

Prevalence of extended spectrum beta-lactamase (ESBL) *Escherichia coli* recovered from ICU and non-ICU at dr. Zainoel Abidin General Hospital

Wilda Mahdani¹, Asha Albela², Zinatul Hayati¹, and Suhartono Suhartono^{2*}

¹Department of Microbiology, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia

²Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh 23111, Aceh, Indonesia

Abstract. Research conducted at Zainoel Abidin General Hospital in Banda Aceh between January 2022 and July 2023, focused on the prevalence and susceptibility patterns of extended-spectrum beta-lactamases (ESBL)-producing *Escherichia coli* associated with nosocomial infections. This study employed a retrospective descriptive methodology to analyze clinical specimens from both the intensive care unit (ICU) and non-ICU settings. Of the 520 isolates examined, 365 (70.19%) were identified as ESBL-producing *Escherichia coli*. The age group of 56-65 had the highest prevalence (28.21%), with female patients contributing 52.50% of the isolates. Both ESBL-producing and non-ESBL-producing *E. coli* had almost equivalent prevalence among patients treated in the ICU and non-ICU, accounting for 67%. Antibiotic susceptibility testing revealed high levels of resistance to carbapenem. Conversely, polymyxin B showed complete susceptibility (100%) and cefoxitin exhibited an 85% susceptibility rate. These findings underscore the alarm resistance levels of ESBL-producing *Escherichia coli* isolates.

1 Introduction

Escherichia coli strains harboring Extended-Spectrum Beta-Lactamases (ESBLs) have garnered considerable recognition due to their substantial role as pathogenic agents, contributing significantly to the incidence of infections across global healthcare settings and the general populace. Notably, the distribution of ESBL-encoding *E. coli* displays geographical variability, as substantiated by multiple research investigations conducted in various nations. Extensive evidence points to the identification of ESBL-producing *E. coli* in diverse contexts within the Indonesian settings, encompassing healthcare facilities [1], dairy farming establishments [1-3], and the surrounding ecological milieu [1]. These discerned occurrences emphasize the potential for interspecies transmission of ESBL-producing *E. coli*, thereby warranting heightened attention and investigation [1].

* Corresponding author: suhartono@usk.ac.id

Within the healthcare environment, ESBL *E. coli* has been frequently observed in close association with urinary tract infections (UTIs), as substantiated by antecedent research investigations [4-10]. Moreover, distinct studies have elucidated its connection with bacteriuria [11], while others have discerned its implication in various categories of infectious ailments [12]. It is important to note that the occurrence of infections associated with ESBL *E. coli* is markedly impacted by recent hospital admissions and the prior utilization of antibiotics [13].

The development and dissemination of ESBLs are predominantly reported in the Enterobacteriaceae family, with a specific emphasis on *Klebsiella pneumoniae*, *K. oxytoca*, and *E. coli* [5]. The prevalence of ESBL-producing *E. coli* exhibits notable variation across numerous scientific investigations and ecological settings. Furthermore, introducing and disseminating ESBL-producing *E. coli* provides substantial obstacles in infection control and therapeutic interventions. These isolates frequently resist many categories of antibiotics, which restricts the range of available treatment choices [14]. Moreover, infections related to ESBL-producing *E. coli* are linked to longer hospitalization periods, higher healthcare expenditures, and greater fatality rates than infections caused by non-ESBL-producing *E. coli* [15]. Hence, the implementation of efficient surveillance and infection control strategies is of utmost importance in mitigating the widespread of ESBL-producing *E. coli* in healthcare facilities and broader communities.

The primary objective of this investigation was to assess the prevalence of both ESBL-producing and non-ESBL-producing strains of *E. coli* within clinical specimens obtained from patients undergoing treatment in the Intensive Care Unit (ICU) and non-ICU wards, at Zainoel Abidin General Hospital.

2 Material and Methods

The clinical specimens utilized in this research investigation were obtained from patients admitted and discharged from Zainoel Abidin General Hospital in Banda Aceh, Indonesia, between January 2022 and July 2023. The specimens gathered comprised an assortment of sample categories, such as swabs, blood, urine, sputum, and other bodily fluids. To verify the accuracy of the data, each sample was subjected to a thorough review, and all essential details like sample type, collection time, and patient gender were documented. Faculty of Medicine, Universitas Syiah Kuala, granted ethical approval for this study.

