Title: Genetic Background of β-Lactamase Genes in Extraintestinal Pathogenic Escherichia coli ST131 in Indonesia Based on Whole-Genome Sequencing (WGS) Sequences

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Original Article

Title: **Genetic Background of β-Lactamase Genes in Extraintestinal Pathogenic *Escherichia coli* ST131 in Indonesia Based on Whole-Genome Sequencing (WGS) Sequences**

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Body of the main article: *(up to 3500 words)*

Introduction

Escherichia coli is a rod-shaped Gram negative bacteria comprised in the *Enterobacteriaceae* family and is important in causing several diseases in animals and humans. Based on its location in the disease development, *E. coli* is divided into Enteropathogenic *E. coli* (EPEC) and Extraintestinal pathogenic *E. coli* (ExPEC). Although *E. coli* is classified as normal flora in the human's digestive system, *E. coli* can also cause infection outside the host's intestine due to their niche reservoir. ExPEC causes infections in the urinary tract, kidney, bloodstream, and prostate.[1] ExPEC is considered as a pathogen that can spread in either community or hospital-acquired.[2] ExPEC is also increasing for antibiotic resistance, making it a limited option for treatment. One of the important diseases caused by ExPEC is bacteremia and sepsis. ExPEC is a common cause of bacteremia in high-income countries, more than other bacteria that also cause bacteremia such as *Staphylococcus aureus* and *Streptococcus pneumoniae*. ExPEC is also a common cause of meningitis and neonatal infection.[3,4] Patients with ExPEC infection have high morbidity and mortality rates because this bacterium may induce a strong host inflammation response and then trigger sepsis.[5]

B-lactam antibiotic is the primary class of antimicrobial that has been used extensively in poultry, farm, moreover for infectious disease treatments. The overuse of β-lactam antimicrobials triggers resistance to this class of antibiotic. β-lactam-resistant *E. coli* becomes challenging to treat since β-lactam is still the first option and is an effective antimicrobial to diminish the wild-*type E.coli*. The resistance mechanism of β-lactam antimicrobial is frequently mediated by acquired resistance genes. These genes encode an enzyme that can degrade the β-lactam antibiotics called the β-lactamase enzyme. Extended-spectrum β-lactamase (ESBL) is considered a class A β-lactam, including 3rd generation β-lactam such as cephalosporins and aztreonam.[6] TEM and SHV ESBLs was the first discovered and became the dominant genes in 2000 present in β-lactam-resistant *E. coli*.[7] Nowadays, the common ESBL genes are *bla*$_{\text{CTX-M-1}}$, *bla*$_{\text{CTX-M-14}}$, *bla*$_{\text{TEM-52}}$, and *bla*$_{\text{SHV-12}}$, together with another variant of *bla*$_{\text{CTX-M}}$, *bla*$_{\text{TEM}}$, dan *bla*$_{\text{SHV}}$.[8] The dissemination of ESBL genes between human is driven by horizontal gene transfer factors. ESBL genes are often associated with a transferable element such as insertion sequence (IS) ISEcp1, ISCR1, IS26, and IS10, also a transposable element (transposon) including Tn2, and integron.[9] Most ESBL genes in *E. coli* isolated from humans are located integrated with a plasmid.[10] The plasmid has a unique sequence in terms of replication function called replicon. *E. coli* with ESBL genes have been discovered in the plasmid, such as plasmid IncF, IncHI2, IncH1, IncN, and Inc1, another replicon also may contributed.

Sequence type (ST) is a group of bacterial clonality that compares specific sequences of several housekeeping genes. The certain ST types identified can be either unique phenotype characteristics in virulence or antibiotic resistance accessory. According to a review and meta-analysis of 217 studies, ExPEC ST131 was the most common in the late 2000s.[11] The study above depicts the importance of ST131 in ExPEC. Meanwhile, only a few studies have been conducted on ExPEC in Indonesia, particularly on whole-genome sequencing (WGS) sequences. Recently, Paramitha et al. 2020 [12] analyzed the bacteremia ExPEC using WGS data.

This study explained the epidemiology of ST type, virulence genes and resistance genes. Using this WGS in National Center for Biotechnology Information (NCBI) database, we explored more about the β-lactamase genes with their genetic background comparison. Furthermore, we also predicted the origin of β-lactamase genes from Indonesian ExPEC.

