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ORIGINAL ARTICLE

Effects of aging on colony count and antibiotic susceptibility patterns of *Escherichia coli* isolated from male Wistar rats

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ABSTRACT

BACKGROUND
Physiological and immunological changes in the elderly may contribute to the composition and patterns of the gut microbiota, including *Escherichia coli*, as well as antimicrobial susceptibility patterns. The research was conducted to assess the effect of aging on *Escherichia coli* growth and susceptibility to several antibiotics.

METHODS
An experimental laboratory study using 7 young (6-month-old) (YWR) and 7 old (24-month-old) Wistar rats (OWR) as subjects. Rat stool specimens were used as representing the subjects. Antibiotic susceptibility was determined using the Kirby-Bauer method. The independent t-test and Mann-Whitney test were used to analyse the data.

RESULTS
All fecal specimens collected from both groups were positive for *E. coli* growth. The YWR showed a low density of *E. coli*, while the OWR showed a statistically significant increase in the population of *E. coli* ($p=0.001$). Overall, the *E. coli* isolates showed a high proportion of resistance to erythromycin (100%), ampicillin (86%), and oxytetracycline (58%). The isolates collected from YWR were significantly more resistant to streptomycin (83% vs. 19%, $p<0.001$) and tetracycline (47% vs. 3%, $p<0.001$) than the isolates from OWR. Young Wistar rats also had a higher resistance rate than OWR to the most frequently used antibiotics, such as ampicillin (97% vs. 76%, $p<0.001$).

CONCLUSION
Our study demonstrated an increase in the fecal *E. coli* population with increasing age of the Wistar rats. In addition, the results of susceptibility tests for several antibiotics showed that age may not be associated with an increase in the population of a resistant strain of *E. coli*.

Keywords: Aging, *Escherichia coli*, colonization, antibiotic resistance, Wistar rats

INTRODUCTION

It may reasonably be stated that more than one thousand and presumably even more bacterial species from various phyla colonize the digestive tract.[1] Of the dominant phyla that have been identified, the *Firmicutes* and *Bacteroidetes* are the two most common bacterial phyla in the human gastrointestinal tract, accounting for 90% of the phylogenetic tree.[2] However, some non-commensal bacteria are also found, especially from *Proteobacteria* such as *Enterobacteriaceae*. *Proteobacteria* tend to be the least stable throughout the life of their host compared to other phyla, and an increase in *Proteobacteria* has been considered a sign of gastrointestinal dysbiosis.[3]

Enterobacteriaceae are a family of *Proteobacteria.* As symbionts, *Enterobacteriaceae* are present in small numbers in the mammalian intestine because their presence is suppressed and maintained at low densities (significantly less than 10^8 CFU/g) by obligate anaerobic bacteria (phylum *Firmicutes* and *Bacteroidetes*) that predominantly colonize the normal mammalian intestine. However, growth of *Enterobacteriaceae* increases under dysbiotic conditions, which then contributes to various pathological conditions related to inflammation, such as inflammatory bowel disease, obesity, colorectal cancer, and celiac disease.[4]

Among *Enterobacteriaceae, Escherichia coli* is the most common pathogenic species. Despite being present in minor numbers as commensal bacteria in a healthy human gut, a significant increase in *E. coli* can be highly relevant for the gastrointestinal tract. The properties of lipopolysaccharides (LPS) in *E. coli* could potentially contribute to the pathogenesis of disease progression. The endotoxic activity of LPS is a triggering factor for inflammation and obesity in rat models. Diet-induced metabolic endotoxemia is an important factor in the development of many chronic diseases in animals and humans. A high-fat diet increases the concentration of LPS in the blood, causes endotoxemia and systemic inflammation, and initiates processes leading to obesity and diabetes.[5]