The processing of clinical samples involved their inoculation onto blood and MacConkey agar (Merck, Germany) plates. Blood specimens underwent a preliminary culturing step using BacT/ALERT® 3D following the manufacturer's protocols before inoculation onto agar plates. These agar plates were then subjected to an incubation period at 37°C lasting 24 hours, subsequently undergoing Gram staining and macroscopic as well as microscopic examination under 1000x magnification for morphological characterization.

The identification of bacterial species and their susceptibility to antimicrobial agents was performed using the VITEK® 2 Compact system (Biomeriux, Lyon, France). Isolates of pure bacterial colonies retrieved from clinical samples were suspended in 0.45% NaCl, adjusted to match the turbidity of a 1.8-2.2 McFarland Standard solution, and subsequently introduced into appropriate cassettes tailored for both identification and antimicrobial susceptibility assessment. Antimicrobial susceptibility testing adhered to the guidelines delineated by the Clinical and Laboratory Standards Institute (CLSI) and encompassed an array of antibiotics.

The distribution of *E. coli* isolates across different species was subjected to descriptive analysis, considering variables such as the clinical sample type, the gender and age of the associated patients, and the wards. Data were organized using Microsoft Excel to generate informative tables and charts for descriptive representation. For statistical analyses, the chi-

square test or Fisher's exact test was employed, as appropriate, with all statistical tests conducted using XLStat 2021.

3 Results and Discussion

During the investigation, 520 *E. coli* isolates were recovered from clinical specimens. Among them, 70.18% (n=365) were identified as producers of ESBLs with the patients's profiles are tabulated in Table 1.

Table 1. The patients' profiles (ages and genders) of ESBL and non-ESBL *E. coli* obtained from clinical specimens during the period of January 2022 to July 2023

Age Groups (years)	ESBL <i>E. coli</i> (n=365)		Non- ESBL <i>E. coli</i> (n=155)	
	Males	Females	Males	Females
0-5	5	5	3	6
6-11	4	1	4	1
12-16	2	4	3	0
17-25	4	2	5	4
26-35	10	8	3	4
36-45	31	18	7	9
46-55	41	59	16	16
56-65	45	58	24	27
>65	35	33	9	14

The high prevalence of ESBL *E.coli* in the present research aligns with a prior study, which reported a prevalence of ESBL *E. coli* accounting for 51.6% of the total clinical *E. coli* isolates (n=455) obtained from healthcare facilities in northeast Iran [16]. In this current research, the predominant source of ESBL- *E. coli* isolates was urine, comprising 145 isolates (71.42%) out of 203 from infected urine cultures, followed by pus and blood, accounting for 118 (32.32%) and 56 isolates (15.34%), respectively (Figure 1). This underscores the significance of ESBL *E. coli* as the primary causative agent in urinary tract infections (UTIs). Multiple studies have established the association of ESBL *E. coli* with both community- and hospital-acquired UTIs [17-19]. In community settings, ESBL-producing *E. coli* has emerged as a notable etiological factor for UTIs [19]. A study conducted in China revealed a high prevalence of ESBL-producing *E. coli* among adults afflicted with community-acquired UTIs [17]. In hospital environments, ESBL *E. coli* has been linked to hospital-acquired infections [18]. Notably, a study conducted in Saudi Arabia also reported a substantial prevalence of ESBL-producing *E. coli* in both communities- and hospital-acquired UTIs [4].

