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Original Research Article

Prevalence and Risk Factors of ESBL-producing Enterobacteriaceae in The Community

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Abstract

Background: World Health Organization (WHO) data collection of *Escherichia coli* resistant to cephalosporin generation III already confirmed in 86 countries. Incredibly high carrier prevalence rates were also widely developed in Thailand, Egypt, and China. The Faculty of Medicine's research team at Jenderal Soedirman University, Purwokerto, discovered at the beginning of 2018 that Extended Spectrum β -Lactamase (ESBL) *E. coli* carriers throughout the 2015 class students were 26.8 percent.

Objective: This research investigated the prevalence and associated factors of ESBL-producing *Enterobacteriaceae* (EPE) asymptomatic carriers in the community.

Methods: The participant fill a questionnaire, and samples were taken from rectal swabs using Amies transport medium (Labware Charuzu), and then models were analyzed using HiCrome™ ESBL Agar Base (Himedia, India). Analysis of its Prevalence and Resistance Predictors used IBM SPSS Statistics Version 22.0 for Windows (Armonk, NY: IBM Corp).

Results: The prevalence of EPE asymptomatic carriers in the community in Purwokerto was 66.7%. In the bivariate analysis, subjects who took antacids in the last eight weeks, history of hospitalization in the previous 12 months, the habit of consuming milk, yogurt, cheese, meat, seafood, and raw vegetables did not show any significant difference. Frequent chicken and freshwater fish consumption tended to be a risk factor for ESBL-producing *Enterobacteriaceae* with PR 1.462, 95% CI (1.115-1.918); PR 1.666, 95% CI (0.936-2.966); however, in the multivariate logistic regression analysis, this was not significant.

Conclusion: The prevalence of EPE asymptomatic carriers in the community in Purwokerto is 66.7%. None of the studied variables was correlated with ESBL-producing *Enterobacteriaceae*. However, ESBL remains an emerging antimicrobial resistance.

Keywords: Carrier; Resistance; Risk Factors; Enterobacteriaceae; ESBL

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INTRODUCTION

Extended-spectrum β -Lactamase (ESBL) is an enzyme made by some bacteria. That enzyme can hydrolyze penicillin, first, second, third-generation cephalosporins, aztreonam (except cepharmycin and carbapenem).¹ The bacteria that produce the most ESBL are bacteria from the *Enterobacteriaceae* family, especially *Escherichia coli* and *Klebsiella pneumoniae*.² Based on substrate specificity the β -lactamase family split into four functional groups: penicillinase, ESBL, carbapenemase, and Amp-C enzyme.^{3,4} The beta-lactamase (bla) gene is usually found on chromosomes,

although it would also found in plasmids.⁵ The ESBL coding gene residing in the plasmid is easily transferred to other germs, thereby accelerating resistance.⁶ Community-acquired infections due to ESBL-producing *Enterobacteriaceae* (EPE) have increased. The flora fecal in the community is a vast potential reservoir for ESBL.⁷ Based on a survey conducted by the CDC in 2013, each year, there are 26,000 infections caused by EPE, and around 1,700 of them die.

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Asymptomatic carriers of ESBL-producing bacteria in communities reported in several countries and continents with large differences in carrier rates between geographic areas and population characteristics studied. Incredibly high carrier prevalence rates were also widely developed in Thailand (66%), Egypt (63%), and China (50%).⁸ Low carrier levels shown from Sweden (3%).⁹ In Indonesia as its own, a community carrier study by Ulla et al. published that 26.8% of healthy Unsoed medical participating students were carriers of ESBL-producing *Escherichia coli*. Prevalence of ESBL *Escherichia coli* in fecal specimens of 457 traveler diarrhea patients in Spain who have traveled to tropical and subtropical regions is 17.9% of patients (82/457). India seems to be the most visited region and revealed a larger number of positive samples. (37.4%).¹⁰

The current study on the prevalence and risk factors of EPE carriers in Indonesia's healthy communities is rare. Each result of this study provides evidence for both the prevention and control of EPE in the population. Based on this context, specific, transparent, observable, and sustainable efforts need to be made in a single health approach across sectors, including the public health, livestock, fisheries, and agriculture sectors, to resolve this issue. These efforts involve critical surveillance data and strict implementation of field regulations to break the chain of EPE deployments. Therefore, prevalence data and related risk factors must establish firm policies to control antimicrobial resistance, especially EPE.

