	<i>Salmonella enterica</i>	R621a
3	AB021078.1	<i>Shigella sonnei</i>	ColIb-P9
4	CP001118.1	<i>Salmonella enterica</i>	pSL476_91
5	CP009566.1	<i>Salmonella enterica</i>	22462_pCVM22462
6	CP012929.1	<i>Salmonella enterica</i>	p12-4374_96
7	CP018104.1	<i>Escherichia coli</i>	pMR0716_tem1
8	CP019207.1	<i>Salmonella enterica</i>	pCFSAN004174
9	CP019215.2	<i>Escherichia coli</i>	pI1_WCHEC050613
10	CP023370.1	<i>Escherichia coli</i>	p96
11	CP027415.1	<i>Salmonella enterica</i>	320_unnamed1
12	CP004087.1	<i>Salmonella enterica</i>	pSEEH1578_1
13	CP013224.1	<i>Salmonella enterica</i>	PDM04
14	CP013221.1	<i>Salmonella enterica</i>	PDM02
15	CP027409.1	<i>Salmonella enterica</i>	317_unnamed
16	CP005389.2	<i>Salmonella enterica</i>	pCFSAN002069_1
17	CP014662.1	<i>Salmonella enterica</i>	pSAN1-2010K-2577
18	CP016585.1	<i>Salmonella enterica</i>	pSH14-009_99
19	CP018625.1	<i>Escherichia coli</i>	44_pFORC44_1
20	CP015996.1	<i>Escherichia coli</i>	pS51_1
21	CP002489.1	<i>Salmonella enterica</i>	TY474p2
22	HE654725.1	<i>Salmonella enterica</i>	pCol1B9_SL1344
23	CP027127.1	<i>Escherichia coli</i>	374_unnamed2
24	CP022064.2	<i>Salmonella enterica</i>	312_unnamed4
25	CP016865.1	<i>Salmonella enterica</i>	2010K-1587_pSTY2-2010K-1587
26	CP016522.1	<i>Salmonella enterica</i>	pSA02DT09004001_101
27	CP012923.1	<i>Salmonella enterica</i>	pSA02DT10168701_99
28	CP016516.1	<i>Salmonella enterica</i>	p11-004736-1-7_99
29	CP016568.1	<i>Salmonella enterica</i>	pAMR588-04-00318_99
30	CP012936.1	<i>Salmonella enterica</i>	pN13-01290_98
31	CP016572.1	<i>Salmonella enterica</i>	pAMR588-04-00320_99
32	CP024854.1	<i>Escherichia coli</i>	6_tig00000311
33	CP023385.1	<i>Escherichia coli</i>	p87

34	CP023356.1	<i>Escherichia coli</i>	p95
35	CP023376.1	<i>Escherichia coli</i>	p92
36	CP023365.1	<i>Escherichia coli</i>	p92
37	CP016533.1	<i>Salmonella enterica</i>	pSA01AB09084001_92
38	CP023382.1	<i>Escherichia coli</i>	p95
39	CP021208.1	<i>Escherichia coli</i>	Z247_p2474-3
40	CP026726.1	<i>Escherichia coli</i>	2_p266917_2_3
41	CP023362.1	<i>Escherichia coli</i>	p85
42	CP010142.1	<i>Escherichia coli</i>	B
43	CP018975.1	<i>Escherichia coli</i>	545_pEC545_1
44	CP023534.1	<i>Escherichia coli</i>	403_unnamed1
45	CP003290.1	<i>Escherichia coli</i>	pESBL-EA11
46	CP025339.1	<i>Salmonella enterica</i>	p113k
47	CP021845.1	<i>Escherichia coli</i>	pEC1515-1
48	CP021841.1	<i>Escherichia coli</i>	pEC974-1
49	CP019173.1	<i>Salmonella enterica</i>	pCFSAN004175
50	CP019205.1	<i>Salmonella enterica</i>	pCFSAN004173
51	CP002186.1	<i>Escherichia coli</i>	pRK1
52	CP002517.1	<i>Escherichia coli</i>	pEKO1101
53	LN890525.1	<i>Salmonella enterica</i>	2
54	CP024152.1	<i>Escherichia coli</i>	p14EC033e
55	CP018110.1	<i>Escherichia coli</i>	pMRSN346595_1203
56	CP018122.1	<i>Escherichia coli</i>	pMRSN346355_1203
57	CP018116.1	<i>Escherichia coli</i>	pMRSN346638_1193
58	CP006641.1	<i>Escherichia coli</i>	PCN061p5
59	CP027535.1	<i>Escherichia coli</i>	81_unnamed1
60	CP016520.1	<i>Salmonella enterica</i>	pCE-R2-11-0435_92
61	CP012627.1	<i>Escherichia coli</i>	pSF-468-2
62	CP024292.1	<i>Escherichia coli</i>	unnamed3
63	CP018946.1	<i>Escherichia coli</i>	224_pEC224_2
64	CP014494.1	<i>Escherichia coli</i>	pMVASt0167_2
65	LT838199.1	<i>Escherichia coli</i>	isolate_pWI1-incI1
66	CP024146.1	<i>Escherichia coli</i>	p14EC029e
67	CP002731.1	<i>Escherichia coli</i>	pUMNK88_91
68	CP010317.1	<i>Escherichia coli</i>	pAPEC-O78-2

