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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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ABSTRACT

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. 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. Indonesian ExPEC ST131 isolates had bla_{OXA-1} with an identical genetic background of *E. coli* originating from China and Austria with *aac (6')-Ib-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. Our findings demonstrate the global spread of β-lactamase genes detected in Indonesian ExPEC ST131. These genes were identical to those isolated from some countries around the world.

1. Introduction

Escherichia coli is a rod-shaped Gram-negative bacteria of the Enterobacteriaceae family that causes various 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 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 (Poolman and Wacker 2016). ExPEC is considered a pathogen that can spread infections in either community or hospital environments (Mellata 2013). ExPEC is also increasing antibiotic resistance, limiting the options for its 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 (Johnson *et al.* 2003; Bonacorsi and Bingen 2005). Patients with ExPEC infection have high morbidity and mortality rates because this bacterium may induce a strong host inflammation response and trigger sepsis (Miajlovic and Smith 2014).

The β-lactam antibiotic is the primary class of antimicrobial that has been used extensively in poultry, farm, and infectious disease treatments.

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The overuse of β-lactam antimicrobials triggers resistance to this class of antibiotic. Treatment of β-lactam-resistant *E. coli* is challenging since β-lactam is still the first option and is an effective antimicrobial agent 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 (Rawat and Nair 2010). TEM and SHV ESBLs were the first discovered and became the dominant genes in 2000 present in β-lactam-resistant *E. coli* (Karim *et al.* 2001; Pitout *et al.* 2005). 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_{TEM}, and bla_{SHV} (Ejaz *et al.* 2021). Horizontal gene transfer factors drive the dissemination of ESBL genes between humans. 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 (Poirel *et al.* 2008; Canton *et al.* 2012; Schink *et al.* 2013). Most ESBL genes in *E. coli* isolated from humans are integrated with a plasmid (Carattoli 2013; Ferreira *et al.* 2014; Guenther *et al.* 2017). 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 may also contribute (Carattoli 2013).

Sequence type (ST) is a group of bacterial clonality that compares specific sequences of several housekeeping genes. The particular ST types identified may be unique phenotype traits related to virulence or antibiotic resistance. According to a review and meta-analysis of 217 studies, ExPEC ST131 was the most common in the late 2000s (Manges *et al.* 2019). *E. coli* ST131 with multidrug-resistant (MDR) accessories is becoming a high threat worldwide, with the most frequent being ExPEC type. This ST131 also been known to spread extensively worldwide (Mathers *et al.* 2015; Pitout and DeVinney 2017). Dissemination of *E. coli* ST131 is common among the patients, and food contamination (Platell *et al.* 2011). This clonality group can infect humans and animals and causes infections in the urinary tract and bloodstream (Kanamori *et al.* 2017; Johnson *et al.* 2017). Recently, *E. coli* ST131 has been the main clonal spread of MDR with CTX-M ESBL compared with another clone (Doi *et al.* 2017). 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. Paramitha *et al.* (2020) recently discovered 22 ExPECs from Indonesian bacteremia patients using WGS data. This study explained the epidemiology of ST type, virulence genes and antimicrobial resistance genes. The most frequent ST type is ST131, among others. Using this approach, we found out more about the β-lactamase genes with their genetic background comparison from WGS data deposited in the National Center for Biotechnology Information (NCBI) database. Furthermore, we also predicted the origin of β-lactamase genes from Indonesian ExPEC origin.

2. Materials and Methods

All *E. coli* ST131 in the Paramitha *et al.* (2020) study were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/), with accession number PRJNA596854. We collected an entire fasta file of each isolate in one file. This entire WGS file comprises many contigs. In finding the β-lactamase genes, we submitted each WGS fasta file in Resfinder 4.1 server on 16th November 2021 with the *Escherichia coli* database as the setting (Zankari *et al.* 2017; Clausen *et al.* 2018; Bortolaia *et al.* 2020). Plasmid replicon sequence was detected in PlasmidFinder 2.1 on 17th November 2021 (Clausen *et al.* 2018; Carattoli and Hasman 2020) with setting as follows; *Enterobacteriaceae* database, 95% minimum identity threshold, and 60% minimum coverage identity threshold. Several sequence hits 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 homolog search optimized for 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 on 21st November 2021 with Prokka annotation, GC content, contig information, and GC skew (Seemann 2014).

