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RESEARCH

PHYLOGENETIC PROFILE OF *ESCHERICHIA COLI* CAUSING BLOODSTREAM INFECTION AND ITS CLINICAL ASPECT

(Profil Filogenetik Escherichia coli Penyebab Infeksi Aliran Darah dan Aspek Klinisnya)

Osman Sianipar[1], Widya Asmara[2], Iwan Dwiprahasto[3], Budi Mulyono[1]

ABSTRAK

Escherchia coli merupakan satu dari bakteri yang paling sering ditemukan dalam infeksi aliran darah. Tujuan penelitian ini adalah untuk mengeksplorasi profil filogenetik E. coli yang menyebabkan infeksi aliran darah dan aspek klinisnya. Ini merupakan penelitian observasional yang melibatkan 12 subjek yang menderita infeksi aliran darah yang disebabkan oleh E.coli. Isolat klinis E.coli serta hasil uji kepekaan antimikroba diperoleh dari metode kaldu microdilution otomatis. Data klinis diperoleh dari rekam medis dan analisis filogenetik yang dilakukan dengan polymerase chain reaction menggunakan gena chuA dan YjaA. Data dianalisis dengan menggunakan statistik deskriptif. Sumber infeksi ini berasal dari saluran kemih, paru-paru, saluran pencernaan dan kulit yang ditemukan dalam 7 kasus. Namun, sumber infeksi tidak diketahui dalam 5 kasus. Sebagian besar subjek adalah pria dewasa dengan keganasan sebagai penyakit yang mendasarinya. Escherichia coli sebagai etiologi infeksi aliran darah sebagian besar (75%) menghasilkan enzim ESBL dan resistensinya terhadap antimikroba seperti ampicilin, ampicilin/sulbactam, ceftazidime, ceftriaxon, cefepime, aztreonam, ciprofloxacin dan trimetropim-sulfamethoxazol yang cukup tinggi. Kelompok filogenetik dari isolat klinis ini sebagian besar (75%) adalah grup B2 dan grup D yang dikenal sebagai strain virulen ekstraintestinal. Isolat klinis yang tersisa (25%) dapat digolongkan sebagai kelompok filogenetik A atau B1 dimana kelompok A dikenal sebagai strain komensal.

Kata kunci: *Filogenetik, Escherichia coli, infeksi aliran darah*

ABSTRACT

Escherchia coli is one of the most frequent bacteria found in bloodstream infections. The objective of the study was to explore the phylogenetic profile of *E.coli* causing bloodstream infection and its clinical aspect. This was an observational study that involved 12 subjects who suffered from bloodstream infection caused by *E.coli*. Clinical isolates of *E.coli* as well as result of antimicrobial susceptibility test were obtained from an automatic microdilution broth method. Clinical data was obtained from medical records and phylogenetic analysis done by colony polymerase chain reaction using *chuA* and *YjaA* genes. Data was analyzed using descriptive statistics. Sources of this infection originated from urinary tract, lung, gastrointestinal tract and skin which were found in 7 cases. On the other hand, source of infection was unknown in 5 cases. Most of subjects were adult male with malignancies as the underlying disease. *Escherichia coli* as the etiology of bloodstream infection mostly (75%) produced ESBL enzyme and its resistance against antimicrobials such as ampicillin, ampicillin/sulbactam, ceftazidime, ceftriaxone, cefepime, aztreonam, ciprofloxacin and trimethoprim-sulfamethoxazole was high enough. Phylogenetic group of these clinical isolates mostly (75%) were group B2 and group D of which known as extraintestinal virulent strain. The remaining clinical isolates (25%) could be classified either as phylogenetic group A or B1 in which group A was known as a commensal strain.

Key words: Phylogenetic, *Escherichia coli*, bloodstream infection

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INTRODUCTION

Bloodstream infection is one of the global health problems due to a high morbidity and mortality. It is defined by the present of bacterial growth in blood culture with clinical signs and symptoms of infection in which contamination could be excluded. Gram-negative rod bacteria is the most common cause of infection.[1,2] *Escherichia coli* is one of the most frequently isolated among gram negative rod bactria in blood culture.[3] It is reported that 8.3% to 23.1% of bloodstream infection is due to *E.coli*.[4,2] Although usually a commensal, some strains of *E. coli* has become pathogenic and not only related with a diarrhoeal disease but also infections in extra-intestinal organs or systems included bloodstream infection.