69	CP015916.1	<i>Escherichia coli</i>	pSLy4
70	CP010130.1	<i>Escherichia coli</i>	A
71	CP018993.1	<i>Escherichia coli</i>	AZ147_pECAZ147_2
72	CP010233.1	<i>Escherichia coli</i>	B
73	AP009241.1	<i>Escherichia coli</i>	pSE11-1
74	CP024822.1	<i>Escherichia coli</i>	pCREC-591_1
75	CP025751.1	<i>Escherichia coli</i>	pCV839-06-p1
76	CP014622.1	<i>Salmonella enterica</i>	pSAN1-1727
77	CP014972.2	<i>Salmonella enterica</i>	pSTY1-1898
78	CP016387.1	<i>Salmonella enterica</i>	p931IncI1
79	CP018638.1	<i>Salmonella enterica</i>	pSE69-3861-1
80	CP019905.1	<i>Escherichia coli</i>	56_unnamed6
81	CP021882.1	<i>Escherichia coli</i>	137_tig00001287_pilon
82	CP027135.1	<i>Escherichia coli</i>	369_unnamed3
83	CP024918.1	<i>Klebsiella pneumoniae</i>	pKPNH542
84	CP015161.1	<i>Escherichia coli</i>	pECO-93a
85	CP020494.1	<i>Salmonella enterica</i>	pSE08-00436-2
86	CP012835.1	<i>Salmonella enterica</i>	pCFSAN001588_2
87	CP013193.1	<i>Escherichia coli</i>	31_pFORC313
88	CP000797.1	<i>Escherichia coli</i>	pETEC_73
89	CP023350.1	<i>Escherichia coli</i>	unnamed1
90	CP024241.1	<i>Escherichia coli</i>	unnamed1
91	CP023352.1	<i>Escherichia coli</i>	unnamed3
92	CP019220.1	<i>Klebsiella pneumoniae</i>	pKp1756
93	CP021739.1	<i>Escherichia coli</i>	150_tig00002897alt
94	CP021693.1	<i>Escherichia coli</i>	151_tig00001252
95	CP021533.1	<i>Escherichia coli</i>	149_tig00000220
96	CP023957.1	<i>Escherichia coli</i>	448_unnamed3
97	CP015838.1	<i>Escherichia coli</i>	pMS6198D
98	CP023900.1	<i>Escherichia coli</i>	433_unnamed6
99	CP024233.1	<i>Escherichia coli</i>	unnamed1
100	CP024270.1	<i>Escherichia coli</i>	unnamed1
101	CP024253.1	<i>Escherichia coli</i>	unnamed4
102	CP008736.1	<i>Escherichia coli</i>	pESBL-283
103	CP008737.1	<i>Escherichia coli</i>	pESBL-305

