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Distribution of Antibiotic-Resistant *Escherichia coli* Bacteria in Various Malls in Makassar City

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Abstract

Bacterial resistance to antibiotics is known as antibiotic resistance. Public places like shopping centres (malls) may serve as locations for spreading antibiotic-resistant bacteria in large cities by transferring bacteria from humans to surfaces and vice versa. We investigated the presence of antibiotic-resistant *Escherichia coli* (*E. coli*) in six major malls in different parts of Makassar City. We collected *E. coli* isolates using sterile swabs on toilet seats, toilet flush buttons, faucets, door handles, and escalators. We randomly selected twenty of these isolates from each mall and tested their resistance against five classes of antibiotics using the Kirby-Bauer Disk Diffusion method. The antibiotics tested are amoxicillin-clavulanate (B-lactams), amikacin (aminoglycosides), chloramphenicol (chloramphenicol), norfloxacin (quinolones), and trimethoprim (inhibitor de-hydrolat reductase). The determination of resistance followed EUCAST standards. *E. coli* resistant was found in all malls. Of the isolates, 55% showed resistance to one or more antibiotics: 5% were resistant to trimethoprim, 21% to amoxicillin-clavulanate, 19% to chloramphenicol, 14% to norfloxacin, and 11% to amikacin. Twenty-six percent of isolates were resistant to more than two antibiotics. In one of the largest and busiest malls, 60% of its isolates were resistant, with one isolate being resistant to all antibiotics. Our data indicates the potential transmission of antibiotic-resistant bacteria through malls; and big and busy malls tend to show a higher risk of transmission.

Keywords: *antibiotic-resistant bacteria, E. coli, mall, multi-drug resistant, Indonesia.*

1. Introduction

Antibiotic-resistant bacteria are becoming a significant health problem, such as causing severe, life-threatening, and difficult diseases to overcome due to limited options as therapy that can cause infection. The contamination and spread of bacteria comes from daily activities and are often not realized by humans. Daily activities that can be a source of bacterial spread include gardening, washing, eating and drinking, touching objects, and many other activities.

Public places visited by many people from various circles, namely shopping centres, have developed rapidly [1]. A shopping centre is a place for buying and selling transactions or trade that can create the city's dynamism or local environment besides being a place of recreation and entertainment for the community. Microorganisms can spread through touch, so objects that are most often touched or touched by the surface of other things tend to have the

potential to be contaminated with microorganisms, especially bacteria [2]. Buying, playing, eating, and interacting in the mall can be a pattern of spreading bacteria, for example, on the escalator handle.

In addition to the escalator handrail, bacteria can also be found in the mall toilets on toilet seats, toilet flush buttons, water taps and toilet door handles. The spreading of bacteria can be caused by individuals who have used the toilet not washing their hands thoroughly when they have used the toilet. Although every mall has provided janitors, the officers only clean places that look dirty without cleaning the faucet area, toilet rim and flush button. [3]. This study will identify the distribution of antibiotic-resistant E.coli bacteria in several malls and determine the resistance pattern of E.coli isolates from Makassar City malls to five classes of antibiotics.

2. Methods

2.1 Sample location

Figure 1. Sampling locations

2.2 Sampling and Dilution

Samples of escalator surfaces, toilets, door handles, and toilet flush buttons were taken using the swab method, then the cotton swab was inserted into a sterile screw cap tube containing 0.85% saline solution. The samples were then brought to the Analytical Chemistry and Environment laboratory for analysis. Samples taken are filtered using a vacuum pump and membrane filter, then inoculated on a CCA medium and incubated at 37 °C for 18-24 hours.

2.3 Cultivation

After growing the samples in cromocult coliform agar, twenty well-isolated colonies of the same morphological type were selected. Touch the top of each colony was touched with a toothpick and transferred to a tube containing tryptic soy broth media. After that, the tubes were incubated at 37^{o}C for 18-24 hours. Then, the bacterial culture was diluted to 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}, with sterile distilled water, and the colony was counted in each dilution. Furthermore, the diluted culture was spread on MHA media and incubated at 37^{o}C for 18-24 hours. [CLSI, 2012] for use in the resistance test.