Initially phylogeny was determined through by Unweighted Pair Group Method with Arithmetic Mean (UPGMA) using data of Multi-Locus Enzyme Electrophoresis (MLEE) from 35 enzyme loci, defined six main phylogenetic groups, classified as A, B1, B2, C, D and E. It was also done by the neighbour-joining method using data from 38 enzyme loci and defined four main groups A, B1, B2 and D, with a few unclassified sequences that are some-times classified as group E.[5] The previous study on phylogenetic analysis used triplex polymerase chain reaction (PCR) *E.coli* strains were classified into 4 main phylogenetic groups: A, B1, B2 and D in which Group B2 and D strains carried various VF and caused different extraintestinal infections.[6] The objective of this study was to explore phylogenetic profile of *E.coli* causing bloodstream infection and its clinical aspect.

METHODS

Clinical isolates of *E.coli* were isolated from blood culture. Blood samples were inoculated into aerobic bottle culture media and then incubated into an automatic incubator. After the growth of bacteria was detected, then it was sub-cultured onto both blood agar and Mc Conkey media. Those samples which grew in these media were then further processed for identification and antimicrobial susceptibility test using microdilution broth (Vitek 2). *Escherichia coli* isolates were inoculated in nutrient agar vertically and kept up ready to run a polymerase chain reaction (PCR) test. Before PCR test, isolates from nutrient agar were sub-cultured onto both blood agar and Mc Conkey media. These laboratory works were conducted at the Clinical Laboratory of the Dr. Sardjito Hospital Yogyakarta. Clinical data of these patients who suffered from bloodstream infection were obtained from medical records.

The primers used in this study were as follows: 5'-GACGAACCA ACGGTCAGGAT-3' (forward) and 5'-TGCCGCCAGTACC AAAGACA-3' (reserve) were used for *chuA* gene. Whereas primers for *yjaA* gene were 5'-TGAAGTGTCAGGAGACGCT G-3' (forward) and 5'-ATGGAGAATGCGTTCCTCAAC-3' (reserve).[6-8] The control used in this reaction was E.coli ATCC 25922.[8,9]

Polymerase chain reactions were conducted as follows: pre-denaturated at 95°C for 4 minutes, denaturated at 95°C for 15 seconds, annealing at 59°C for 15 seconds, elongation at 72°C for 30 seconds and extension at 72°C for 5 minutes. This PCR test was conducted in 30 cycles. Data were analyzed using descriptive statistics.

RESULT AND DISCUSSION

Total number of study subjects was 12 patients consisting of 9 males (75%) and 3 females (25%). Proportion of females who suffered from bloodstream infection due to *E.coli* was 72.2%[10], whereas another research reported as high as 58.4%.[11] According to the age group the study subjects comprised of 4 children, 6 adults and 2 elderly persons. In another study, it was reported that proportion of subjects were equal or more than 65 years who suffered from bloodstream infection due to *E.coli* was 48.6%.[10] Similarly, 79.5% subjects aged more than 50 years was found in people who suffer from bloodstream infection caused by *E.coli*.[11]

Seven subjects were taken care in the hospital equal or less than 2 days up to suffered from a bloodstream infection. It reflected most probably community-acquired infection. The remaining 5 subjects were already taken care in the hospital more than 2 days, therefore tt could be assumed as hospital acquired infection. Community and hospital-acquired bloodstream infection due to *E.coli* were 68.4% and 31.4%, respectively.[12] Another study reported that hospital-acquired bloodstream infection caused by *E.coli* was 40.9%.[13] It was reported that 35.6% of bloodstream infection due to this bacteria was categorized as health care-associated infections. Bloodstream infection with community onset episodes were categorized into health care-associated infection if fulfilled any of the following criteria: had a history as in patient more than 48 hours during last 90 days, hemodialysis, medicated intravenously, home care of wound during last 30 days, or stayed either in a long-term care facility or a nursing home.[10] A study on *E.coli*

bacteremia with urinary tract as the origin found that the cases of community-acquired and hospital-acquired were 89.3% and 10.7%, respectively.[9] Hospital-acquired bloodstream infection due to *E.coli* was reported as high as 40.9%.[13]