104	CP008738.1	<i>Escherichia coli</i>	pESBL-315
105	KT779550.1	<i>Escherichia coli</i>	p369
106	KF787110.1	<i>Escherichia coli</i>	pIFM3804
107	KJ484638.1	<i>Escherichia coli</i>	pC49-108
108	KJ484637.1	<i>Escherichia coli</i>	pC59-112
109	KJ484635.1	<i>Escherichia coli</i>	pC60-108
110	KM052220.1	<i>Escherichia coli</i>	pH18-Hel20-TF1
111	KJ484630.1	<i>Escherichia coli</i>	pH1519-88
112	KJ484629.1	<i>Escherichia coli</i>	pH2291-112
113	KM377238.1	<i>Escherichia coli</i>	pHV114
114	KM377239.1	<i>Escherichia coli</i>	pHV292
115	KM377240.1	<i>Escherichia coli</i>	pHV295
116	CP007651.1	<i>Escherichia coli</i>	pTCN40607
117	MF152729.1	<i>Escherichia coli</i>	pCTXM1-MU2
118	KJ563250.1	<i>Escherichia coli</i>	pTF2
119	EU935740.1	<i>Escherichia coli</i>	pEK204
120	CP008735.1	<i>Escherichia coli</i>	pESBL-12
121	GU371927.1	<i>Escherichia coli</i>	pEC-Bactec
122	KU355874.1	<i>Escherichia coli</i>	pFAM22871-2
123	HE610900.2	<i>Escherichia coli</i>	pHUSEC2011-1
124	KJ406378.1	<i>Shigella sonnei</i>	pSH4469
125	MK238490.1	<i>Salmonella enterica</i>	pSPA440915
126	KJ866866.1	<i>Escherichia coli</i>	pSKLX3330
127	KP987217.1	<i>Klebsiella pneumoniae</i>	p628-CTXM
128	LM651376.1	<i>Escherichia coli</i>	pV404
129	MH674341.1	<i>Escherichia coli</i>	pUR-EC07
130	LN735558.1	<i>Escherichia coli</i>	pC193
131	LN735561.1	<i>Escherichia coli</i>	pC271
132	KT282968.1	<i>Escherichia coli</i>	pEC012
133	LN735559.1	<i>Escherichia coli</i>	pM105
134	LN735560.1	<i>Escherichia coli</i>	pV408
135	CM007643.1	<i>Escherichia coli</i>	pEC1333
136	KY964068.1	<i>Escherichia coli</i>	pLV23529-CTX-M-8
137	AP017892.1	<i>Escherichia coli</i>	pN23
138	AP017893.1	<i>Escherichia coli</i>	pS11

139	KJ125070.2	<i>Escherichia coli</i>	pHNAH4-1
140	KP198616.1	<i>Escherichia coli</i>	pCTXM123

Table 2. 11 plasmids analyzed in this study

Plasmid					Host <i>E. coli</i> strain				
No.	Plasmid		<i>bla</i> _{CTX-M} subtype	pMLST	Strain	ST	Source	Year	Accession No.
	name	Size (bp)							
141	p15A37	106153	CTX-M-1	pST318	15A37	ST48	<i>Homo sapiens</i>	2015	LC567050
142	pS12-1	105327	CTX-M-1	pST3	S12-1	ST1582	Chicken meat	2015	LC567056
143	pS40-2	104027	CTX-M-1	pST3	S40-2	ST117	Chicken meat	2015	LC567057
144	pS43-1	108830	CTX-M-1	pST3	S43-1	ST1684	Chicken meat	2015	LC567058
145	pS83-1	106223	CTX-M-1	pST3	S83-1	ST117	Chicken meat	2015	LC567060
146	pN603	109957	CTX-M-1	pST3	N603	ST117	<i>Homo sapiens</i>	2010	LC567083
147	pS13	109827	CTX-M-1	pST3	S13	ST155	Chicken meat	2010	LC567084
148	pS74	108158	CTX-M-1	pST3	S74	ST155	Chicken meat	2010	LC567090
149	pS124	113140	CTX-M-1	pST3	S124	ST1594	Chicken meat	2010	LC567093
150	pS127	107498	CTX-M-1	pST3	S127	ST1594	Chicken meat	2010	LC567094
151	pS292	114151	CTX-M-1	pST3	S292	ST2491	Chicken meat	2010	LC567097
152	pP16-1	91143	CTX-M-15	pST31	P16-1	ST1011	<i>Sus scrofa</i>	2015	LC567054
153	pN367	87540	CTX-M-15	pST31	N367	ST349	<i>Homo sapiens</i>	2010	LC567068
154	pN441	88536	CTX-M-15	pST31	N441	ST11218	<i>Homo sapiens</i>	2010	LC567071
155	pN600	91115	CTX-M-15	pST16	N600	ST3524	<i>Homo sapiens</i>	2010	LC567081
156	pN601	91134	CTX-M-15	pST16	N601	ST1193	<i>Homo sapiens</i>	2010	LC567082
157	pS68	93395	CTX-M-15	pST31	S68	ST57	Chicken meat	2010	LC567089
158	pS122	86016	CTX-M-15	pST323	S122	ST88	Chicken meat	2010	LC567092