Methods

Paramitha et al.[12] discovered 22 ExPECs from Indonesian bacteremia patients. They analyzed the genotype of virulence and antimicrobial resistance genes using the sequences of whole-genome sequencing. The most frequent ST type is ST131, among others. All *E. coli* ST131 in that study were downloaded from National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/). Their WGS were submitted with accession number PRJNA596854 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA596854). We collected an entire fasta file of each isolate in one file. This entire WGS file comprises many contigs. In finding a β-lactamase gene, we submitted each WGS fasta file in Resfinder 4.1 server on 16th November 2021 (https://cge.cbs.dtu.dk/services/ResFinder/) with *Escherichia coli* database as the setting. Plasmid replicon sequence was detected in PlasmidFinder 2.1 on 17th November 2021 (https://cge.cbs.dtu.dk/services/PlasmidFinder/) with setting as follows; Enterobacteriacec database, 95% minimum identity threshold, and 60% minimum coverage identity threshold. Several databases that have unique sequences compared with our *E. coli* ST131 β-lactamases were downloaded from NCBI on 17th November 2021 and then analyzed in BlastN with a program set as a highly similar sequence. A Genomic mapping was generated manually with the raw mapping from BlastN mapping. Circular map comparison of genomic data from all *E. coli* ST131 was visualized using CGView server (cgview.ca) on 21st November 2021 with Prokka annotation,[13] GC content, contig information, and GC skew.

Results

We chose EC_0406 as a reference due to containing more number of β-lactamase genes including *bla*$_{DHA-1}$, *bla*$_{OXA-10}$, *bla*$_{SHV-120}$, and *bla*$_{TEM-1B}$. A whole sequence of EC_0406 had around 5.5 megabase pairs length. The circular genome visualization of these isolates has a brief annotation, GC skew and GC content (Fig. 1). We enlarged the circular genome from CGView to observe more detail about the genetic background or surrounding DNA Sequences of β-lactamase genes in all ExPEC ST131. Since we used CGView for generating the circular genome visualization, we need to confirm the precise annotation using ResFinder 4.1. Only EC_0406 had *bla*$_{OXA-10}$ (in CGView circular genomic mapping, it is annotated by bla_3) from all ExPEC ST131 in this position (Fig. 2A). The *bla*$_{OXA-10}$ was the only protein-coding sequence (CDS) in its contig. Interestingly, EC_0406 putatively had the same copy of the *bla*$_{OXA-10}$ presented in another position (Fig. 2B is annotated by bla_7 and bla_8; and Fig. 3B is annotated by bla_3). EC_0406 genome had *bla*$_{TEM-1}$ as the only CDS in its contig. EC_0406 was also the only one harboured *bla*$_{TEM-1}$ in this position (Fig. 2B). In figure 2C, only EC_0406 had *bla*$_{OXA-17}$ (annotated by bla_5). The upstream of the *bla*$_{OXA-17}$ was *emrE_3* and *folP_3,* which were located in the same contig. Interestingly, EC_0911 and EC_1833 had these upstream genes without *bla*$_{OXA\_17}$. In figure 2D, all ST131 isolates had *bla*$_{DHA-1}$. The upstream and downstream of *bla*$_{DHA-1}$ (annotated by ampC_4) in EC_0406 were hypothetical protein and *gcvA_3*,

respectively, in one contig. However, none of the rest isolates contained those genes. There were other two copies of *bla*$_{DHA-1}$ with identical upstream and downstream patterns in each isolate (Fig. 3A and Fig. 3C). According to figure 3A, only EC_0406 had *bla*$_{SHV-120}$ (annotated by bla_4) with hypothetical protein in the downstream and *ygbJ_3* in the upstream. Also, there was another copy of *bla*$_{SHV-120}$ with identical surrounding genes in another location (Fig. 3C). In figure 3D, we found *bla*$_{TEM-1}$ (annotated by bla_1) in EC_0406 and EC_1833. Interestingly, in EC_0406, *bla*$_{TEM-1}$ was surrounded by IS21 family transposase IS1326 downstream and IS1182 family transposase ISCfr1 upstream. While in EC_1833, only the upstream was closely related to EC_0406 of bla$_{TEM-1}$ surrounding genes (Fig. 3D).

