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# The Antibiotic resistance of lactic acid bacteria isolated from kefir made from Etawah goat milk

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**Abstract.** The objectives of the study were to determine the antibiotic resistance of Lactic Acid Bacteria (LAB) isolated from kefir made from Etawah goat milk. A total of 25 isolates were isolated from etawah goat milk, yogurt and kefir. LAB isolates were resistant to Ceftazidime (Caz, 100%), Ceftriaxone (Cro, 100%), Clindamycin (Da, 100%), Lincomycin (Li, 100%), Rifampicin (Rd, 100%) and Tobramycin (Tob, 100%). The bacteria were susceptible to Sulphamethoxazole-Trimethoprim (Sxt, 60%) and Tetracycline (Te, 40%). The percentage of antibiotic resistance of LAB was found to be in the range between 40 to 100%. The presence of antibiotic resistance in good bacteria may introduce the horizontal genetic transfer to bad bacteria which has the potential to disrupt human health.

## 1. Introduction

The COVID-19 pandemic that has hit the world since 2020 until now has killed more than 4 million people with the number of positive cases of COVID 19 reaching more than 200 million people [1]. The strategy used to reduce cases is with 3M, namely wearing masks, maintaining distance and washing hands [2]. Preventive efforts that are currently being intensively carried out are the provision of vaccines for the entire human population on earth to be able to bring up herb immunity [3]. Another effort made to increase immunity is by consuming foods that contain probiotics [4][5].

Various studies have examined the possibility of giving probiotics to prevent and reduce the risk caused by COVID 19. This happens because probiotics contain good live microorganisms so that they are able to fight bad microorganisms that live in the human digestive area. The competition between the good and bad microorganisms may resulted the reduction in the population of bad microorganisms. This is expected to increase human immunity and reduce the risk of COVID 19 [2, 4, 5].

However, there is another concern that arises in the natural conditions of probiotics, namely the existence of resistance to antibiotics. This may be due to the over-administration of antibiotics in animals [6]. The dairy products are a good natural medium for the growth of probiotics. This has an impact on various products produced from milk processing. Antibiotics carried in the animal's metabolic system will affect the genetic properties of the probiotics that live in it [6]. These resistance properties can be transmitted vertically or horizontally between microorganisms [7][8][9][10]. If these properties are transmitted to bad microorganisms, it will cause big problems for healing a disease in humans [11]. Etawah goat's milk and various processed products are products that are known to contain high nutrition and can increase human immunity and can cure various digestive diseases in humans [12]. One of the processed goat milk products is kefir [13]. Kefir is a fermented product that contains probiotics with a very large variety of microorganisms such as lactic acid bacteria [14]. The concern that arises is the



existence of resistance properties in lactic acid bacteria which can be transferred to bad microorganisms such as pathogenic bacteria and viruses [6]. Until now there have been limited studies that provide information about the nature of antibiotic resistance in kefir made from Etawah goat milk. Therefore, it is necessary to do this research. The objectives of the study were to determine the antibiotic resistance of Lactic Acid Bacteria (LAB) isolated from kefir made from Etawah goat milk

## 2. Material and methods

### 2.1. Sample

A total of 25 samples of Lactic acid bacteria sp. isolated from kefir made from etawah goat milk were obtained from 7 local markets in Jember and Surabaya (May 2020 to October 2021).

### 2.2. Disc diffusion assay

By using disc diffusion assay, the antimicrobial activity was measured as the method of Bauer [15][16] for Ceftazidime (10 µg), Clindamycin (2 µg), Ceftriaxone (5 µg), Lincomycin (2 µg), Rifampicin (30 µg), Spectinomycin (10 µg), Sulphamethoxazole-Trimethoprim 19:1 (25 µg), Tetracycline (30 µg). The antibiotics discs were obtained from Oxoid (UK). Fresh cultures were prepared in Luria Broth medium (LB, Himedia, India) which were incubated in a shaker incubator for 16 h at 37 °C. Prior to the observation of the antibiotic resistance test, the turbidity of the diluted culture was equivalent to 0.5 on the McFarland scale. For disc diffusion observations, cultures were grown on Mueller Hinton Agar (Oxoid, England) media by swabbing method. The discs of antibiotics were placed on top of the culture. This observation was made after the sample was incubated at 37°C for 18-24 hours and repeated for 3 times. The inhibition zone measurement is expressed in millimeters. These experiments use negative control by dripping 15 µL of 10% DMSO onto sterile disc paper. Penicillin G (10 units/ml) was used as a positive control.