3. Results

We chose EC_0406 as a reference against other isolates in circular genomic mapping since it contains more β-lactamase genes, including bla_{DHA-1}, bla_{OXA-10}, $bla_{SHV-120}$, and bla_{TEM-1B}. An entire sequence of EC_0406 had a length of about 5.5 megabase pairs. The circular genome visualization of these isolates includes a brief annotation, GC skew and GC content (Figure 1). We enlarged the circular genome of the CGView to provide more details about the genetic background or surrounding DNA sequences of β-lactamase genes in all ExPEC ST131. Since we used CGView to generate the circular genome visualization, we need to confirm the precise annotation using ResFinder 4.1. Only EC_0406 had blaOXA-10 (in CGView circular genomic mapping, it is annotated by bla_3) from all ExPEC ST131 in this position (Figure 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 (Figure 2B is annotated by bla_7 and bla_8, and Figure 3B is annotated by bla_3). EC_0406 was the only one that harboured bla_{TEM-1} (annotated by bla_6 in Figure 2B) as the only CDS in its contig. 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 (Figure 3A and C). 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 (Figure 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 (Figure 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 had 100% identity with those of *E. coli* 142 plasmid p142-A-OXA-181, *Shigella sonei* FC1428 chromosome, *S. sonei* 6207 plasmid pM2901, and *Shigella flexneri* M2901 pM2901 (Figure 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 harbored IncFIA, IncFIB, and INCFII, except IncFIA was absent in EC_1833.

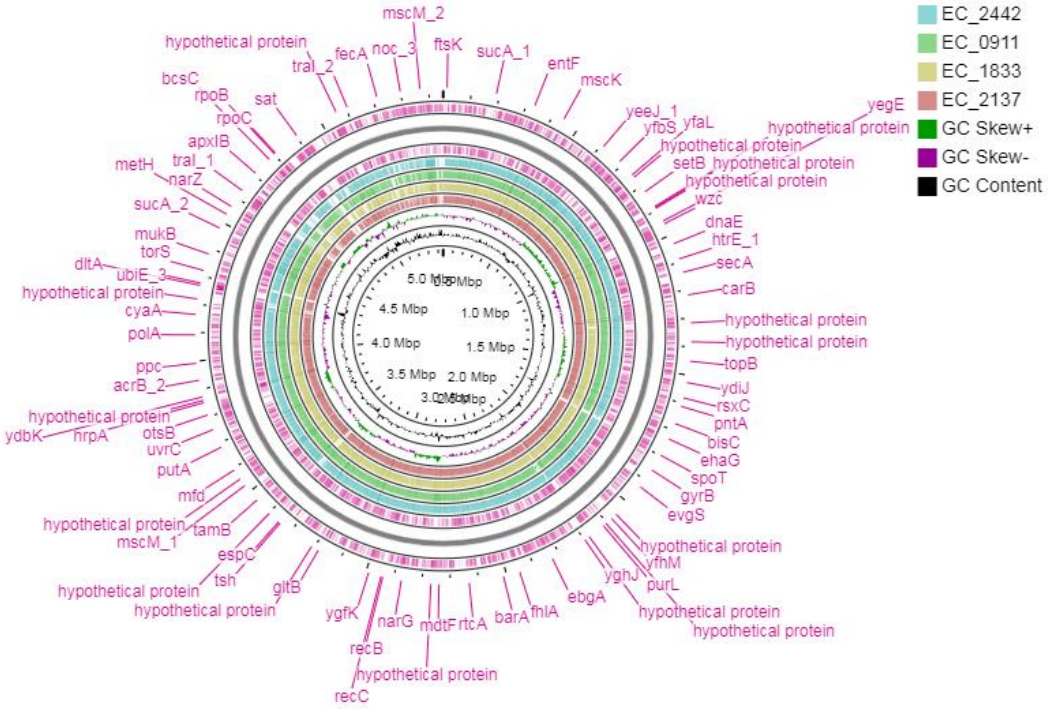

Figure 1. The circular genomic mapping of WGS EC_0406 as a reference against other isolates. Notes from outermost circle to innermost are as follows; purple: forward transcription direction of EC_0406, purple: reverse transcription direction of EC_0406, the blue circle is EC_2442 sequence, the green 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