Nine subjects (75%) were infected by ESBL producing *E.coli*, whereas the other 3 patients infected by non-ESBL producing *E.coli*. A study on clinical implications, risk factors and mortality following community-onset bacteremia reported that only 4.7% subjects were infected by ESBL producing *E.coli*.[11]

Table 1. Clinical characteristics of the subject

Variable	n	%
Gender		
Male	9	75.0
Female	3	25.0
Age		
28 days – 17 years	4	33.3
> 17 years – 65 years	6	50.0
> 65 years	2	16.7
Length of stay up to bacteremia determined		
≤ 2 days	7	58.3
> 2 days	5	41.7
ESBL producer	9	75.0
Non-ESBL producer	3	25.0
Bacteremia		
Primary	5	41.7
Secondary	7	58.3

Source of infection was found in seven subjects (secondary bloodstream infection), but unfortunately the other 5 patients the source of infection (primary bloodstream infection) was not known. Source of infection of 7 cases of secondary bloodstream infection were as follows urinary tract in 3 cases, lung in 2 cases, gastrointestinal tract and skin each 1 case. Source of infection of bloodstream infection caused by *E.coli* as reported in another study mostly came from urinary tract (43.6%), followed by biliary tract/liver (23.0%), intra-abdominal (6.9%), pneumonia (5.4%) and other (5.4%). Thirty-two cases (15.7%) the origin of bloodstream infection was not known.[14]

Underlying disease/comorbid of those infected by either phylogenetic group A or B1 were as follows: Rectosigmoid adenocarcinoma, active lung tuberculosis; Mixed leukemia, granuloma pyogenicum, pneumonia; Malignant melanoma stage 4, anemia, acute kidney injury, Disseminated Intravascular Coagulation (DIC). Those subjects whom were infected by phylogenetic group B2 also suffered from underlying disease/comorbid were as follows: Rectosigmoid adenocarcinoma, diabetic ulcer, acute kidney injury, urinary tract infection; Acute lymphoblastic leukemia; Breast cancer stage 4 with brain metastase; Rectosigmoid carcinoma with anemia; Histiocytosis, marasmic, anemia, acute watery diarrhea; Burn stage 2-3.22% with anemia; Retinoblastoma with epistaxis. Underlying disease/comorbid of those infected by phylogenetic group D of *E.coli* were: Pancreatic head cancer with thrombocytopenia; Cervical cancer with chronic kidney disease, urinary tract infection.

Diabetes mellitus, liver disease, renal disease, solid tumor were reported as the underlying disease of patients suffering from community-acquired bloodstream infections caused by strains of Extended-Spectrum β-Lactamase (ESBL)-producing *E.coli*. In addition, some surgical or medical intervention such as recent operation, immunosuppressant use, central venous catheterization, indwelling urinary catheter and percutaneous tube was reported as a comorbid condition.[10] Another study reported that malignancies (solid tumor, hematological disease and stem cell transplant) were the underlying disease of bacteremia caused by ESBL producing *E.coli*. Mechanical ventilation, solid organ transplant, chronic liver disease, chronic renal disease, diabetes, dialysis, neutropenia, corticosteroid use, immunosuppressant use, central venous catheter, indwelling Foley catheter,

Table 2. Underlying disease/comorbid

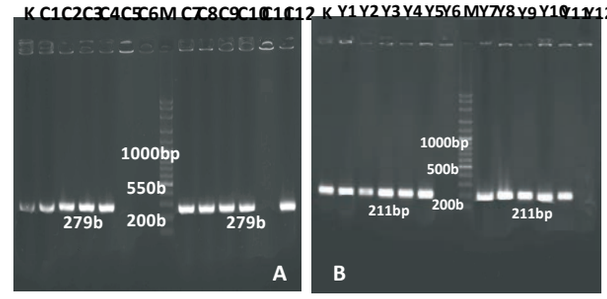
Phylogenetic group	Underlying disease/comorbid
B1/A	Rectosigmoid adenocarcinoma, active lung tuberculosis Mixed leukemia, granuloma pyogenicum, pneumonia Malignan melanoma stage 4, anemia, acute kidney injury, DIC
B2	Rectosigmoid adenocarcinoma, diabetic ulcer, acute kidney injury, urinary tract infection Acute lymphoblastic leukemia Breast cancer stage 4 with brain metastases Rectosigmoid carcinoma with anemia Histiocytosis, marasmic, anemia, acute watery diarrhea Burn stage 2-3, 22% with anemia Retinoblastoma with epistaxis
D	Head pancreatic cancer with thrombocytopenia Cervical cancer with chronic kidney disease, urinary tract infection