Various conditions can provide growth advantages to *Enterobacteriaceae*, including environmental and nutritional changes resulting from intestinal inflammation. In addition, physiological and immunological changes in the elderly are likely to be contributing factors. With age, there are substantial changes in both innate and adaptive immunity.[6] These changes contribute to the increased frequency of some infectious diseases in older individuals. *Several* intrinsic and extrinsic factors such as diet, drugs, sedentary behavior, and chronic health conditions also drive changes in gut microbiota in the elderly, characterized by loss of beneficial commensal microbes.[7] Overall microbial diversity decreases while certain bacterial groups associated with frailty increase.[1]

In this study, we assessed the potential impact of aging on *E. coli* growth, which may, in turn, contribute to the development of gut microbial dysbiosis and render older individuals susceptible to *E. coli* infection. Several factors, such as ethical issues, environmental and social factors, and natural life expectancy, can constrain the use of humans in aging research. However, all organisms undergo the aging process, and it is possible to use animal models to study the effects of aging. Research on rodents such as rats has been commonly carried out in studies related to aging, considering several advantages, including a good background understanding of this animal model, capacity to control environmental factors, and genetic manipulation.[8] The relatively short life expectancy compared to humans makes this animal model easier to study in aging research.

Escherichia coli is inherently sensitive to nearly all therapeutically relevant antibiotics. However, this species can accumulate resistance genes, including the capacity to produce extended-spectrum beta-lactamase (ESBL) encoding resistance to all beta-lactams except the carbapenem group.[9] Another point of concern is that identifying and characterizing commensal *E. coli* based on the 16S rRNA sequencing approach often poses difficulties, considering the high homology of 16S rRNA gene sequences between the different strains.[10] Therefore, we used a selective culture media approach to isolate and identify commensal *E. coli* strains from laboratory Wistar rats. In addition to identifying and counting the number of *E. coli* colonies in stool specimens obtained from young and old Wistar rats, we assessed the susceptibility of *E. coli* to several antibiotics, including the detection of possible ESBL-producing strains.

Several studies on antimicrobial resistance in *E. coli* isolated from rats have been conducted, such as the study by Huy et al.[11] using brown rats and house shrews in the market area, Bogor, Indonesia, which found the presence of multi-drug

resistant (MDR) *E. coli*, indicating the potential contamination of antimicrobial resistant (AMR) *E. coli*. There is also the study by Anuwong et al.[12] which found the presence of ESBL *E. coli* (ESBL-EC) in city areas and animal farms in Hong Kong. The uniqueness of our study that distinguishes it from other studies is the focus on the aging process represented by young and old Wistar rats, to assess the effect of the aging process on the presence of MDR *E. coli* and ESBL-EC.

We suspected a relationship between the aging process and the growth of *E. coli* in the gastrointestinal tract. Therefore, the objective of this study was to assess the impact of aging on *Escherichia coli* growth and susceptibility to several antibiotics.

METHODS

Research design

The study design was that of a cross-sectional laboratory experiment. The research was conducted at the Microbiology Laboratory of the Central Laboratory of Padjadjaran University, from September 2022 to February 2023.

Study subjects

The study involved two groups of subjects, namely the groups of young Wistar rats (YWR) and old Wistar rats (OWR). The specimens used in this study were the stools of the YWR and OWR obtained by consecutive sampling.

Sample size determination

Based on Mead's equation methods,[13] with k (the number of research population groups) of 2, the sample size was determined s follows: The minimum number of subjects per group n = 10/k + 1 = 10/2 + 1 = 6 rats (six subjects collected for each group). To each group was added 10% to anticipate the possibility of dropping out during the study, therefore the number of subjects required for each group was seven rats. The minimum total number of research subjects N = minimum n x k = 7 x 2 = 14 rats.

Preparation of young and old Wistar rats

The equivalent age of rats in comparison to humans refers to an average of 16.4 days in rats, equivalent to 1 year in humans.[14] The inclusion criteria were as follows: (1) male Wistar rats, (2) six months and 24 months old (equivalent to 18 and 60 years of age), and (3) being in good health and able to move actively. Seven male Wistar rats aged six months (YWR) and seven rats aged 24 months (OWR), weighing 350-450 grams, were purchased from PT. Biofarma, Bandung, Indonesia. The rats were placed in standard cages and were fed a pelleted rat diet (Prospect Rat Standard Chow Diet, Indonesia), with free access to drinking water (ad libitum). The rats were housed in the Veterinary Laboratory of the Faculty of Medicine, Universitas Padjadjaran. The environmental conditions included a 12-hour dark and light cycle, an ambient temperature of 22°C-24°C, and a stable humidity of 60%. The rats were acclimatized to the set environment for two weeks.