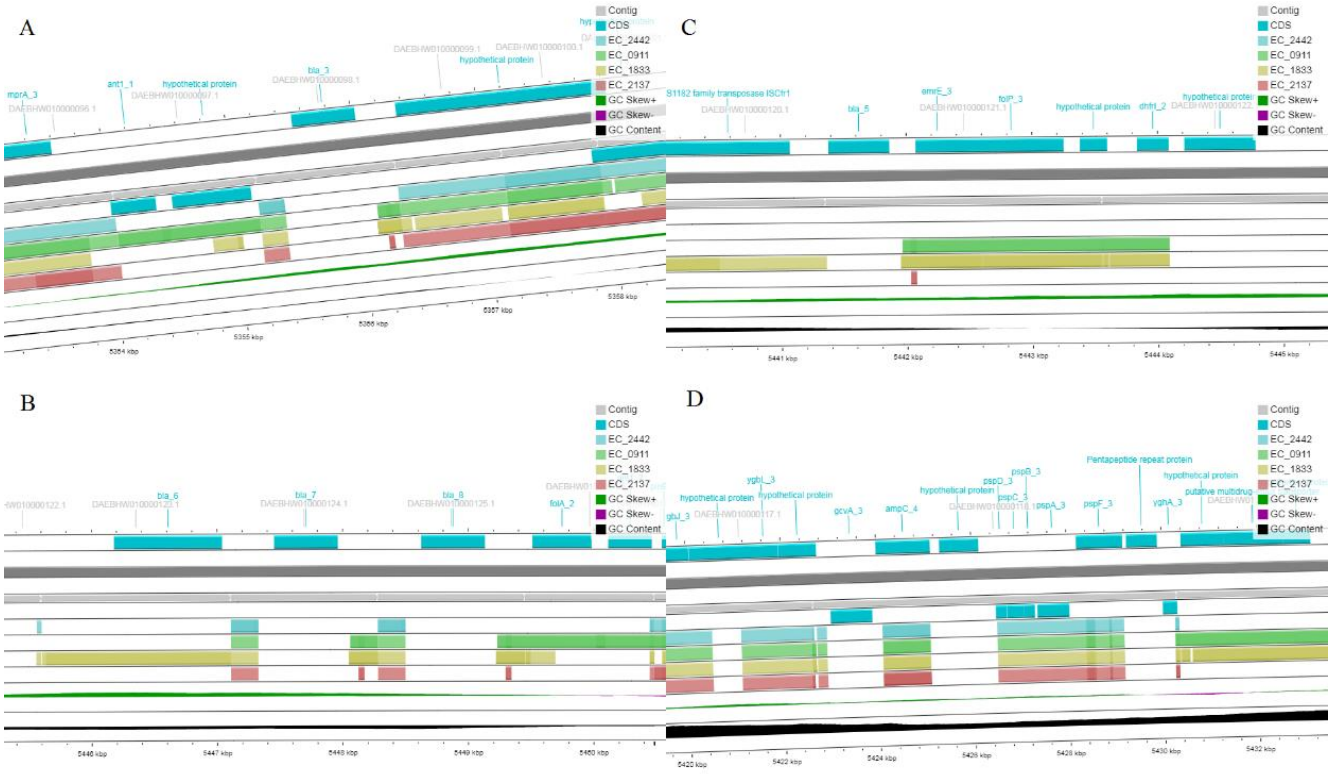

Figure 2. Insert appearance of circular genomes from CGView of all ExPEC ST131 β-lactamase genes. (A) Visualization of the bla_{OXA-10} (Annotated by bla_3), (B) the bla_{TEM-1} (bla_6), and the bla_{OXA-10} (bla_7 and bla_8), (C) the bla_{OXA-17} (bla_5), (D) the bla_{DHA-1} (ampC_4)