2.4 Resistance Test

Three to five bacterial colony was suspended to 0.85% saline solution. The sample suspension was adjusted with MacFarland 0.5. This bacterial suspension was gently applied to the entire surface of the MHA plate using a sterile cotton swab. Antibiotic discs with a concentration of 5 µg for trimethoprime antibiotic, 10 µg norfloxacin, and 30 µg for amikacin, chloramphenicol, and amoxicilin-clavulanate antibiotics were used. These were aseptically placed on the surface of agar plates and incubated at 37^{o}C for 18-24 hours. The diameter of the inhibition was measured using a ruler around the antibiotic disc three times.

2.5 Indicator of Resistance

The diameter of the inhibition zone formed determines the sensitivity or resistance of a bacterium to an antibiotic. Inhibition will be seen as an area that shows no bacterial growth around the disk. This inhibition zone depends on the type of antibiotic and the bacterial strains. We follow the EUCAST protocol to determine resistance strains.

2.6 Data Analysis

We calculated the resistance rate (RR), which is the ratio of resistant strain over the total strains at location. The resistance rate obtained was visualized in histograms using Excel software (Microsoft Office version 2021) to compare the resistance rate between sampling sites.

2.7 Results and Discussion

Bacterial sampling was carried out at five mall locations in Makassar city. The bacteria *E. coli* used in this study were selected using CCA media.

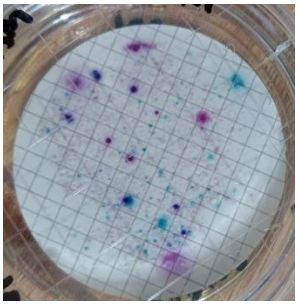

Figure 1. Bacteria in *CCA* Media

Table 1: Number of Colonies on Each Mall

Mall Code	Average Colonies Sampling location						Total coli	Percent *E. coli*
	Toilet seat (n=6)		Door Handle (n=6)		Escalator (n=2)			
	E.coli	Other *coli*	*E.coli*	Other *coli*	*E.coli*	Other *coli*		
Mall A	17	35	3	7	0	2	64	31
Mall B1	70	78	52	10	2	0	212	58
Mall B2	60	53	50	11	2	0	176	64
Mall C	77	59	72	76	2	10	296	51
Mall D	67	23	38	1	3	2	134	81
Mall E	34	54	40	9	6	7	150	53

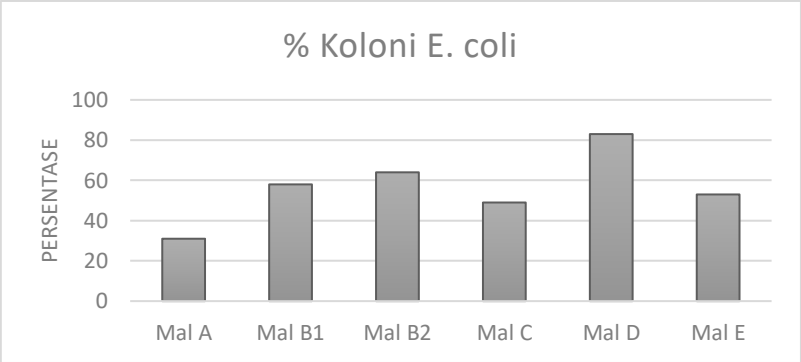

Figure 2. Percent E. coli colonies in each mall

We found more E. coli colonies in the toilet seats than in the door handles and escalators (Table 1); that could be due to several factors, such as E. coli coming from human faeces, the

toilet having high humidity, and is not always cleaned properly. These conditions are an ideal breeding ground for E. coli (Hendrayana, 2023).

The results of the antibiotic inhibition test against E. coli bacteria found in the mall can be seen in Figures 3 and 4. We found that 55% of isolates resisted one or more antibiotics.