Only several β-lactamase genes, including *bla*$_{OXA-1}$ and *bla*$_{DHA-1}$ were visualized due to the read sequence limitation since these genomes are derived from short-read sequencing. In figure 4, all of Indonesian ExPEC ST131 had *bla*$_{OXA-1}$ with AAC (6')-1b-cr5 in the downstream and Cab83 in the upstream with 100% identity. This region also had 100% identity with those in *E. coli* AH01 plasmid pAH01-4, *E. coli* NDM4 plasmid unnamed1, *Klebsiella pneumoniae* INF142 plasmid4, and *Salmonella enterica* S146 chromosome. One *Pseudomonas aeruginosa* genome also had this region, yet different only in AAC(6')-1b-cr5 with 99% identity (lighter grey). While *E. coli* EC42 chromosome and *K. pneumoniae* pKP112 harboured *bla*$_{OXA-1}$ with different surrounding genes than Indonesian ExPEC ST131 isolates. The differences were Class I integrin integrase IntI1 downstream and ANT(3'')-Ia upstream.

Specifically for *bla*$_{DHA-1}$, only EC_0406 was detected from all Indonesian ExPEC ST131. The surrounding genes of this region were 100% identity with those *E. coli* 142 plasmid p142-A-OXA-181, *Shigella sonei* FC1428 chromosome, *S. sonei* 6207 plasmid pM2901, and *Shigella flexneri* M2901 pM2901 (Fig. 5). *Acinetobacter indicus* B18 pB18-2 had an identical only in Globulin in the upstream and LysR in the downstream. Moreover, *bla*$_{DHA-1}$ with LysR downstream of EC_0406 was identical to those in *Providencia rettgeri* YPR31 pYPR31 and *Enterobacter cloacae* BSI034 pBSI034. The presence of plasmid backbone in their genomes was detected using PlasmidFinder. All Indonesian ExPEC ST131 harboured IncFIA, IncFIB and INCFII, unless IncFIA was absent in EC_1833.

Discussion

The β-lactams antibiotic is a broad-spectrum antibiotic that commonly produced by microorganisms, with a unique structure of four rings called β-lactam ring. Some of them has fused with another ring (penicillins, cephalosporins, and carbapenems), while another is formed monocyclic (monobactams). This ring is responsible for antibiotic activity by inhibiting bacterial cell wall synthesis by binding to penicillin-binding proteins. (PBPs). This PBPs is the obligatory enzyme in building a bacterial cell wall called peptidoglycan.[14] Overuse of many β-lactam antibiotics all over the field increases bacterial resistance. Many resistance mechanisms have been studied, such as efflux pump, change in antibiotic target, and β-lactam-degraded enzyme.[15] The β-lactamase has been widespread all over the world recently. Moreover, this gene can be transferred because it is often found in one frame with transposable element and transferable plasmid.[16] So far, β-lactamase has four different classes such as class A (TEM, SHV, CTX-M, and KPC), class B (NDM and VIM), class C (CMY and ADC), and class D or

called oxacillinase (OXA).[17] The OXA-10 has been considered to have a weak carbapenemase activity. The OXA-10 is also commonly found in *P. aeruginosa* that can be expressed to association with class I integron or transposons.[18] It makes OXA-10 can spread among *Enterobacteriaceae* due to horizontal gene transfer. In the Indonesian ExPEC ST131 genome, all *bla*$_{\text{OXA-10}}$ were in the short contig. Thus we could not evaluate the surrounding genes among the blaOXA-10.

The *bla*$_{\text{OXA-17}}$ only has been detected in EC_0406. The *bla*$_{\text{OXA-17}}$ is another variant of extended-spectrum β-lactamase OXA-10, which is different in serin that replaces asparagine in amino acid position number 73. OXA-17 transformation is confirmed to cause a higher resistance to cefotaxime and cefepime than that of OXA-10 transforman. The OXA-17 extracted enzyme had more activity than that of OXA-10 enzyme against oxacillin and cefotaxime. In EC_0406, the upstream gene of *bla*$_{\text{OXA-17}}$ was *emrE* and *folP*. The *emrE* encodes transporter in bacterial membran that is belonged to Small Multidrug Resistance (SMR) transporter family. It has been known to imply osmotic stress response,[19] biofilm formation,[20] and resistance to acriflavine (a topical cationic antiseptic compound). TEM-1 is an enzyme from class A β-lactamase that was firstly found in *E. coli* and *Salmonella*.[21] Unfortunately, it can spread among Enterobacteriaceae and responsible for β-lactam resistance dissemination. The TEM-1 has a high catalytic activity against penicillins and cephalosporins. Another class A β-lactamase is SHV enzyme. The SHV enzyme is not easily spread compared to that of CTX-M enzyme. However, The SHV has been found in Enterobacteriaceae, including *E. coli* and *K. pneumoniae*. We found SHV-120 in these WGS of ExPEC ST131. Liakopoulos et al. 2016 [22] published the different types of schematic representation of *bla*SHV. Although, there was no *bla*$_{\text{SHV-120}}$ in the mentioned scheme. Based on its genetic background, Indonesian ExPEC ST131 EC_0406 was identical to that of IncL_M plasmid-containing *bla*$_{\text{SHV-5}}$. In its downstream, there was *lacY* gene and also *ygb* gene in the upstream. This surrounding gene pattern is identical with EC_0406 surrounding gene of *bla*$_{\text{SHV-120}}$.