MATERIALS AND METHODS

Population and sampling methods

The population in this study is the healthy people in Purwokerto. This research has received approval from the Faculty of Medicine's ethics committee, Universitas Jenderal Soedirman (Ref: 182/KEPK/VIII/2020). The sampling method is consecutive sampling. The minimum sample size for this analysis was 75. We conducted this research in 2020 for eight months at Purwokerto.

Questionnaire

Participants must fill out a questionnaire containing informed consent, name, age, sex, medical records (prior intake of antibiotics or antacids, initial hospitalization), consumption (milk, yogurt, cheese, meat, freshwater fish, seafood, chicken, etc.), and use of public toilets.

Sampling method

Amies transport medium (Labware Charuzu) (non-nutritive medium) tubes to collect all swab specimens.

Isolation and identification

Samples isolated in HiCrome™ ESBL Agar Base (Himedia, India) + HiCrome™ ESBL agar supplement and incubated for 24 hours at 37° C. Chromogenic mixture is used to distinguish the pigment ESBL-producing organisms. HiCrome™ ESBL Selective Supplement (FD278) allows suppressing some contaminating microorganisms. ESBL *E.coli* grow as either pink or purple colonies. ESBL producing members of the KESC group have bluish-green colonies; *Proteus*, *Morganella*, and *Providencia*. (Table. 1).

Table 1. Identification of ESBL producing bacteria

Organism	Inoculum (CFU)	Growth	Recovery	Color of Colony
<i>Escherichia coli</i> NCTC 13351	50-100	luxuriant	>=50%	pink to purple
<i>Klebsiella pneumoniae</i> ATCC 700603	50-100	luxuriant	>=50%	bluish green

Note: (*) Corresponding WDCM numbers.
NCTC: National Collection of Type Cultures
ATCC: American Type Culture Collection

Statistical analysis

This research uses an analytic observational study with a cross-sectional approach, which emphasizes measuring or observing data one time based on the dependent variable and the independent variable. Univariate analysis of the independent and dependent variables to obtain a descriptive picture of the variables studied in presentation distribution. Bivariate analysis to see the relationship between independent and dependent variables uses the Chi-Square or Fisher Exact test for categorical variables and the Mann-Whitney U test for numerical data. Multivariate analysis conducted with multiple logistic regression. Statistical analysis of Prevalence and determinants of resistance uses IBM SPSS Statistics Version 22.0 for Windows (Armonk, NY: IBM Corp). The analysis results described as Prevalence Risk (PR), p-value, and 95% Confidence Interval (CI). All p-values were 2-tailed, and p <0.05 would be considered significant.

RESULTS

The prevalence of asymptomatic carriers of *Enterobacteriaceae* ESBL from a total of seventy-five respondents who participated in this study was 50 (66.7%). The most common isolate was *Escherichia coli* 50 (66.7%) were two strains of organisms (*Escherichia coli* and *Klebsiella pneumoniae*) in one respondent were more often found with a percentage of 29 (38.7%) compared to one strain (*Escherichia coli*) 21 (28%). Most of the respondents had a senior high school education level, namely 32 (42.7%). (Table 2). Participants in this study, 43 (52.3%) were women, and 32 (42.7%) were men, with the mean age of the respondents was 32.35 ± 10.93 years. Women had a 67.4% higher frequency of ESBL colonization (29/43) than men 65.6% (21/32), but there was no statistically significant difference.

Table 2. Respondent Characteristics

Characteristic	Total n(%)
Culture result	
ESBL +	50 (66.7%)
ESBL -	25 (33.3%)
Organism	
<i>Escherichia coli</i>	21 (28%)
<i>Escherichia coli</i> and <i>Klebsiella pneumoniae</i>	29 (38.7%)
Sterile	25 (33.3%)
Last education	
Primary school	4 (5.3%)
Junior High	6 (8%)
High school	32 (42.7%)
Vocational High School	3 (4%)
D3	5 (6.7%)
S1	17 (22.7%)
S2	7 (9.3%)
S3	1 (1.3%)