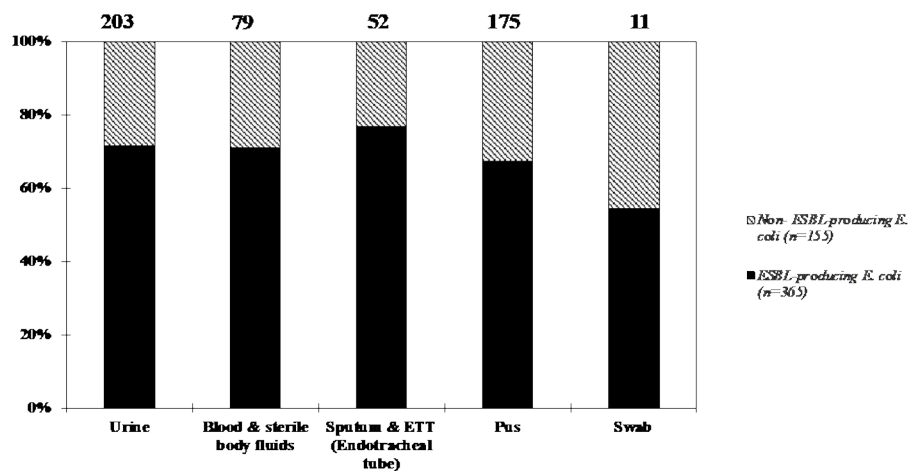


Fig. 1. The distribution and prevalence percentages (%) of ESBL *E. coli* (n=365) and non-ESB *E. coli* (n=155) isolates in relation to clinical specimens during the period spanning from January 2022 to July 2023. The numerical values within each column denote the total count of isolates. Analysis employing the Chi-square test for independence revealed no significant association between the types of clinical specimens and the presence of ESBL in *E. coli* ($P = 0.522$; $\chi^2 = 3.218$).

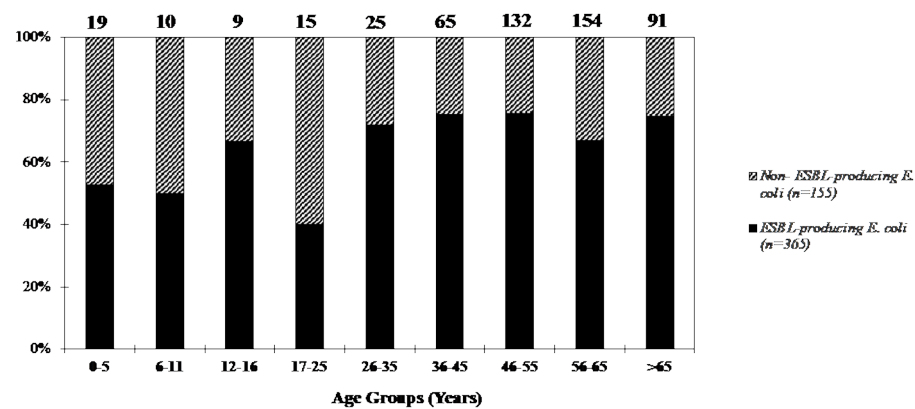


Fig. 2. The distribution and prevalence percentages (%) of ESBL *E. coli* (n=365) and non-ESBL *E. coli* (n=155) isolates categorized by patients' age groups during the timeframe spanning from January 2022 to July 2023. The numerical values provided in each column signify the total count of isolates. Employing the Chi-square test for independence, it was determined that a statistically significant association exists between the types of clinical specimens and the presence of ESBL in *E. coli* ($P = 0.044$; $\chi^2 = 15.868$).

Regarding patient age groups, as depicted in Figure 2, the highest occurrence of ESBL *E. coli* was observed among senior patients, specifically in the 56-65 age group, comprising 103 isolates (28.21%), and the 46-55 age group, consisting of 100 isolates (27.39%). Notably, this distribution pattern revealed that the highest prevalence of ESBL-producing *E. coli* was observed within patients aged 46-56 years [20]. In line with our findings, this study documented a prevalence of 33.3% for ESBL-producing *E. coli* among clinical uropathogenic isolates in Riyadh, Saudi Arabia. Furthermore, other research endeavors have

identified age as an independent risk factor associated with mortality in patients afflicted with ESBL *E. coli* bacteremia [21]. These observations imply that age may influence the distribution and the clinical outcomes of infections caused by ESBL *E. coli*.

In the present study, both ESBL and non-ESBL *E. coli* displayed nearly equivalent prevalence rates among patients receiving treatment in both the Intensive Care Unit (ICU) and non-ICU settings, constituting 67% of the total cases, as indicated in Figure 3. Although the prevalence of extended-spectrum beta-lactamase (ESBL)-producing *E. coli* is notably higher within Intensive Care Units (ICUs), it is important to acknowledge that non-ICU patients are also exposed to significant risks of colonization or infection, especially individuals residing in long-term care facilities (LTCFs) or those with a history of recurrent hospitalizations [8, 22, 23]. These non-ICU patients may exhibit similar risk factors as their ICU counterparts, including prolonged hospital stays and extensive antibiotic exposure [8, 22, 23]. In LTCFs, the proximity of patients and frequent utilization of antibiotics can contribute to the dissemination and selection of ESBL-producing strains [23, 24]. Additionally, recurrent hospitalization can heighten the susceptibility to ESBL-producing *E. coli* colonization or infection [25].