Specimen collection

A total of 14 stool specimens were collected from 14 Wistar rats, placed in small sterilized jars, and stored in a cabinet at 4 °C. Isolation and identification of *E. coli* were carried out at the Microbiology Laboratory of the Faculty of Medicine, Universitas Padjadjaran.

Isolation and identification of *E. coli*

Fourteen YWR and OWR stool specimens were diluted first with 0.85% physiological saline to a dilution of 10^{-8}. They were then streaked on eosin methylene blue (EMB) media (Oxoid) using the scatter method and incubated for 24 hours at 37°C. Morphologically typical colonies producing a green metallic sheen were considered presumptive *E. coli*. Simultaneously, colonies suspected of being *E. coli* were picked from the agar plate and stained with Gram's stain. Likewise, a single colony of each isolate was fixed on a clean slide to examine morphological characteristics under a light microscope. *E. coli* bacteria stained with Gram stain will be seen as red-colored short rods.

The colonies confirmed by Gram staining are then subjected to different biochemical tests using the IMVIC procedure for the definitive diagnosis of *E. coli*. The indole test was done by growing *E. coli* in a sulfide indole motility media (SIM) tube, the methyl red and Voges-Proskauer tests were conducted with MR-VP medium, and the citrate test with Simon's citrate. IMVIC tubes were incubated for 24 hours at 37°C, except for MR-VP, which was incubated for 2-5 days. Positive results of the indole test were indicated by the formation of a pink indole ring after adding

Kovac's reagent. A red or orange-colored solution characterizes the observation results for the positive Methyl Red test, while yellow means negative. The Voges Proskauer (VP) test was negative for *E. coli* because *E. coli* ferments carbohydrates into acidic products and does not produce neutral products. The citrate test was negative for *E. coli* because *E. coli* does not utilize citrate as a carbon source, as indicated by no color change in the citrate test medium.

Enumeration of *E. coli*

To count *E. coli* colonies from the specimens, we used the total plate count (TPC) method based on the following steps: (1) take a stool specimen with a weighing spoon to the amount of 1 gram; (2) place the specimen in 9 ml of diluent solution (sterile distilled water) and homogenize to a dilution of 10^{-1}; (3) from the 10^{-1} dilution, take 1 ml, add to 9 m of sterile distilled water and homogenize (as the 10^{-2}) dilution and so on until the 10^{-5} dilution; (4) plate the bacteria in dilutions of 10^{-4} and 10^{-5} (pour plate), by taking 1 ml of each suspension and putting it in a petri dish; (5) fill each petri dish with Plate Count Agar (PCA) media at 45°C, homogenize and wait until solid; (6) incubate at 37°C for 2x24 hours; (7) observe the growth of colonies on each plate and count with a colony counter, and (8) the number of colony-forming units per gram wet weight (CFU/g) of the stool specimen is calculated from the readable range dilution. Calculation of the number of colonies is as follows: N = number of colonies per plate x 1/dilution factor, where dilution factor = dilution x grown; N = number of produced colonies (colonies per ml or gram). Note: if the number of colonies per plate is significantly more than 250 in the entire dilution, report the result as overcounting.