gastrointestinal tube, percutaneous drainage, recent surgery, invasive procedures within 72 hours, prior antibiotics within 1 month third or fourth generation cephalosporins were reported as comorbidines.[15]

Result of antimicrobial susceptibility test showed that resistance rate of *E.coli* presented considerably high against ampicillin, ampicillin/sulbactam (β-lactam/β-lactamase inhibitor), ceftazidime, ceftriaxone, cefepime, aztreonam, ciprofloxacine and trimetroprim/sulfamethoxazol. Clinical isolates of *E.coli* were considerably susceptible against a combination piperacillin/tazobactam (β-lactam/β-lactamase inhibitor), ertapenem, meropenem, amikacin and tigecycline.

In year 2014, resistance of *E.coli* against ampicilin, ampicilin/sulbactam, third-generation cephalosporin, ciprofloxacin, trimethoprim/sulfametoxazole was 58.4%, 56.7%, 6.6%, 24.5%, and 29.2% respectively. Among Gram-Negative Bacteria (GNB) causing community-acquired infections, it was reported that the rate of resistance to 3rd generation cephalosporins had increased. The global spread of community acquired infection was considered due to the spread of CTX-M type of ESBLs, especially in *E.coli*. Global spread of *E.coli* clone ST131 was also thought out a potential cause of the development of ESBL-producer of *E.coli* in the community.[16]

Another prospective cohort study which compared effectiveness of antimicrobial empiric and definitive treatment in bloodstream infection due to ESBL producing *E.coli* between a combination β-lactam/β-lactamase inhibitor (BLBI) versus carbapenem with a mortality in 30 days observation as the outcome. The β-lactam/β-lactamase inhibitor used in this study were amoxicillin/clavulanic acid (AMC) and piperacillin/tazobactam (PTZ). In empirical treatment, mortality among those treated with BLBI and carbapenem were 9.7% and 19.4%, respectively. A similar finding was also found in definitive antimicrobial treatment in which mortality in those treated with BLBI was 9.3%, whereas in those treated with carbapenem 16.7%.[17]



K= control (*E. coli* ATCC 25922); **M**= Marker 100bp; **C1-12**= sample

K= control (*E. coli* ATCC 25922); **M**= Marker 100bp;**Y1-12**= sample

Figure 1. Electrophoresis of polymerase chain reaction product of *E.coli*. A, PCR product to detect *chuA* gene. B, PCR product to detect *YjaA* gene

Resistance rate of *E.coli* against ciprofloxacin in this study was considerebly high namely 75%. A study in Brazil reported that in 2014 resistance rate of *E.coli* isolated from urine samples was 36%.[18] A systematic review and meta-analysis reported that resistance of *E.coli* against ciprofloxacin in hospital-acquired urinary tract infection was 38% (95% CI: 36%-41%), whereas in community-acquired urinary tract infection was significantly lower i.e 27% (95% CI: 27%-31%). Resistance significantly varied by region and country with the highest resistance observed in developing countries.[19]