All Indonesian ExPEC ST131 isolates had OXA-1 where either different strains or different species had this OXA-1 with an identical genetic background. Interestingly, we found that *E. coli* originating from China and Austria had an identical genetic background to Indonesian ExPEC ST131. Moreover, the *E. coli* from China was associated with plasmid-mediated colistin resistance. Colistin is the last line agent for treating Multidrug resistance bacteria. At the same time, *E. coli* from Austria contained NDM-5 and was resistant to cefiderocol.[23] *Klebsiella pneumoniae* INF142 plasmid originating from Australia and *S. enterica* S146 chromosome from China were other species with identical genetic background. Meanwhile, OXA-1 with different genetic background was detected in *E. coli* EC42 chromosome from Ghana and *K. pneumoniae* KP112 from France (country origin of isolates were provided by NCBI).

The *bla*$_{\text{DHA-1}}$ was harbored by EC_0406, the only Indonesian ExPEC ST131 isolate. The genetic background of *bla*$_{\text{DHA-1}}$ was identical to either *E. coli* 142 plasmid p142-A-OXA181 or even other species, including *Shigella sonei* FC1428, *S. sonei* 6207, and *S. flexneri* M2901. In India, *S. sonei* FC1428 had been isolated from stool specimens with novel chromosome integration in IncFII plasmid-containing *mphA* gene.[24] Expression of *mphA* gene has been

known to be able to inactivate azithromycin antibiotics. Interestingly, this *bla*$_{DHA-1}$ was present in the chromosome instead of the plasmid. An isolate with identical genetic background, *S. flexneri* M2901, was known to cause an outbreak in Northern Australia with multidrug resistance accessories in 2016-2019.[25] This isolate was the first report for MDR *Shigella* sp. in Australia that was not associated with men who have sex with men. This isolate also contained *bla*$_{DHA}$ with IncFII plasmid backbone.

Conclusion

We reported a genetic background of β-lactamase genes of Indonesian ExPEC ST131 from WGS sequences. *bla*$_{OXA-1}$ gene with its genetic background from all Indonesian ExPEC ST131 were identical with *E. coli* isolated from Austria and China. This *bla*$_{OXA-1}$ is also identical with other species, including *K. pneumoniae* from Australia and *S. enterica* from China. At the same time, *bla*$_{DHA-1}$ with its genetic background from Indonesian EC_0406 was identical with *E. coli* and other species, including *Shigella sonei* isolated from India and *S. flexneri* isolated from Australia.

Authors' contributions statement:

The authors NAI, MI, SN were involved in the conceptualization and design of the study. NAI was collected WGS sequences from NCBI. S and MY were engaged in giving deep analysis about genomic mapping comparison. All authors were involved in reviewing and approving the manuscript.

Conflicts of interest and source of funding statements:

All authors – none to declare

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Abstract

Introduction Extraintestinal pathogenic *Escherichia coli* (ExPEC) is a group of pathogens that can colonize the outside of the intestine, such as the kidney, urinary tract or bloodstream. *E. coli* ST131 has been reported as a critical ST type among the ExPECs. *E. coli* is an opportunistic pathogen frequently found in Indonesia and worldwide, with many of them being resistant to β-lactam antibiotics.

Methods This study analyzed more about the genetic background of β-lactamase genes among Indonesian ExPEC ST131. Whole-genome sequencing (WGS) sequences from the National Center for Biotechnology Information (NCBI) of Indonesian ExPEC ST131 were taken, then analyzed. Circular genomic mapping and genomic comparison of surrounding genes of β-lactamase in these isolates were generated. *bla*$_{OXA-1}$ and *bla*$_{DHA-1}$ were analyzed deeply due to their presence in a relatively long contig, making them available for analysis.