Antibiotic susceptibility test (AST)

Antibiotic susceptibility testing of isolates was performed using the Kirby-Bauer method according to the Clinical and Laboratory Standards Institute (CLSI, 2023) guidelines. First, each nutrient agar plate for *E. coli* isolates from stool specimens of 14 Wistar rats was divided into four quadrants. Next, we took 2-3 colonies from each quadrant to obtain 10 colonies per nutrient agar plate, so the total number of colonies for the antibiotic susceptibility test was 140. The bacterial colonies were then added to the 0.9% NaCl solution until a turbidity of 0.5 MacFarland was reached (equivalent to 1.5×10^8 cells/ml), and the suspension was inoculated into Mueller-Hinton agar. The following antibiotics were used: tetracycline (TE, 30 µg), trimethoprim/sulfamethoxazole (SXT, 5 µg), nalidixic acid (NA, 30 µg), ampicillin (AMP, 10 µg), azithromycin (AZM, 15 µg), erythromycin (E, 15 µg), streptomycin (S, 10 µg), chloramphenicol (C, 30 µg), oxytetracycline (OXT, 30 µg), and ciprofloxacin (CIP, 5 µg). The presence of ESBL was identified using a combination disk test (CDT) as described by CLSI (2023). The CDT uses a ceftazidime CAZ (30 µg) and cefotaxime CTX (30 µg) disk, to which clavulanic acid (CLA, 10 µg) has been added. If the inhibition zone diameter was ≥5 mm larger with CLA than without, the test was considered positive for ESBL production. Isolates are considered multidrug-resistant (MDR) when resistant to three or more antibiotics from different categories.[15]

Statistical analysis

Data were entered in SPSS 20.0 (IBM Inc., Chicago, IL, United States). Data cleaning, statistical analysis, and graphical presentation were done in Stata 13.0 (College Station, TX, United States) and R-3.4.2 (R Core Team, 2014). The Kolmogorov-Smirnov test was used to determine the normality of the data distribution, then the homogeneity of the data was tested with the Levene test. Based on the results of the normality and homogeneity tests, both the independent t-test and the Mann-Whitney test were used for comparisons between groups, respectively for normally distributed and non-normally distributed data. Differences were considered statistically significant when $p<0.05$.

Ethical clearance

Animal procedures and treatments were performed following laboratory animal guidelines and were approved by the Padjadjaran University Research Ethics Committee (approval no. 127/UN6. KEP/EC/2022).

RESULTS

Fecal carriage of *Escherichia coli* in Wistar rats

All 14 stool specimens collected from Young Wistar rats [YWR] and Old Wistar rats [OWR] showed positive growth for *E. coli*. The mean count ± standard deviation for total *E. coli* in YWR was $2.6 \pm 2.2\ 10^4$ CFU/g, while the mean count of *E. coli* in OWR was $3.4 \pm 4.0\ 10^4$ CFU/g,

the difference being statistically significant (p=0.001).

Antibiotic susceptibility of *Escherichia coli*

A total of 140 *E. coli isolates* were tested for susceptibility against a panel of 13 antibiotics. A high proportion of *E. coli isolates* were resistant to erythromycin (100%), ampicillin (86%), and oxytetracycline (58%). Only 5% of all *E. coli* isolates were resistant to azithromycin. No isolate was resistant to ceftazidime, ceftazidime + clavulanic acid, cefotaxime, or cefotaxime + clavulanic acid (Figure 1).

The susceptibility patterns of *E. coli* to the 13 antibiotics tested in this study showed that the rate of antibiotic resistance of YWR (n=70) was significantly higher to streptomycin (83% vs. 19%, p=0.000) and tetracycline (47% vs 3%, p=0.000) when compared with those of OWR (n=70). Young Wistar rats also had higher resistance rates than OWR for most commonly used antibiotics such as ampicillin (97% vs 76%, p=0.000) (Table 1).