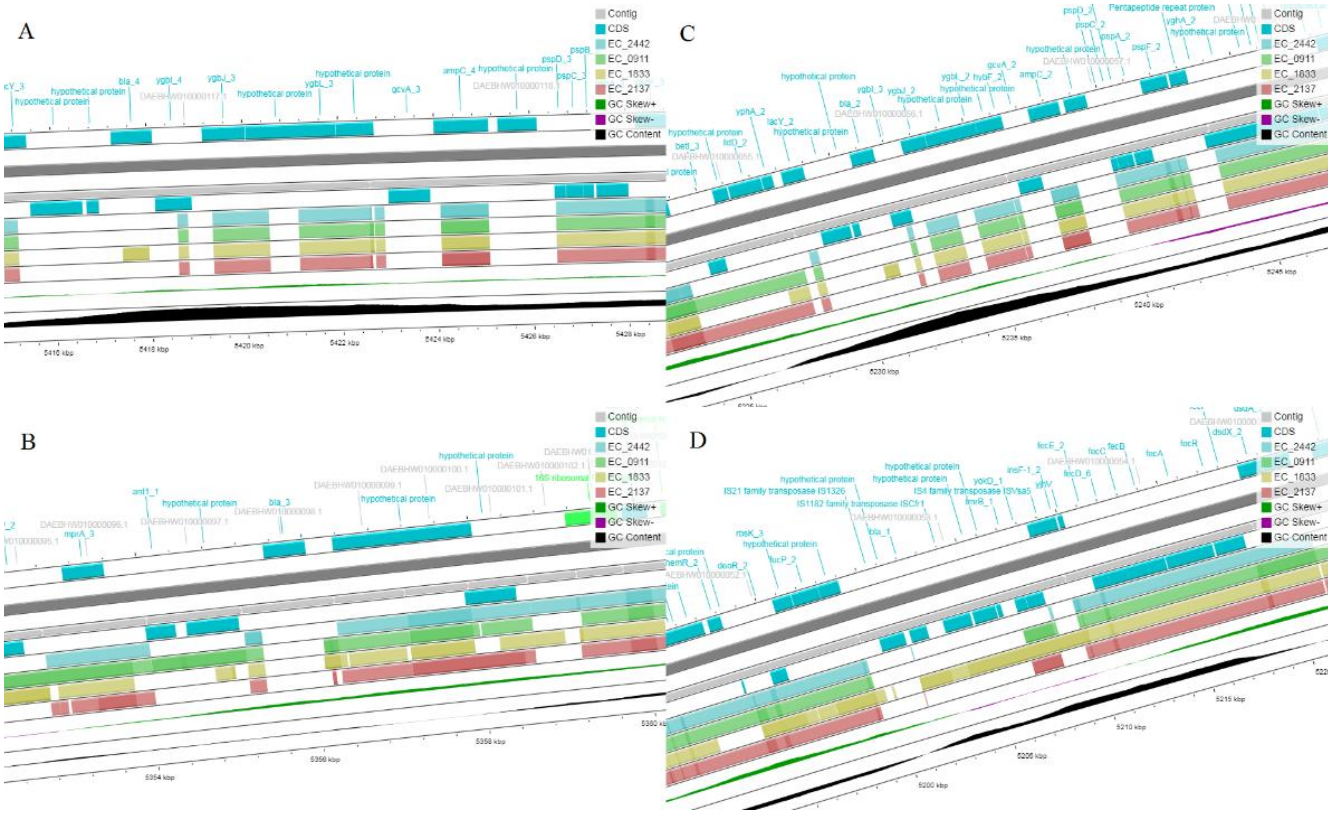

Figure 3. Insert appearance of other β-lactamase genes. (A) The *bla*$_{SHV-120}$ (annotated by bla_4) and the *bla*$_{DHA-1}$ (ampC_4), (B) the *bla*$_{OXA-10}$ (bla_3), (C) the *bla*$_{SHV-120}$ (bla_2), (D) the *bla*$_{TEM-1b}$ (bla_1)