159	pS261	88544	CTX-M-15	pST31	S261	ST93	Chicken meat	2010	LC567096
160	pN83	86191	CTX-M-55	pST16	N83	ST6756	<i>Homo sapiens</i>	2010	LC567063
161	pN126	86191	CTX-M-55	pST16	N126	ST6756	<i>Homo sapiens</i>	2010	LC567065
162	p15A44	116105	CTX-M-14	pST80	15A44	ST156	<i>Homo sapiens</i>	2015	LC567051
163	p15A63	99602	CTX-M-14	pST80	15A63	ST410	<i>Homo sapiens</i>	2015	LC567052
164	pP819-1	92584	CTX-M-14	pST319	P819-1	ST410	<i>Sus scrofa</i>	2015	LC567055
165	pS62-1	97933	CTX-M-14	pST320	S62-1	ST162	Chicken meat	2015	LC567059
166	pS84-3	112637	CTX-M-14	pST162	S84-3	ST162	Chicken meat	2015	LC567061
167	pN11	87299	CTX-M-14	pST275	N11	ST131	<i>Homo sapiens</i>	2010	LC567062
168	pN270	87298	CTX-M-14	pST275	N270	ST131	<i>Homo sapiens</i>	2010	LC567066
169	pN333	87299	CTX-M-14	pST275	N333	ST131	<i>Homo sapiens</i>	2010	LC567067
170	pN410	87299	CTX-M-14	pST275	N410	ST131	<i>Homo sapiens</i>	2010	LC567070
171	pN537	101264	CTX-M-14	pST321	N537	ST156	<i>Homo sapiens</i>	2010	LC567074
172	pN557	87340	CTX-M-14	pST275	N557	ST11219	<i>Homo sapiens</i>	2010	LC567075
173	pN567	86970	CTX-M-14	pST275	N567	ST131	<i>Homo sapiens</i>	2010	LC567076
174	pN580	88656	CTX-M-14	pST275	N580	ST448	<i>Homo sapiens</i>	2010	LC567077
175	pN581	87307	CTX-M-14	pST275	N581	ST10	<i>Homo sapiens</i>	2010	LC567078
176	pN583	101632	CTX-M-14	pST322	N583	ST70	<i>Homo sapiens</i>	2010	LC567079
177	pN84	123379	CTX-M-65	pST71	N84	ST224	<i>Homo sapiens</i>	2010	LC567064
							<i>Canis lupus</i>		
178	pP44	89476	CTX-M-8	pST113	P44	ST410	<i>familiaris</i>	2015	LC567053
179	pHU485	86204	CTX-M-8	pST114	N485	ST23	<i>Homo sapiens</i>	2010	LC567072

180	pHU493	94912	CTX-M-8	pST113	N493	ST1170	<i>Homo sapiens</i>	2010	LC567073
181	pHU590	91926	CTX-M-8	pST113	N590	ST2526	<i>Homo sapiens</i>	2010	LC567080
182	pCH41	89476	CTX-M-8	pST113	S41	ST351	Chicken meat	2010	LC567085
183	pCH42	89147	CTX-M-8	pST113	S42	ST88	Chicken meat	2010	LC567086
184	pCH49	89476	CTX-M-8	pST113	S49	ST224	Chicken meat	2010	LC567087
185	pCH56	92049	CTX-M-8	pST113	S56	ST131	Chicken meat	2010	LC567088
186	pCH110	97607	CTX-M-8	pST235	S110	ST4576	Chicken meat	2010	LC567091
187	pCH365	108776	CTX-M-8	pST132	S365	ST602	Chicken meat	2010	LC567098
188	pCH407	89476	CTX-M-8	pST113	S407	ST101	Chicken meat	2010	LC567099
189	pN373	129994	CTX-M-2	pST12	N373	ST2223	<i>Homo sapiens</i>	2010	LC567069
190	pS241	129370	CTX-M-2	pST12	S241	ST602	Chicken meat	2010	LC567095