Results Indonesian ExPEC ST131 isolates had *bla*$_{OXA-1}$ with an identical genetic background of *E. coli* originating from China and Austria with *aac (6')-1b-cr5* in the downstream and *cab83* in the upstream. The *bla*$_{OXA-1}$ was also detected in other species, including *Klebsiella pneumoniae* INF142 originating from Australia and *Salmonella enterica* S146 from China. While, *bla*$_{DHA-1}$ in EC_0406 had an identical genetic background to *E. coli* 142 and other species such as *Shigella sonei* FC1428 from India and *S. flexneri* M2901 from Australia, with Globulin-encoded gene in the upstream and *lysR* in the downstream.

Conclusion Our findings demonstrate the global spread of β-lactamase genes detected in Indonesian ExPEC ST131. These genes were identical with those isolated from some countries around the world.

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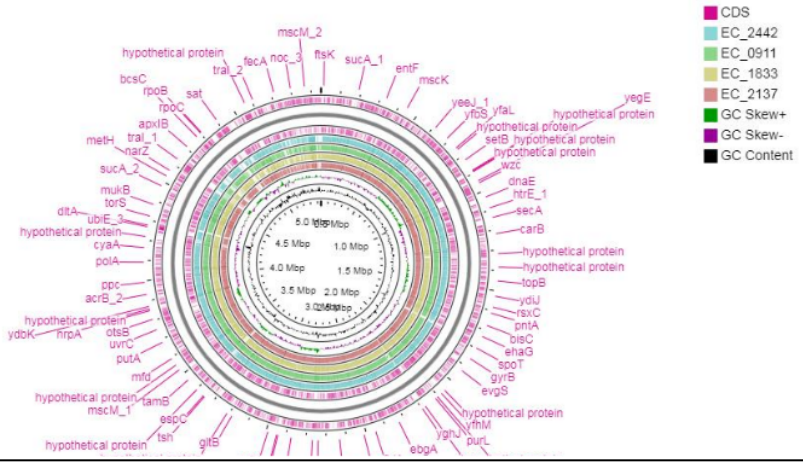

Figure 1. The circular genomic mapping of WGS EC_0406 as a reference against other isolates. Notes from outermost circle to innermost are as follows; purple: forward transcription direction of EC_0406, purple: reverse transcription direction of EC_0406, the blue circle is EC_2442 sequence, the green circle is EC_0911 sequence, the grey circle is EC_1833, the pink circle is EC_2137. Annotation was performed using Prokka annotation from CGView Server.

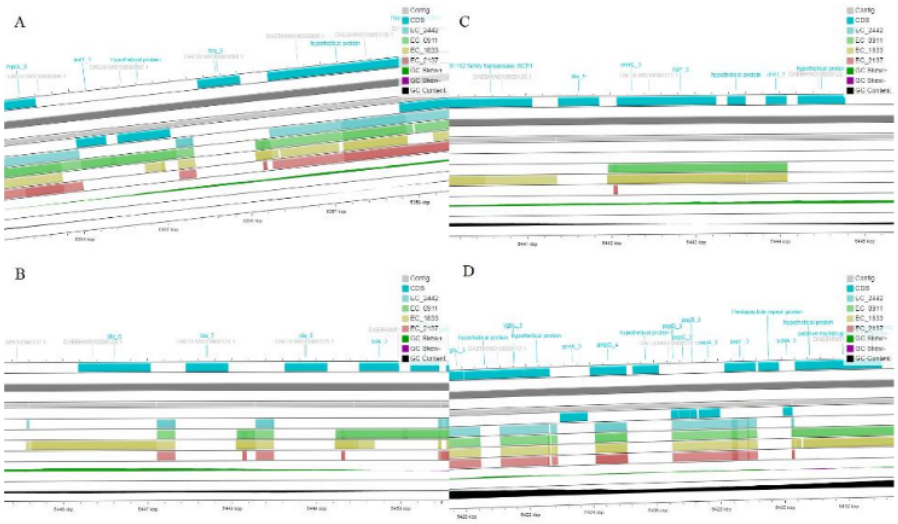

Figure 2. Insert appearance of circular genomes from CGView of all ExPEC ST131 β-lactamase genes. A) Visualization of the *bla*$_{\text{OXA-10}}$ (Annotated by bla_3), B). The *bla*$_{\text{TEM-1}}$ (bla_6) and the *bla*$_{\text{OXA-10}}$ (bla_7 and bla_8), C). The *bla*$_{\text{OXA-17}}$ (bla_5), D). The *bla*$_{\text{DHA-1}}$ (ampC_4)