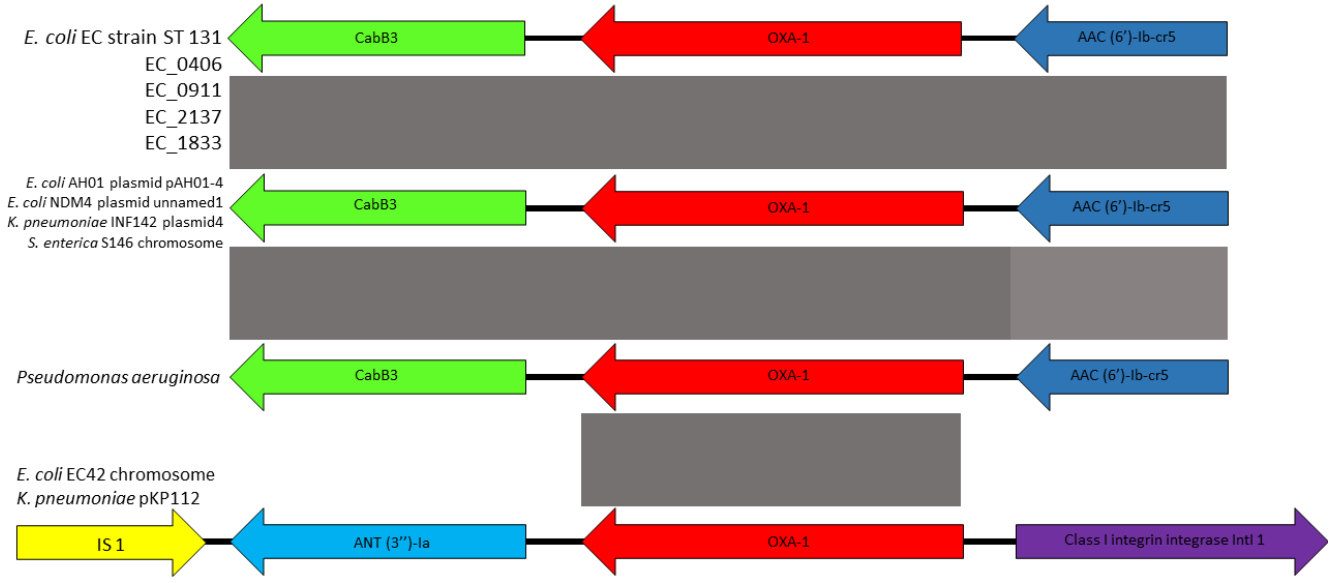

Figure 4. Genomic mapping of *bla*$_{OXA-1}$ surrounding genes of several strains compared to *E. coli* ST131 isolates. The *bla*$_{OXA-1}$ was denoted with red bars. The mapping was visualized with protein abbreviations

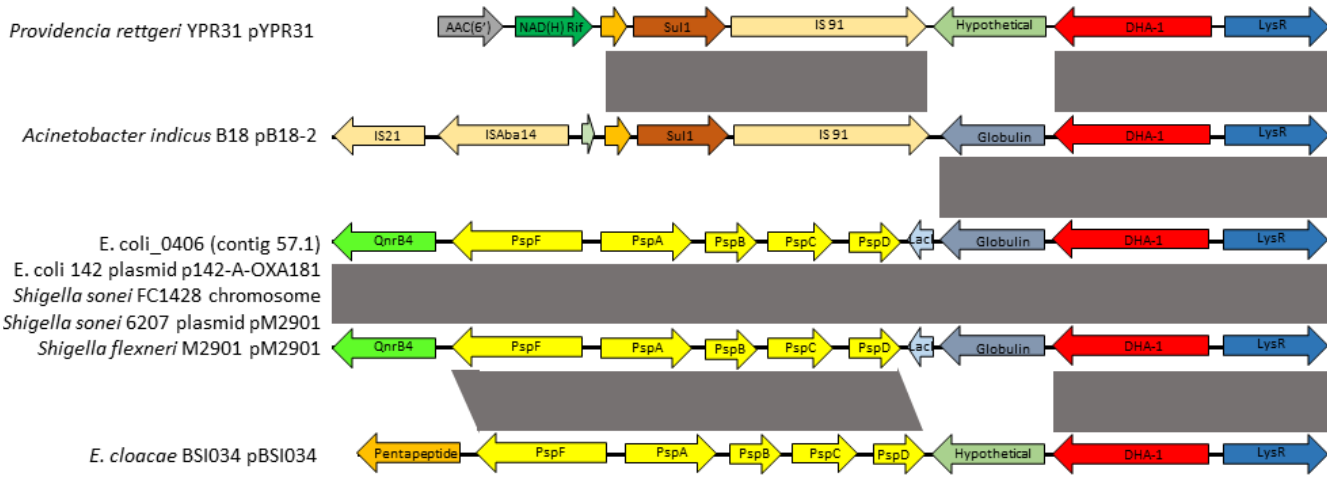

Figure 5. Genomic mapping of bla_{DHA-1} surrounding genes from several strains compared to *E. coli* EC_0406. The bla_{DHA-1} was denoted with red bars. The mapping was visualized with protein abbreviations

4. Discussion

The β-lactams antibiotic is a broad-spectrum antibiotic commonly produced by microorganisms, with a unique structure of four rings called β-lactam ring. Some have 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 through binding to penicillin-binding proteins (PBPs). This PBPs is the obligatory enzyme in building a bacterial cell wall called peptidoglycan (Bush and Bradford 2016). 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 (Tang *et al.* 2014). 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 elements and transferable plasmid (Canton *et al.* 2012; Rozwandowicz *et al.* 2018; Darphorn *et al.* 2021). 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) (Tooke *et al.* 2019).