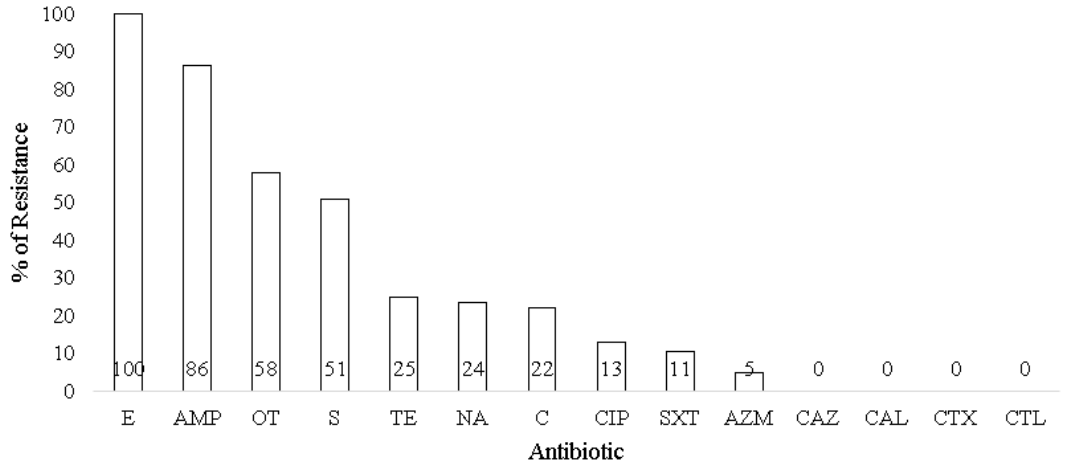

Figure 1. Prevalence of *E. coli* resistant to different classes of antibiotics in Wistar rats aged 6 months (n=70) and 24 months (n=70). Tet, Tetracycline; Sxt, Trimethoprim/Sulfamethoxazole; Nal, Nalidixic Acid; Amp, Ampicillin; Azm, Azithromycin; E, Erithromycin; C, Chloramphenicol; S, Streptomycin; Oxt, Oxytetracycline; Cip, Ciprofloxacin; Caz, Ceftazidime; Ctx, Cefotaxime; Cal, Ceftazidime + Clavulanic Acid; Ctl, Cefotaxime + Clavulanic Acid

Table 1. Antibiotic resistance rates of *Escherichia coli* isolated from Wistar rats

Antibiotic	Antibiotic resistance (n,%)		p value
	YWR (n=70)	OWR (n=70)	
E†	70 (100)	70 (100)	0.071
AMP†	68 (97)	53 (76)	0.000‡
OT†	43 (61)	38 (54)	0.015‡
S†	58 (83)	13 (19)	0.000‡
TE†	33 (47)	2 (3)	0.000‡
NA†	31 (44)	2 (3)	0.000‡
C†	30 (43)	1 (1)	0.000‡
CIP†	18 (26)	0 (0)	0.155
SXT*	15 (21)	0 (0)	0.000‡
AZM*	5 (7)	2 (3)	0.299
CAZ*	na	na	na
CAL*	na	na	na
CTX*	na	na	na
CTL*	na	na	na

Note : MDR : multiple drug resistance; YWR : young Wistar rats; OWR : old Wistar rats; E: Erythromycin; AMP: Ampicillin; OT: Oxytetracycline; S: Streptomycin; TE: Tetracycline; NA: Nalidixic Acid; C: Chloramphenicol; CIP: Ciprofloxacin; SXT: Trimethoprim/Sulfamethoxazole; AZM: Azithromycin; CAZ: Ceftazidime; CAL: Ceftazidime + Clavulanic Acid; CTX: Cefotaxime; CTL: Cefotaxime + Clavulanic Acid; na: not applicable. *Normal distribution (p>0.05), continued with a significance test using an independent t-test. †Distribution is not normal (p<0.05), continued with a significance test using the Mann-Whitney test. ‡Significant data (p<0.05).

Table 2. Antibiotic resistance patterns for the *Escherichia coli* isolated from Wistar rats