Table 3. Meta data of I1 plasmids carrying *bla*_{CTX-M} genes

No.	Plasmid	Source	Geographic location	Collection year	<i>bla</i> CTX-M subtype	pMLST
39	Z247_p2474-3	<i>Homo sapiens</i>	China	2015	CTX-M-55	pST167
42	B				CTX-M-55	pST16
43	545_pEC545_1	<i>Homo sapiens</i>	Viet Nam	2011	CTX-M-15	pST16
44	403_unnamed1	<i>Homo sapiens</i>			CTX-M-15	pST31
45	pESBL-EA11				CTX-M-15	pST31
61	pSF-468-2	<i>Homo sapiens</i>	USA	2010	CTX-M-14	pST166
74	pCREC-591_1	<i>Homo sapiens</i>	South Korea	2015	CTX-M-123	pST108
82	369_unnamed3				CTX-M-15	pSTUT01
102	pESBL-283	<i>Sus scrofa</i>			CTX-M-1	pST7
103	pESBL-305	<i>Gallus gallus domesticus</i>			CTX-M-1	pST3
104	pESBL-315	<i>Gallus gallus domesticus</i>			CTX-M-1	pSTUnknown
105	p369	<i>Gallus gallus domesticus</i>			CTX-M-1	pST3
106	pIFM3804	<i>Sus scrofa</i>	UK	2009	CTX-M-1	pST108
107	pC49-108	<i>Gallus gallus domesticus</i>	Switzerland	2013	CTX-M-1	pST3
108	pC59-112	<i>Gallus gallus domesticus</i>	Switzerland	2013	CTX-M-1	pST3
109	pC60-108	<i>Gallus gallus domesticus</i>	Switzerland	2013	CTX-M-1	pST3
110	pH18-Hel20-TF1		Denmark		CTX-M-1	pST49
111	pH1519-88	<i>Homo sapiens</i>	Switzerland	2013	CTX-M-1	pST145
112	pH2291-112	<i>Homo sapiens</i>	Switzerland	2013	CTX-M-1	pST3

113	pHV114	<i>Gallus gallus domesticus</i>	Switzerland	2013	CTX-M-1	pST3
114	pHV292	<i>Gallus gallus domesticus</i>	Switzerland	2013	CTX-M-1	pST3
115	pHV295	<i>Gallus gallus domesticus</i>	Switzerland	2013	CTX-M-1	pST3
116	pTCN40607	<i>Gallus gallus domesticus</i>	USA	2012	CTX-M-1	pST3
117	pCTXM1-MU2	<i>Sus scrofa</i>	Australia		CTX-M-1	pST3?*
118	pTF2		Denmark	2007	CTX-M-1	pST49
119	pEK204				CTX-M-3	pST16
120	pESBL-12	<i>Homo sapiens</i>			CTX-M-15	pST37
121	pEC-Bactec	<i>Equus caballus</i>	Belgium		CTX-M-15	pST31
122	pFAM22871-2				CTX-M-15	pST37
123	pHUSEC2011-1	<i>Homo sapiens</i>	Germany	2011	CTX-M-15	pST31?
124	pSH4469	<i>Homo sapiens</i>	Korea	2008	CTX-M-15	pST16
125	pSPA440915	<i>Homo sapiens</i>			CTX-M-15	pST31
126	pSKLX3330	<i>Homo sapiens</i>	China		CTX-M-55	pST16
127	p628-CTXM	<i>Homo sapiens</i>	China	2010	CTX-M-55	pST16
128	pV404	<i>Homo sapiens</i>	Bolivia		CTX-M-14	pST107
129	pUR-EC07	<i>Homo sapiens</i>	Uruguay		CTX-M-14	pST80
130	pC193		Bolivia	2011	CTX-M-65	pST71
131	pC271		Bolivia	2011	CTX-M-65	pST71
132	pEC012	<i>Gallus gallus domesticus</i>	China		CTX-M-65	pST71
133	pM105	<i>Homo sapiens</i>	Bolivia	2011	CTX-M-65	pST71
134	pV408	<i>Homo sapiens</i>	Bolivia	2011	CTX-M-65	pST71

135	pEC1333	sewage	Brazil	2009	CTX-M-8	pST113
136	pLV23529-CTX-M-8	<i>Sus scrofa</i>	Portuga	2015	CTX-M-8	pST113
137	pN23	<i>Homo sapiens</i>	Japan		CTX-M-8	pST113
138	pS11	chicken meat	Japan		CTX-M-8	pST113
139	pHNAH4-1	<i>Gallus gallus domesticus</i>			CTX-M-123	pST108
140	pCTXM123		China		CTX-M-123	pST108