The class A β-lactamases enzyme is the most studied of β-lactamases including TEM (from patient Temoneira), SHV (Sulfhydryl type, has similar activity to TEM, discovered in the chromosome of *K. pneumoniae* and mobilized into plasmids), CTX-M (Cefotaximase, active against cefotaxime antibiotic), KPC (*K. pneumoniae* carbapenemase) (Tooke *et al.* 2019). With their variants, TEM, SHV, and CTX-M are globally distributed and present in plasmids and mobile genetic elements among Gram-negative pathogens, such as *Enterobacteriaceae*. TEM is the β-lactamase that is carried by plasmid in Gram-negative bacteria and is active against penicillins and some cephalosporins. Carbapenems, such as imipenem, meropenem, and ertapenem, are considered a last-line agent for combating multidrug resistant bacteria. Unfortunately, a new variant of β-lactamases could interfere with the carbapenems. The most crucial enzyme with carbapenemase activity is KPC enzymes (Yigit *et al.* 2001). Recently, this enzyme has been found globally due to its genetic flexibility (Rapp and Urban 2012).

The class B β-lactamases is a zinc-dependent enzyme called Metallo- β-lactamases (MBLs). MBLs are differentiated with metal motifs in their core structure. The class B β-lactamases have a global distribution, including NDM (New Delhi MBL) and VIM (Verona Imipenemase) (Tooke *et al.* 2019). NDM is commonly found in *Enterobacteriaceae*, and VIM is mainly in non-fermenting bacteria, such as *P. aeruginosa*. The class C β-lactamase is the group that resides in the chromosome. This enzyme, annotated as amps, which is in normal conditions, could not be expressed. It was found in *E. coli*. This group is including CMY and DHA enzymes and is often found in mobile genetic elements in *Enterobacteriaceae* and non-fermenting bacteria, including *P. aeruginosa* (Cardoso *et al.* 2021).

The OXA enzyme is considered as the class D β-lactamase. The OXA-10 have a weak carbapenemase activity than other OXA type. The OXA-10 is also commonly found in *P. aeruginosa* that can be expressed as an association with class I integron or transposons (Partridge *et al.* 2002; Antunes and Fisher 2014). It makes OXA-10 can spread among *Enterobacteriaceae*

due to horizontal gene transfer. In the Indonesian ExPEC ST131 genome, all bla_{OXA-10} were in the short contig. Thus we could not evaluate the surrounding genes among the bla_{OXA-10}. The bla_{OXA-17} only has been detected in EC_0406. The bla_{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 OXA-10 transforman. The OXA-17 extracted enzyme had more activity than the OXA-10 enzyme against oxacillin and cefotaxime (Danel *et al.* 1999). In EC_0406, the upstream gene of bla_{OXA-17} was *emrE* and *folP*. The *emrE* encodes transporter in bacterial membrane that is belonged to Small Multidrug Resistance (SMR) transporter family. It has been known to imply osmotic stress response (Bay *et al.* 2017), biofilm formation (Matsumura *et al.* 2011), 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* (Palzkill 2018). Unfortunately, it can spread among *Enterobacteriaceae* and is responsible for β-lactam resistance dissemination. The TEM-1 has a high catalytic activity against penicillins and cephalosporins (Palzkill 2018). Another class A β-lactamase is SHV enzyme. The SHV enzyme is not easily spread compared to the CTX-M enzyme. However, The SHV has been found in *Enterobacteriaceae*, including *E. coli* and *K. pneumoniae* (Canton *et al.* 2012). We found SHV-120 gene in these WGS of ExPEC ST131. Liakopoulos *et al.* (2016) published the different types of the schematic representation of bla_{SHV}. Although, there was no $bla_{SHV-120}$ in the mentioned scheme. Based on its genetic background, Indonesian ExPEC ST131 EC_0406 was identical to that of IncL_M plasmid-containing bla_{SHV-5}. In its downstream, there was *lacY* gene and also ygb gene in the upstream. This surrounding gene pattern is identical to EC_0406 surrounding gene of $bla_{SHV-120}$.

All Indonesian ExPEC ST131 isolates exhibit OXA-1, with either different strains or different species of 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 (Simner *et al.* 2021). *Klebsiella pneumoniae* INF142 plasmid originating from Australia and *S. enterica* S146 chromosome from China were other species with identical genetic backgrounds. Meanwhile, OXA-1 with different genetic background was detected in *E. coli* EC42 chromosome from Ghana and *K. pneumoniae* KP112 from France (NCBI provided country origin of isolates).

The *bla*DHA-1 was exhibited by EC_0406, the only Indonesian ExPEC ST131 isolate. The genetic background of blaDHA-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 (Muthuirulandi *et al.* 2021). The 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 (Guglielmino *et al.* 2021). 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.

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 was identical with *E. coli* isolated from Austria and China. This bla_{OXA-1} is also identical to 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.

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