Table 3. Result of antimicrobial susceptibility test and phylogenetic group

Antimicrobia	Phylogenetic group B1/A			Phylogenetic group B2			Phylogenetic group D		
	R	I	S	R	I	S	R	I	S
Ampicillin	3	-	-	7	-	-	1	-	1
Ampicillin/sulbactam	-	1	2	6	-	1	-	1	1
Piperacillin/tazobactam	-	-	3	1	1	5	-	-	2
Ceftazidime	3	-	-	6	-	1	-	-	2
Ceftriaxone	3	-	-	6	-	1	-	-	2
Cefepime	3	-	-	6	-	1	-	-	2
Aztreonam	3	-	-	6	-	1	-	-	2
Ertapenem	-	-	3	-	-	7	-	-	2
Meropenem	-	-	3	-	-	7	-	-	2
Amikacin	-	-	3	-	-	7	-	-	2
Gentamicin	2	-	1	-	-	7	-	-	2
Ciprofloxacin	3	-	-	6	-	1	-	-	2
Tigecycline	-	-	3	-	-	7	-	-	2
Trimethoprim/Sulfamethoxazole	2	-	1	7	-	-	1	-	1

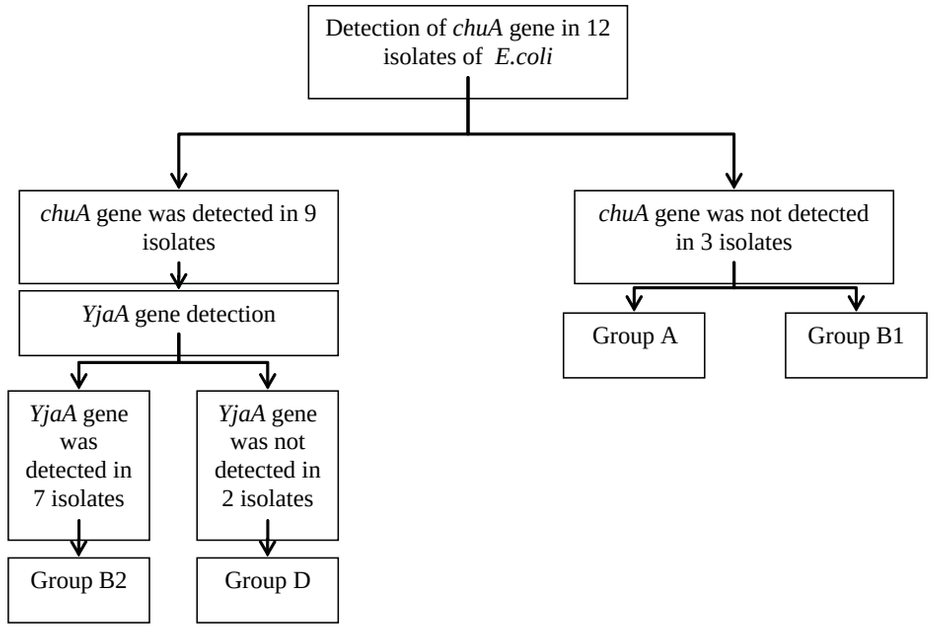


Figure 2. Phylogenetic analysis of 12 clinical isolates of *E.coli*

The results of PCR colony examination on 12 *E. coli* isolates showed that 9 isolates expressed *chuA* gene and 3 isolates did not express *chuA* gene. Three isolates (25%) that did not express *chuA* gene were likely to be a phylogenetic group A or B1 known as commensal bacteria. Seven of the nine isolates (58.3%) who expressed *chuA* gene, also expressed the *YjaA* gene and corresponded to the B2 phylogenetic group. Whereas two isolates (16.7%) expressing *chuA* gene but not expressing *YjaA* gene were phylogenetic group D. The phylogenetic group B2 and D were phylogenetic virulent *E.coli*.[6]

A previous study used triplex PCR to conduct rapid phylogenetic analysis of *E.coli* by examining the presence or absence of *chuA* genes. This gene existed in group B2 and D strains but not in both groups B1 and A. The gene of *chuA* presence together with *yjaA* gene existed in group B2. Phylogenetic group D was identified by the presence of *chuA* gene but absence of *yjaA* gene. The DNA fragment TSPE4.C2 existed in group B1 strains but not in group A.[6] Phylogenetic analysis also could be done by unweighted pair group method with arithmetic mean (UPGMA) using MLEE data from 35 enzyme loci, or by the neighbour-joining method using data from 38 enzyme loci resulted four main groups A, B1, B2 and D, with some of unclassified sequences which were grouped as E.[5] The reference method for phylogenetic grouping actually were multilocus enzyme electrophoresis or ribotyping but unfortunately both of these reference techniques are complex and time-consuming and also require a collection of typed strains.[6]

Six out of 7 phylogenetic group B2 *E.coli* isolates produced ESBL enzyme. They were isolated patients suffering from rectal cancer (2 isolates), breast cancer (1 isolate), acute lymphoblastic leukemia (1 isolate), retinoblastoma (1 isolate), histiocytosis (1 isolate) and burn (1 isolate).