Subjects who took antacids in the last eight weeks and hospital admission in the previous 12 months had a higher incidence rate of ESBL carriers than other respondents (Table 3). However, the bivariate analysis showed no significant differences between these variables. In the bivariate analysis, the habits of consuming milk, yogurt, cheese, meat, seafood, and raw vegetables did not show any significant differences. In contrast, for subjects who took antibiotics over the last eight weeks, the overall incidence was 100 percent (11/11), significantly higher than several other respondents who were also asymptomatic carriers of *Enterobacteriaceae* ESBL 60.9 percent (39/64). Where PR 0.609, 95% CI (0.501-0.741), which has the meaning of this variable, is not a risk factor for the *Enterobacteriaceae* ESBL carriers or is protective; still, in multivariate logistic regression analysis, this becomes insignificant. Excessive consumption of chicken and freshwater fish appeared to become a risk factor for *Enterobacteriaceae*-producing ESBL with Prevalence risk, respectively, PR 1.462, 95% CI (1.115-1.918); PR 1.666, 95% CI (0.936-2.966) (Table. 3); however, in the logistic regression multivariate analysis, it was not significant. Participants who do not have the habit of using public toilets found to be higher in asymptomatic carriers of *Enterobacteriaceae* ESBL 68.2% (30/44) than those who habitually use public restrooms 64.5% (20/31). Still, the analysis showed that this was not statistically significant.

DISCUSSION

Research into the asymptomatic carrier of ESBL *E. coli* is essential because it has significant clinical consequences for public health.¹¹ A healthy carrier can develop severe urinary tract, intra-abdominal, or bloodstream infections in the future.^{12,13} Mortality rates following infection with ESBL *E. coli* are considerably higher.^{14,15} The last review on carbapenems might be the only efficient treatment.¹⁶ Often, moderate urinary tract infections occur to remain due to enhanced morbidity.¹⁷ The cumulative combined Prevalence of ESBL *E. coli* carriers in the community (2003-2018) was 16.5%.¹⁸ The combined Prevalence shows an increasing trend from 2.6% in 2003–2005 to 21.1% in 2015–2018. The highest carrier rates were observed in Southeast Asia throughout the period, while Europe was the lowest.¹⁹ In the research we conducted, especially in Purwokerto, there was also an increase in ESBL *E. coli* carriers trends to 66.7% compared to previous studies conducted by a research team from the Faculty of Medicine, General Soedirman Purwokerto University in 2015 class students as much as 26.8%. In some other research, the fecal carrier rate of *Enterobacteriaceae* ESBL is about 62.7% among hospitalized patients and 33.8% among asymptomatic healthy people. A more frequently reported *Enterobacteriaceae* of ESBL seemed to be *Escherichia coli* (70.3% and 78.4%, in both patients and healthy subjects), followed by *Klebsiella pneumoniae* (26.8% and 17.0 %).²⁰ In our study, most isolates found were *Escherichia coli* 50 isolates (66.7%), most of which were found together with *Klebsiella pneumoniae* or two strains in one respondent 29 (38.7%).

Hijazi et al., in their study, found that thirty-one of the 125 participants (24.8%) were carriers of the

Enterobacteriaceae ESBL. In the bivariate test, regular consumption (3 times per week or more) of milk, yogurt, cheese, chicken, or meat showed a significant effect. In multivariate logistic regression analysis, beef and chicken's routine consumption is also significantly related to increased *Enterobacteriaceae* ESBL carriers; the association of dairy products (milk, yogurt, cheese) was not significant.⁷ In our study, frequent consumption of chicken and freshwater fish to be a risk factor for the carriers of ESBL-producing *Enterobacteriaceae* (Table. 3); however, in the logistic regression multivariate analysis, it was not significant. In the research developed by Otter et al., the risk factor for *Enterobacteriaceae* ESBL carriers among 1633 patients was receiving two or more antibiotic courses six months before the examination.²¹ In our study, participants taking antibiotics in the last eight weeks had a 100% higher prevalence rate (11/11) than some other respondents who also were asymptomatic carriers of *Enterobacteriaceae* ESBL 60.9% (39/64) with PR 1.000, 95% CI (0.442-2.263). The above means that this variable is not a risk factor for the *Enterobacteriaceae* ESBL carriers or is protective, but this is not significant in multivariate logistic regression analysis. Shitrit et al. in their study found that of the 525 patients tested, 56 seemed to be positive for ESBL carriers, 4 different risk factors are associated: residency in a nursing facility, history of 1-year previous hospitalization, history of antibiotic treatment, and last ESBL carriers. More than 50% of patients screened have at least one risk factor.²²

CONCLUSION

The prevalence of EPE asymptomatic carriers in the community in Purwokerto was 66.7%. These data are required to establish firm policies to control antimicrobial resistance. Consumption of chicken and freshwater fish is not a risk factor for ESBL-producing *Enterobacteriaceae*. However, ESBL remains an emerging antimicrobial resistance.