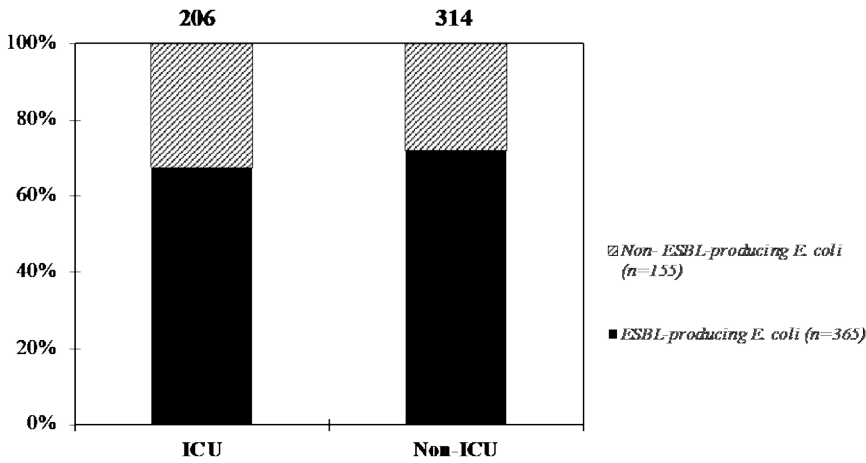


Fig. 3. The distribution and prevalence percentages (%) of ESBL *E. coli* (n=365) and non-ESBL *E. coli* (n=155) isolates in relation to patients' wards during the period spanning from January 2022 to July 2023. The numerical values within each column denote the total count of isolates. Analysis employing the Chi-square test for independence revealed that no significant association between the types of patients' wards and the presence of ESBL in *E. coli* ($P = 0.273$; $\chi^2 = 1.203$).

Concerning the gender distribution of patients, the prevalence of infections attributed to both ESBL and non-ESBL *E. coli* was notably more pronounced among females, constituting 188 isolates (52.50%), compared to 177 isolates (48.49%) in males, as illustrated in Figure 4. This observation aligns with prior research findings that consistently highlight a heightened incidence of ESBL *E. coli* infections in females compared to their male counterparts. For instance, a study conducted in Saudi Arabia revealed that females exhibited an elevated susceptibility to urinary tract infections caused by *E. coli* strains displaying resistance to various antibiotics, including ESBLs, compared to males [4]. Similarly, a research study conducted in Iran reported a substantially higher incidence rate of ESBL *E. coli* isolates in females, reaching 81.8%, in contrast to a mere 18.2% in males [5]. This gender-based trend was further corroborated by a subsequent investigation in India [26]. However, it is worth noting that there have been instances where studies have indicated a more pronounced prevalence of ESBL *E. coli* infections in males than females [11].

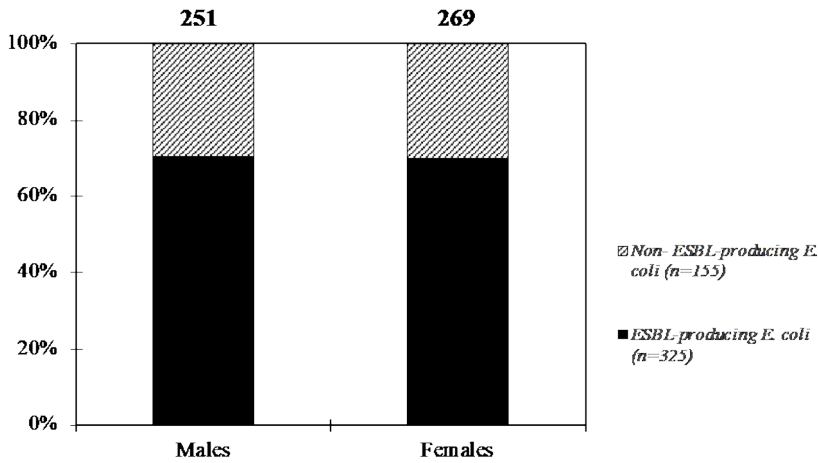


Fig. 4. The distribution and prevalence percentages (%) of ESBL *E. coli* (n=365) and non-ESBL *E. coli* (n=155) isolates in relation to patient's gender during the period spanning from January 2022 to July 2023. The numerical values within each column denote the total count of isolates. Analysis employing the Chi-square test for independence revealed no significant association between the patient's gender and the presence of ESBL in *E. coli* ($P = 0.875$; $\chi^2 = 0.025$).