Two isolates of phylogenetic group D *E.coli* did not produce ESBL enzyme. One of them was an isolated patient suffering from carvical cancer and chronic kidney disease and another was isolated from the patient suffering from pancreatic cancer with thrombocytopenia.

Three isolates of *E.coli* either phylogenetic group A or group B1 were discovered from patients suffering from malignant melanoma stage 4 accompanied by anemia and acute kidney injury, rectosigmoid adenocarcinoma accompanied with lung tuberculosis and mixed leukemia. They all produced ESBL enzyme.

Two out of three patients infected by either phylogenetic group A or group B1 died during 14 days of follow up. These 2 subjects suffered from malignant melanoma stage 4 accompanied by anemia and acute kidney injury, rectosigmoid adenocarcinoma accompanied by lung tuberculosis . One patient suffered from mixed leukemia remained alive up to 14 days follow up. Subject who suffered from malignant melanoma died on day 5, whereas another patient

who suffered from rectosigmoid cancer died on day 2 observation.

ChuA is a gene which is requiered in haem transfer in enterohaemorrhagic *E.coli* O157:H7, whereas *YjaA* originally known in the genome sequence of *E.coli* K-12 and the function is not known exactly.[6] The gene of chuA (heme receptor) is 1 of 5 iron uptake virulence associated genes. The other iron uptake genes are fyuA (yersiniabactin siderophore receptor), ireA (iron-regulated element, siderophore receptor), iroN (salmochelin siderophore receptor) and iutA (aerobactin siderophore receptor).[9] The siderophores excreted by bacteria are able to steal the iron from ferritin or lactoferrin being recaptured by specific bacteria receptors such as *iutA*, *fyuA*or *chuA*.[20]

Phylogenetic group B2 of *E.coli* (58.3%) was the most frequently found in this study. Phylogenetic group D and both phylogenetic group A and B1 together were 16.7% and 25% respectively. Phylogenetic group of *E.coli* causing urinary tract infection 53.5% phylogenetic group B2, 27.1% phylogenetic group A, 10.9% phylogenetic group D and 8.5% phylogenetic group B1.[21] Another study among patients suffering from bacteremia of urinary tract origin found that phylogenetic group of *E.coli* as the etiology were as follows, 67% phylogenetic group B2, 22% phylogenetic group D, 4% phylogenetic group A, 4% phylogenetic group B1, 3% non-typable phylogenetic.[9] A reseacrh on mice to investigate a link between phylogeny and virulence in *E.coli* found that phylogenetic groups A, B1 and D showed capability to kill the mice, their virulence most frequently was associated with the availability of virulence determinants. In addition, the B2 phylogenetic group was a highly virulent strain which killed the mice considerably high and had the highest level of virulent determinants.[22]

CONCLUSION AND SUGGESTION

Sources of bloodstream infection caused by *E.coli* originating from urinary tract, lung, gastrointestinal tract and skin were found in 7 cases. On the other hand, the source of infections was unknown in 5 cases. Most of subjects are adult males with malignancies as the underlying disease.

Escherichia coli as the etiology of bloodstream infection mostly (75%) produce ESBL enzyme and its resistance against antimicrobial such as ampicillin, ampicillin/sulbactam, ceftazidime, ceftriaxone, cefepime, aztreonam, ciprofloxacin and trimethoprim-sulfamethoxazole was high enough. Phylogenetic group of these clinical isolates mostly (75%) were group B2 and group D of which was known as extraintestinal virulent strain. The remaining clinical isolates (25%) can be classified either as phylogenetic group A or B1 in which group A is known as a commensal strain.

By knowing the phylogenetic profile of *E.coli*, the evolutionary history as the cause of bloodstream infection as well as its diversity can be known. Phylogenetic analysis can be performed on *E.coli* or other bacteria as the cause of infection in other systems or organs.

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