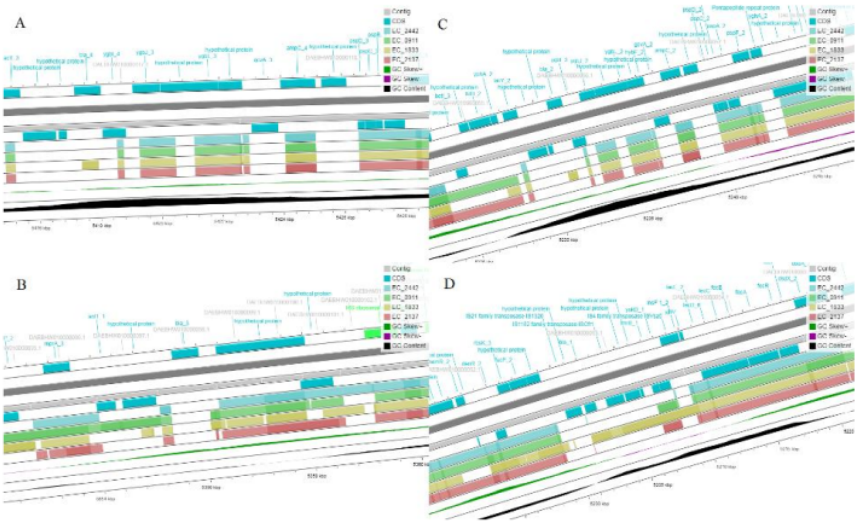

Figure 3. Insert appearance of other β-lactamase genes. A). The *bla*$_{SHV-120}$ (annotated by bla_4) and The *bla*$_{DHA-1}$ (ampC_4), B). The *bla*$_{OXA-10}$ (bla_3), C). The *bla*$_{SHV-120}$ (bla_2), D). The *bla*$_{TEM-1b}$ (bla_1).

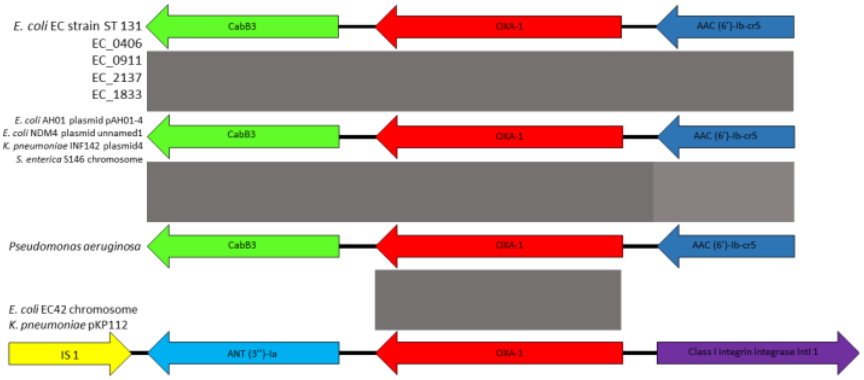

Figure 4. Genomic mapping of *bla*$_{OXA-1}$ surrounding genes of several strains compared to *E. coli* ST131 isolates. The *bla*$_{OXA-1}$ was denoted with red bars. The mapping was visualized with protein abbreviations.

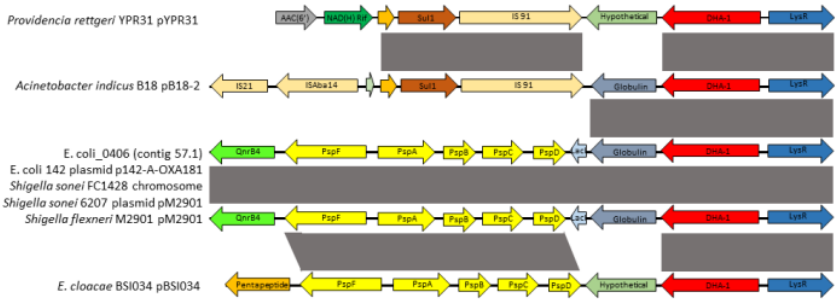

Figure 5. Genomic mapping of *bla*$_{DHA-1}$ surrounding genes from several strains compared to *E. coli* EC_0406. The *bla*$_{DHA-1}$ was denoted with red bars. The mapping was visualized with protein abbreviations.

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