The results of antibiotic susceptibility testing revealed that ESBL *E. coli* displayed resistance to amikacin, ceftadine, colistin, and cefoperazone/sulbactam, as outlined in Table 2. These findings are align with prior research, which has consistently reported resistance of amikacin, ceftazidime, colistin, and cefoperazone/sulbactam against ESBL *E. coli* isolates, as determined through antibiotic susceptibility testing [27]. The emergence of resistance to amikacin in ESBL *E. coli* is a matter of concern, given the widespread utilization of this antibiotic in managing infections stemming from multidrug-resistant Gram-negative bacteria [28]. It is worth noting the challenges posed by the development of resistance to ceftazidime and cefoperazone/sulbactam, as these antibiotics are frequently employed as empirical treatment choices for infections caused by ESBL bacteria [29]. Resistance to colistin is particularly alarming, as this antibiotic is regarded as a last-resort therapy for multidrug-resistant Gram-negative infections [27].

This study showed that ESBL and non-ESBL *E. coli* strains exhibited substantial resistance to ceftriaxone, cefotaxime, doxycycline, and levofloxacin. This observation is consistent with the prevalent occurrence of antibiotic resistance previously documented in *E. coli* isolates [30-33]. The observed heightened resistance levels to ceftriaxone and cefotaxime can be attributed to the widespread distribution of ESBL *E. coli* strains across various settings, including hospitals, communities, and the environment [30, 31, 34]. The increasing prevalence of resistance to doxycycline and levofloxacin is concerning, given their frequent use in managing urinary tract infections and other community-acquired diseases [32, 35].

In contrast, the isolates in the current study showed notable susceptibility to doripenem, imipenem, meropenem, and polymyxin B. Previous studies have demonstrated favorable susceptibility outcomes of these antibiotics against *E. coli* [27]. This is encouraging because of the efficacy of these antibiotics against gram-negative bacteria that are resistant to many drugs, including ESBL *Escherichia coli* [27]. Meticulous monitoring of carbapenems and polymyxins is necessary to mitigate the potential resistance [31].

Table 2. The antibiotic susceptibility profiles of ESBL *E. coli* (n=365) and non-ESBL *E. coli* (n=155) isolates obtained from clinical specimens during the period of January 2022 to July 2023

Antibiotics	ESBL <i>E. coli</i> (n=365)		Non- ESBL <i>E. coli</i> (n=155)		Chi-2 ^a	p-values ^b
	n	%	n	%		
Ampicillin	0	0.00	12	7.74	28.926	<0.0001
Amoxicillin/Clavulanic Acid	74	20.27	52	33.55	10.442	0.001
Amoxicillin	45	12.33	28	18.06	2.966	0.085
Amikacin	42	11.51	57	36.77	45.064	<0.0001
Ceftazidime	35	9.59	68	43.87	80.498	<0.0001
Ceftriaxone	0	0.00	33	21.29	82.975	<0.0001
Colistin	42	11.51	77	49.68	89.824	<0.0001
Cefotaxime	1	0.27	39	25.16	94.90364	<0.0001
Doxycycline	42	11.51	48	30.97	28.790	<0.0001
Doripenem	100	27.40	90	58.06	44.128	<0.0001
Cefoxitin	85	23.29	72	46.45	27.698	<0.0001
Gentamicin	56	15.34	71	45.81	54.702	<0.0001
Imipenem	100	27.40	94	60.65	51.42059	<0.0001
Levofloxacin	12	3.29	35	22.58	49.25692	<0.0001
Meropenem	100	27.40	83	53.55	32.623	<0.0001
Polymyxin B	100	27.40	96	61.94	55.262	<0.0001
Cefoperazone/Sulbactam	42	11.51	94	60.65	136.0186	<0.0001
Tobramycin	61	16.71	71	45.81	48.622	<0.0001
Pipiracillin/Tazobactam	24	6.58	80	51.61	137.9275	<0.0001