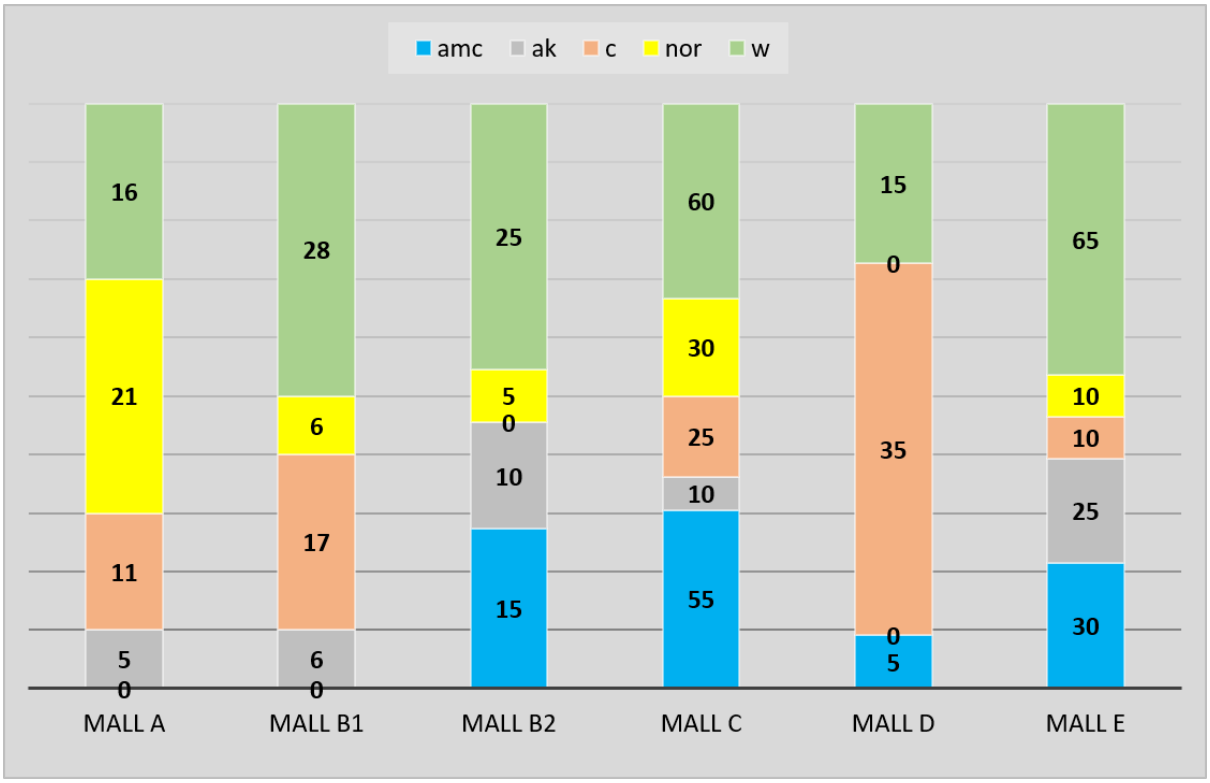

Figure 3 Percent of Colonies that are Resistant to Antibiotics following EUCAST protocol. Notes: amc = amoxicilin-clavulanate, ak = amikacin, c = chloramphenicol, nor = norfloxacin, w = trimethoprim.