Pattern	MDR Pattern	Resistant *E. coli* isolates [n (%)]		
		YWR	OWR	Total
I	S, SXT, AMP, NA, OT, E, CIP, AZM	1 (1)	0	1 (1)
II	S, AMP, NA, OT, E, TE, CIP, C	1 (1)	0	1 (1)
III	S, SXT, AMP, NA, E, CIP, AZM	2 (3)	0	2 (1)
IV	S, SXT, AMP, NA, OT, E, AZM	1 (1)	0	1 (1)
V	S, AMP, NA, OT, E, TE, CIP	12 (17)	0	12 (9)
VI	S, SXT, AMP, OT, E, TE, C	10 (14)	0	10 (7)
VII	S, AMP, NA, OT, E, CIP	1 (1)	0	1 (1)
VIII	S, AMP, NA, OT, E, TE	9 (13)	0	9 (6)
IX	S, SXT, AMP, E, AZM, C	1 (1)	0	1 (1)
X	S, AMP, OT, E, AZM	0	1 (1)	1 (1)
XI	S, AMP, NA, E, CIP	1 (1)	0	1 (1)
XII	S, AMP, NA, E, C	1 (1)	0	1 (1)
XIII	S, AMP, OT, E, C	8 (11)	0	8 (6)
XIV	S, AMP, E, TE, C	1 (1)	0	1 (1)
XV	AMP, NA, OT, E	0	2 (3)	2 (1)
XVI	AMP, OT, E, C	0	1 (1)	1 (1)
XVII	AMP, OT, E, AZM	0	1 (1)	1 (1)
XVIII	S, AMP, OT, E	0	8 (11)	8 (6)
XIX	S, OT, E, TE	0	2 (3)	2 (1)
XX	S, AMP, E, C	10 (14)	0	10 (7)
XXI	AMP, OT, E	0	16 (23)	16 (11)
XXII	S, OT, E	0	2 (3)	2 (1)
XXIII	AMP, NA, E	2 (3)	0	2 (1)
XXIV	S, AMP, E	1 (1)	0	1 (1)
XXV	AMP, E	8 (11)	24 (34)	32 (23)
XXVI	OT, E	0	5 (7)	5 (4)
XXVII	E	0	8 (11)	8 (6)
Cla	Resistance to three or more antibiotics (MDR)	62 (89)	33 (47)	95 (68)
Cla	Resistance to two antibiotics	8 (11)	29 (41)	37 (26)
Cla	Resistance to only one antibiotic	0	8 (11)	8 (6)
Cla	Pan-susceptible	0	0	0

Note : MDR : multiple drug resistance; YWR : young Wistar rats; OWR : old Wistar rats; E: Erythromycin; AMP: Ampicillin; OT: Oxytetracycline; S: Streptomycin; TE: Tetracycline; NA: Nalidixic Acid; C: Chloramphenicol; CIP: Ciprofloxacin; SXT: Trimethoprim/Sulfamethoxazole; AZM: Azithromycin: CAZ: Ceftazidime; CAL: Ceftazidime + Clavulanic Acid CTX: Cefotaxime; CTL: Cefotaxime + Clavulanic Acid; Cla: Classification

Next, we observed the multi-resistant characteristics of the *E. coli* isolated from both groups. Table 2 summarizes antibiotic resistance patterns of *E. coli* isolated from Wistar rats. Sixty-eight percent (95/140) of the *E. coli* isolates were multidrug-resistant and classified as MDR. Resistance to multiple antibiotics was widespread in both groups, but *E. coli isolates* from YWR tended to be more multi-resistant than OWR (89% vs 47%); however, this difference was statistically not significant. Resistance to two antibiotics was more commonly detected in isolates from OWR than those of YWR (41% vs 11%). The most common multi-drug resistance phenotype was ampicillin-resistant erythromycin-resistant (Amp^R E^R) with or without resistance to other antibiotics (123 out of 140 or 88%).

DISCUSSION

This study showed that the average number of *E. coli* in the feces of 6-month-old rats was at low-density level. However, the population of *E. coli* significantly increased in Wistar rats aged 24 months. We compared *E. coli* counts between Wistar rats in the 2 age groups and found a significant difference in the *E. coli* population. It is unclear how the microbial composition shifts from the young adult to the old adult stage. However, several studies on humans showed that gut microbial composition and patterns change with age, and these changes have been associated with decreased immunity, as observed in immunosenescence.[7,16] Despite this view, Klimova et al.[17] stated that old animals are not

inferior to young animals in the immune system's capacity to respond to infectious agents, such as *E. coli*., but use different strategies to elicit an immune response.