^a Chi-square calculated for comparison of susceptibility in *ESBL-producing E. coli* and *non- ESBL-producing E. coli*. ^b P values generated from the chi-square

4 Conclusion

ESBL *E. coli* were predominantly observed in urine and pus specimens, particularly among senior patients aged 46-56 years. Furthermore, these isolates exhibited a higher prevalence among female patients receiving treatment in both ICU and non-ICU settings. The antibiotic susceptibility profile of ESBL *E. coli* isolates underscores the critical need for judicious antibiotic usage and the enforcement of stringent infection control to mitigate the emergence of multidrug-resistant bacterial strains. Subsequent research endeavors should aim to elucidate the mechanisms underpinning resistance and develop innovative therapeutic strategies for addressing ESBL-producing *E. coli* infections

References

1. Widodo, A., et al., Biodiversitas, **24**, 1 (2023)

2. Maulana, K.Y., et al., *Vet. Integr. Sci.*, **19**, 3 (2021)
3. Agatha, T.M., et al., *Vet. World*, **16**, 1 (2023)
4. Abalkhail, A., et al., *Healthcare*, **10**(9), 2022.
5. Shakya, P., et al., *Open Microbiol. J.*, **11**, 2017.
6. Devipalanisamy, D., S. Olaganathan, and M. Marimuthu, *Int. J. Pharm. Investig.* **11**, 1 (2021)
7. Ulfa, M., et al., *J. Med. Invest.*, **67**, (2020)
8. Chen, K., et al., *Clin Exp Obstet Gynecol.*, **48**,4 (2021)
9. Denkel, L.A., et al., *Clin. Microbiol. Infect.*, **26**, 8 (2020)
10. Ukah, U.V., et al., *Epidemiol. Infect.*, **146**, 1 (2017)
11. Raphael, E., M.M. Glymour, and H.F. Chambers, *Antimicrob. Resist. Infect. Control.* **10**, 1 (2021)
12. Mazel, D., et al., *Antimicrob. Agents Chemother.*, **44**, 6 (2000)
13. Frost, L.S., et al., *Nat Rev Micro*, **3**, 9 (2005)
14. Hanberger, H., et al., *BMC infect. Dis.*, **14**, 1 (2014)
15. Brodrick, H.J., et al., *Genome Med.* **9**, (2017)
16. Moghanni, M., et al., **15**, (2018)
17. Jia, P., et al., *Front. Microbiol.*, **12**, (2021)
18. Yasir, M., et al., *Sci. Rep.*, **8**, 1 (2018)
19. Syre, H., et al., *Apmis*, **128**, 3 (2020)
20. Alqasim, A., A.A. Jaffal, and A.A. Alyousef, *Int. J. Microbiol.*, **2018**, (2018)
21. Siregar, M.L., et al., *F1000research*, **11**, (2022)
22. Gorrie, C.L., et al., *Clin. Infect. Dis.*, **65**, 2 (2017)
23. Evins, C., et al., *Antibiotics*, **10**, 5 (2021)
24. Reuland, E.A., et al., *J. Antimicrob. Chemother.*, **71**, 4 (2016)
25. Yu, Y., et al., *Biomed Res. Int.*, **2018**, (2018)
26. Gu, J., et al., *J. Clin. Lab. Anal.*, **36**, 1 (2022)
27. Chang, Q., et al., *Evol. Appl.*, **8**, 3 (2015)
28. Ding, C., et al., *Int. J. Infect. Dis.*, **49**, (2016)
29. Ji, X., et al., *Front. Pharmacol.*, **13**, (2022)
30. Blaak, H., et al., *PLoS ONE*, **10**, 6 (2015)
31. Nakayama, T., et al., *Arch.Microbiol.*, **204**, 2, (2022)
32. Clay, K., et al., *Antimicrob. Agents Chemother.* **65**, 11 (2021)
33. Tamta, S., et al., *Vet. World*, **13**, 2 (2020)
34. de Been, M., et al., *PLoS Genet.*, **10**, 12 (2014)
35. Haseeb, A., et al., *Infect. Drug Resist.*, (2023)