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Table 3. Factors Associated with *Asymptomatic Careers of Enterobacteriaceae* ESBL

Characteristic	Total n (%)	ESBL + n (%)	ESBL - n (%)	Bivariate Analysis		Multivariate Analysis	
				p	PR (95%CI)	p	PR (95%CI)
Gender		50 (66.7%)	25 (33.3%)	0,869 ^a	0.973 (0.703-1.348)		
Male	32 (42.7%)	21 (42%)	11 (44%)				
Women	43 (57.3%)	29 (58%)	14 (56%)				
Hospital admissions in the past six months				0.256 ^a	0.733 (0.534-1.007)		
Yes	8 (10.7%)	7 (14%)	1 (4%)				
No	67 (89.3%)	43 (86%)	24 (96%)				
Taking antibiotics for eight weeks				0.012 ^{a*}	0.609 (0.501-0.741)	0.999 ^c	0.000 (0.000-0.000)
Yes	11 (14.7%)	11 (22%)	0 (0%)				
No	64 (85.3%)	39 (78%)	25 (100%)				
Taking antibiotics without a doctor's prescription within six months				1.000 ^a	1.000 (0.442-2.263)		
Yes	3 (4%)	2 (4%)	1 (4%)				
No	72 (96%)	48 (96%)	24 (96%)				
Taking antacids within eight weeks				0.150 ^a	0.701 (0.531-0.925)		
Yes	10 (13.3%)	9 (18%)	1 (4%)				
No	65 (86.7%)	41 (82%)	24 (96%)				
Drinking milk				0.275 ^b	1.231 (0.818-1.853)		
Often	21 (28%)	12 (24%)	9 (36%)				
Rarely or never	54 (72%)	38 (76%)	16 (64%)				
Drinking yogurt				0.088 ^a	0.632 (0.528-0.758)		
Often	7 (9.3%)	7 (14%)	0 (0%)				
Rarely or never	68 (90.7%)	43 (86%)	25 (100%)				
Eating cheese				1.000 ^a	0.954 (0.608-1.470)		
Often	10 (13.3%)	7 (14%)	3 (12%)				
Rarely or never	65 (86.7%)	43 (86%)	22 (88%)				

Table 3. Continued

Characteristic	Total n (%)	ESBL + n (%) 50 (66.7%)	ESBL - n (%) 25 (33.3%)	Bivariate Analysis		Multivariate Analysis	
				p	PR (95%CI)	p	PR (95%CI)
Eating meat				0.737 ^b	0.946 (0.685-1.306)		
Often	29 (38.7%)	20 (40%)	9 (36%)				
Rarely or never	46 (61.3%)	30 (69%)	16 (64%)				
Eating chicken				0.032 ^{b*}	1.462 (1.115-1.918)	0.088 ^c	4.136 (0.808-21.165)
Often	58 (77.3%)	35 (70%)	23 (92%)				
Rarely or never	17 (22.7%)	15 (30%)	2 (8%)				
Eating seafood				0.709 ^a	0.838 (0.566-1.239)		
Often	9 (12%)	7 (14%)	2 (8%)				
Rarely or never	66 (88%)	43 (86%)	23 (92%)				
Eating freshwater fish				0.028 ^{b*}	1.666 (0.936-2.966)	0.068 ^c	3.536 (0.912-13.708)
Often	16 (21.3%)	7 (14%)	9 (36%)				
Rarely or never	59 (78.7%)	43 (86%)	16 (64%)				
Eating raw vegetables				0.763 ^a	0.918 (0.629-1.340)		
Often	14 (18.7%)	10 (20%)	4 (16%)				
Rarely or never	61 (81.3%)	40 (80%)	21 (84%)				
Sharing toilet				0.740 ^b	1.057 (0.760-1.470)		
Often	31 (41.3%)	20 (40%)	11 (44%)				
Rarely or never	44 (58.7%)	30 (60%)	14 (56%)				

^a Analysis using Chi-square test, ^b Analysis using Fisher's Exact Test, ^c Multiple Logistic Regression Test, * p < 0.05

Often ($\geq 3x$ / week)

Rarely ($\leq 2x$ / week) or Never

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