Concerning the resistance pattern of *E. coli* to the 13 antibiotics, this study shows that antibiotic resistance tends to be more common in *E. coli* isolated from YWR than OWR. *E. coli* isolated from YWR was significantly more resistant to streptomycin and tetracycline when compared to OWR. In addition, *E. coli* from YWR showed a higher degree of resistance than OWR to ampicillin. Ampicillin belongs to the β-lactam antibiotics. Resistance to ampicillin is related to the expression of the β─lactamase gene to produce an enzyme that hydrolyzes the β-lactam ring. There is more antibiotic resistance in these YWR, which is quite interesting. It is often indicated, especially in humans, that antimicrobial patterns and sensitivity change with age, possibly because the elderly are particularly susceptible to infection. Managing elderly patients with a high risk of disease has become one of the significant challenges for health services, as well as increasing the practice of prescribing improper and unnecessary antibiotics, thereby contributing to the proliferation of resistant bacteria among the elderly. However, the subjects of this study were rats that did not receive antibiotics during the procedure and treatment period, so there were no antibiotic factors that could change the intestinal microbial profile.

This study also observed the microbial susceptibility patterns for chloramphenicol and erythromycin. These antibiotics eliminate bacteria by interfering with protein synthesis. They inhibit protein synthesis by binding to the 50S ribosomal subunits. Most of the *E. coli* from the two groups were still sensitive to chloramphenicol. However, all *E. coli* isolates from YWR and OWR were resistant to erythromycin (100%). Bacteria can inactivate chloramphenicol with the chloramphenicol enzyme o-acetyl-transferase produced by the plasmid. In contrast, the target site for erythromycin on the ribosome is modified by the enzyme encoded by the erythromycin ribosomal methylation (erm) gene.[18]

Rats are mammals that are close to everyday human life. The presence of multidrug-resistant *E. coli*, particularly those that produce ESBLs in rats, may cause these resistant bacterial strains to be transferred to humans and pose a risk to human health. Meanwhile, several studies have shown that older age is a risk factor for colonization or infection with ESBL-producing organisms.[19,20] Extended-spectrum beta-lactamase production is one of the most critical risk factors associated with mortality in *Enterobacteriaceae* infection.[21,22] The CDT in this study showed that none of the *E. coli* isolates (n=140) produced ESBL. The absence of ESBL-EC may indicate that the plasmid is not distributed in the rat population and that aging may not be a risk factor for the development of ESBL strains. However, it should be emphasized that there are several other risk factors for ESBL infection besides age, including co-morbidities, previous use of antibiotics, and specific clinical situations such as intensive care. Onanga et al.[23] showed that 34.37% of rats in semi-rural areas of Gabon produce ESBL, which poses a health risk to humans and pets in the Gabon region.

A limitation of this study was the small number of rats included and that they were collected from a hatchery with optimal conditions for rat growth, rather than from the environment. In contrast to the study by Onanga et al, [23 which uses rats from semi-rural areas, our study used healthy rats, which were strictly monitored, including cage conditions and a controlled diet, and never exposed to antibiotics so that we could rule out some other factors that might affect the colonization of ESBL-producing organisms. However, further studies that include analysis of various risk factors for ESBL infection need to be carried out to obtain comprehensive data regarding the effect of age and other factors in increasing the risk of infection or colonization with ESBL-producing organisms.

CONCLUSSION

This study showed an increase in the fecal *E. coli* population with increasing age in Wistar rats. In addition, the results of susceptibility tests for several antibiotics showed that age was not associated with an increase in the population of a resistant strain of *E. coli*.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Data Availability Statement

Data is available from the corresponding author upon request.

Author Contributions

IM: conceptualization, methodology, supervision, formal analysis; PAA: data curation, original draft preparation, visualization; NS: conceptualization, validation, reviewing and editing, All authors have read and approved the final manuscript.

Declaration of Use of AI in Scientific Writing

Nothing to declare.

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