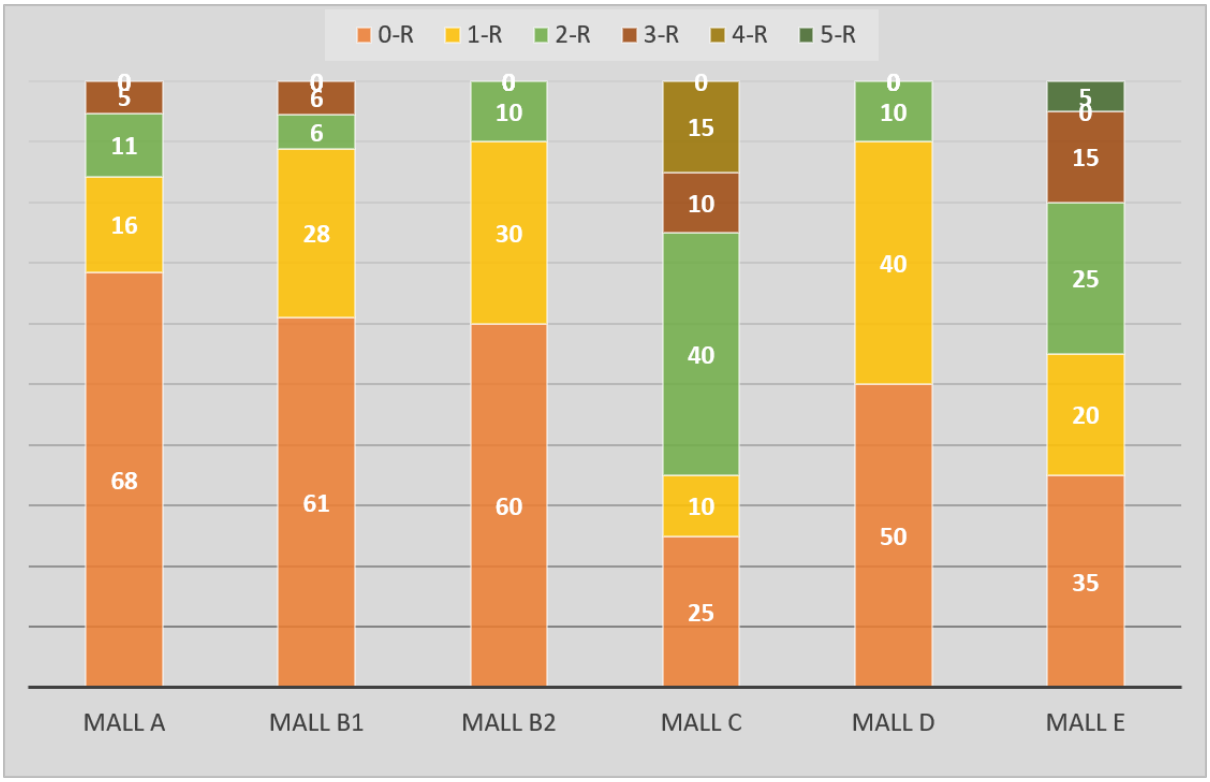

Figure 4. Percent of colonies not resistant (0-R) and resistant to 1 (1-R) or more (2-R, 3-R, 4-R, 5-R) antibiotics.

Each mall showed a different pattern of resistance to antibiotics. Mall A showed the highest E. coli resistance to norfloxacin (21%) (Figure 3) than to other antibiotics. In contrast, Mall C, B, and E all showed higher E. coli resistance to trimethoprim (25 and 28 for B1 and B2, 60 and 65% for Mall C and Mall E) (Figure 3) than to other antibiotics. Mall D showed the highest chloramphenicol resistance and neither norfloxacin nor amikacin. At mall B, the resistance to trimethoprim was higher (25 and 28%), and the second highest was to amoxicillin-clavulanate (17%) (Figure 3).

Mall C showed the highest number of resistant isolates (75%), followed by Mall E (65%) (Figure 3). Both malls are big. The lowest one was Mall A, although it was a big mall as well, but it was not as busy as the other two malls at the time of sampling. We can also see that Mall C and E also had higher E. coli isolates resistant to two antibiotics. In Mall E, we also found one isolate that was resistant to five antibiotics tested.

Based on our discussions above, we know that most E. coli were resistant to trimethoprim at 65% in Mall B and 60% (Figure 3) in Mall E. In comparison, we observed the highest resistance to amoxicillin-clavulanate (55%) at Mall E. These results also showed that these antibiotics may no longer be adequate for E. coli.

Differences in the distribution pattern of antibiotic resistance in each mall can reflect the condition of visitors to each mall. Environmental sanitation conditions and practices of

antibiotic use can affect the resistance of E. coli to antibiotics. In addition, these results may also show antibiotic use, hygiene and sanitation conditions of the population in different areas of Makassar. According to the Central Bureau of Statistics (BPS) of Makassar City, the mall with the most visitors in Makassar in 2023 was Mall D, which also showed the highest percentage of E. coli colonies, and 50% of the colonies were resistant. These results showed that a crowded mall has a more significant potential for spreading E.coli from one individual to another.

3. Conclusion

Based on the level of bacterial resistance to the five antibiotics used, it is possible to observe the distribution of E. coli in Makassar city malls. One of the largest malls in Makassar, Mall E, which is always crowded with visitors, seems to be a hotspot for the spread of resistant E. coli.

References

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