### 2.3. Statistical Analysis

The antimicrobial activity of antibiotics to inhibit the growth of lactic acid bacteria was carried out by using one way ANOVA at a significant level of  $P < 0.05$ .

## 3. Result and Discussion

The antibiotic resistance among lactic acid bacteria isolated from kefir was resistant to Ceftazidime, Ceftriaxone (25/25), Clindamycin (25/25), Lincomycin (25/25), Rifampicin (25/25) and Tobramycin (25/25). Some isolates were susceptible to Sulphamethoxazole-Trimethoprim (15/25) and Tetracycline (10/25)

Ceftazidime and ceftriaxone interferes with inhibition of bacterial cell wall synthesis [17]. In other antimicrobials, this study found that most of *Lactic acid bacteria* isolates were resistant to Lincomycin which inhibit protein synthesis that used for cell structures or ribosomes. This is not surprising that lincomycin might be used heavily in Etawah goat farming system. Sharma *et al.* [18] revealed that clindamycin was used to treat the respiratory tract infections in goat.

Up to 60% of *Lactic acid bacteria* isolated from kefir made from Etawah goat milk were resistant to tetracycline. Acquired tetracycline resistance was attributed to the extensive use of antibiotics in etawah goat farming in some countries to treat the disease [18]. All isolates from kefir made from etawah goat milk were resistant to rifampicin. The action of rifampicin was binding to the specific site on the DNA-dependent RNA polymerase [19]. Other study revealed that the resistance to this antimicrobial was caused by mutation on the  $\beta$  subunit of RNA polymerase [20]. Therefore, the RNA chain initiation and elongation were inhibited [19] and the isolates could decrease the affinity of rifampicin [21].

In this study, the majority of LAB isolated from kefir were susceptible to sulphamethoxazole-trimethoprim (Table 1). These may be initiated by the absence of genes in the chromosomes and plasmids. Therefore, it becomes an option to avoid this antibiotic as a cure for a disease to allow lactic acid bacteria to grow optimally. However these bacteria were resistant to Ceftazidime, Ceftriaxone, Clindamycin, Lincomycin, Rifampicin, Tobramycin and may increase the risk of horizontal transfer gene to pathogenic bacteria.

**Table 1.** Number (%) of *Lactic Acid Bacteria* (LAB) isolated from kefir made from Etawah goat milk resistant to antibiotics

Antibacterial agents	Total isolates n = 25
Ceftazidime (Caz)	25 (100%) <sup>a</sup>
Ceftriaxone (Cro)	25 (100%) <sup>a</sup>
Clindamycin (Da)	25 (100%) <sup>a</sup>
Lincomycin (Lin)	25 (100%) <sup>a</sup>
Rifampicin (Rd)	25 (100%) <sup>a</sup>
Sulphamethoxazole-Trimethoprim (Sxt)	10 (40%) <sup>c</sup>
Tetracycline (Te)	15 (60%) <sup>b</sup>
Tobramycin (Tob)	25 (100%) <sup>a</sup>

Note : <sup>a,b</sup> = different alphabet means significant difference at P<0.05 in the same column

#### 4. Conclusion

The present work highlights on antibiotic resistance of Lactic acid bacteria sp. in kefir made from Etawah goat milk which was resistant to Ceftazidime, Ceftriaxone, Clindamycin, Lincomycin, Rifampicin, Tobramycin and susceptible to Sulphamethoxazole-Trimethoprim and Tetracycline. The antibiotic resistance percentages ranged from 40 to 100%.

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