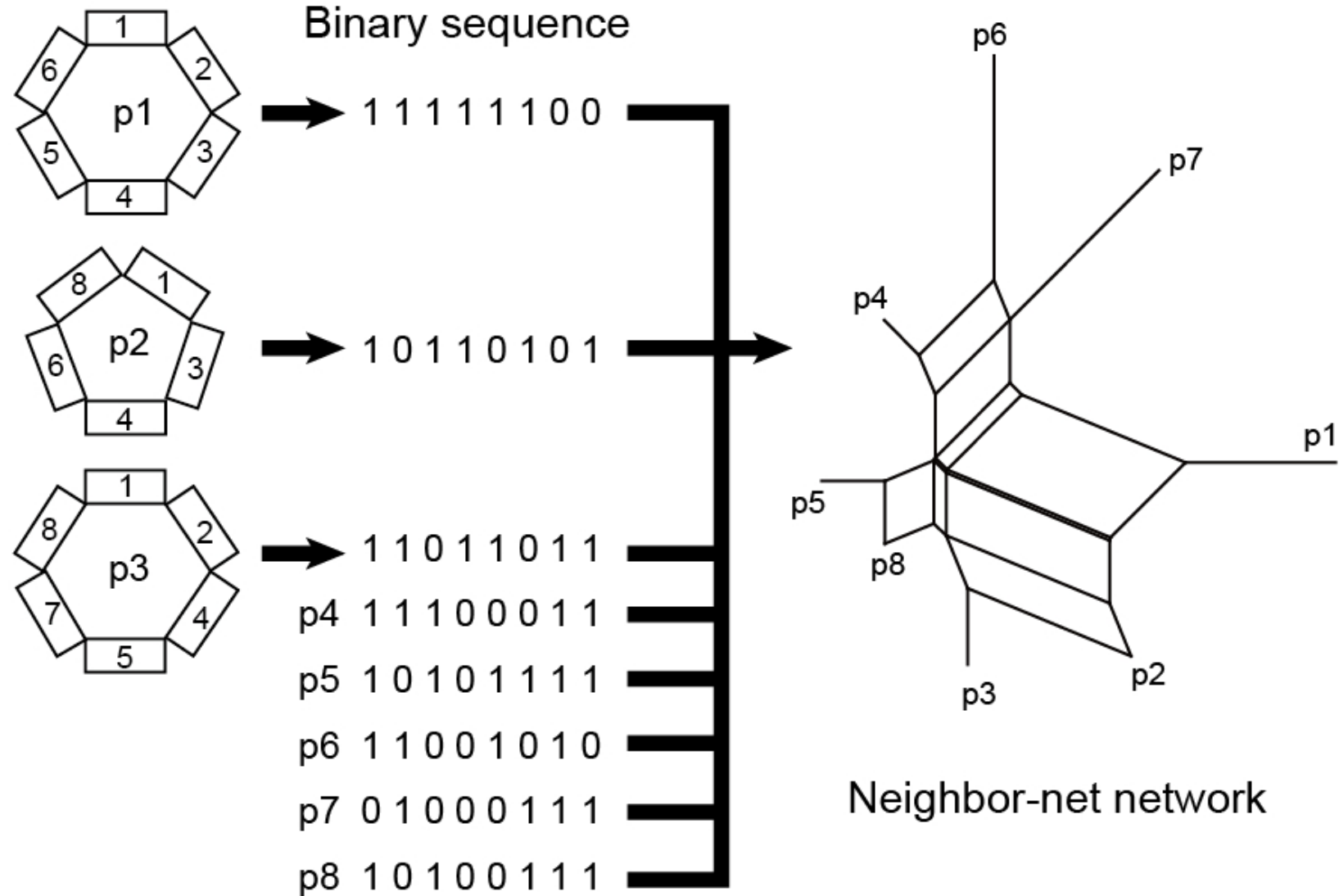


Figure 1
Outline of OSNAp analysis. Plasmid sequences (p1 – p3) are broken down into ORFs (1 – 8) and binarized based on the presence of ORFs. Phylogenetic relationships among plasmids are depicted as neighbor-net network based on the distance matrix of the binary sequences. ORF contents of plasmid 4 to 8 (p4 – p8) are omitted.

