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Antibiotic Sensitivity Pattern of *Staphylococcus aureus* and *Escherichia coli* Isolated from Bovine Fresh Milk

(*POLA SENSITIVITAS ANTIBIOTIK TERHADAP STAPHYLOCOCCUS AUREUS DAN ESCHERICHIA COLI YANG DIISOLASI DARI SUSU SAPI SEGAR*)

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ABSTRACT

The aims of this study were to determine the sensitivity of *S.aureus* and *E. coli* isolated from fresh milk against against several antibiotics and to determine the safety of the milk for human comsumsion. Milk was collected from milking diary cow and was used for the bacterial isolation. *E. coli* were were identified using Total Plate Count (TPC), Gram staining, their growth on Endo Agar and Eosin MethyleneBlue Agar, Biochemical analysis including glucose, lactose, sucrose,maltose, and sorbitol would be followed by Sorbitol Mac Conkey Agar Test for the identification of *E.coli*O157:H7. The isolation and identification of *S.aureus* were performed using Gram stain, TPC, growth on Baird Parker Agar and Mannitol Salt Agar. *S. aureus* and *S. epidermidis* were differentiated by coagulase and catalase tests. The antibiotic sensitivity tests for both *S. aureus* and *E.coli* were carried out using the following antibiotics: ampicillin, bacitracin, vancomycin, cefoperazone, ceftriaxone, cefotamine, cefuroxime, cefepime, cefazoline, ceftazidime, chloramphenicol, tetracycline, doxycycline, amikacin, kanamycin, neomycin, ertapenem, meropenem, imipenem, erythromycin, gentamycin, nalidixic acid, ciprofloxacin, levofloxacine, norfloxacine, ofloxacin, and novobiocin. Fresh milk obtained from the farm was positive for *S.aureus* and *E.coli* and resistant to most antibiotics tested. The best antibiotics for *S. aureus* were imipenem (54.1 mm), ampicillin (42.3 mm), cefazolin (41.6 mm), doxycycline (41.15 mm), and for *E.coli* were Imipenem (30.1 mm), ertapenem (29.5 mm), and meropenem (25.35 mm). The bovine fresh milk examined was contaminated by *S.aureus* and *E.coli* and to some extent, were also resistant to most antibiotics tested.

Keywords: antibiotics, sensitivity, *E.coli*, *S.aureus*

ABSTRAK

Tujuan penelitian ini untuk menentukan sensitivitas beberapa antibiotik terhadap bakteri *Staphylococcus aureus* dan *Escherichia coli* yang diisolasi dari susu sapi,dan juga mengidentifikasi keamanan susu sapi tersebut. Bakteri*S. aureus* dan *E. coli* umumnya sebagai penyebab dari *food borne diseases*.Bakteri *S. aureus* dan *E. coli* merupakan bakteri yang umum mencemari susu. Dalam mengisolasi dan mengidentifikasi *S.aureus* dan *E.coli,* sampel diambil dari peternakan sapi. Sampel untuk isolasi dan identifikasi diambil sebanyak 50 mL per sampel sapi.Uji yang dilakukan untuk identifikasi *E.coli* adalah *Total Plate Count* (TPC),pewarnaan Gram dan pertumbuhan pada *Endo Agar* dan *Eosin Methylene Blue Agar*, uji biokimia dilakukan terhadap glukosa, laktosa, sukrosa, maltosa, dan sorbitol. Uji kemudian dilanjutkan dengan pertumbuhan bakteri pada media *Mac Conkey Agar* untuk identifikasi terhadap *E.coli*. Identifikasi terhadap *S.aureus* dengan pengujian pewarnaan Gram, TPC, pertumbuhan dalam *Baird Parker Agar* dan *Mannitol Salt Agar*. Bakteri *S. aureus* dan *S.epidermidis* dibedakan dengan uji koagulase dan uji katalase. Uji sensitivitas antibiotik untuk *S. aureus* dan *E.coli* dilakukan dengan menggunakan antibiotik: *ampicillin, bacitracin, vancomycin, cefoperazone, ceftriaxone, cefotamine, cefuroxime, cefepime, cefazoline, ceftazidime, chloramphenicol, tetracycline, doxycycline, amikacin, kanamycin, neomycin, ertapenem, meropenem, imipenem, erythromycin, gentamycin, nalidixic acid, ciprofloxacin, levofloxacine, norfloxacine, ofloxacin, dannovobiocin*. Susu yang diambil dari peternakan sapi ternyata positif terhadap *S.aureus* dan *E.coli* kedua bakteri tersebut resisten terhadap uji sensitivitas antibiotik. Antibiotik yang terbaik untuk *S. aureus* adalah *imipenem* (54,1 mm), *ampicillin* (42,3 mm), *cefazolin* (41,6 mm), *doxycycline* (41,15 mm), dan untuk *E.coli* adalah *imipenem* (30,1 mm),*ertapenem* (29,5 mm), dan *meropenem* (25,35 mm). Susu sapi yang diuji ternyata positif terhadap *S.aureus* dan *E.coli* dan resisten terhadap hampir semua antibiotik uji.

Kata-kata kunci: antibiotik, sensitivitas, *E.coli*, *S.aureus*

INTRODUCTION

Milk is a kind of food with complete nutrition. It contains subtances which are needed for the body such as protein, glucose, lipids, minerals, and vitamins. However milk not only has the complete nutrient but also good medium for bacterial growth. Milk can be contaminated either by bacteria in the farm or in the processing (Farzana *et al.* 2004; Kagkli *et al.* 2007).

Recent years to bloom both print and electronic mass media about bacterial contamination of food pathogens issues, which invites the reaction abroad about contamination.Unpasteurized milk is the source of transmission of *Escherichia coli* and *Staphylococcus aureus* to humans. *Escherichia coli* contamination has been documented in Europe (Sharp, 1987; *Sospedra et al*., 2009; Turner*et al.,* 2011) and America (Sharp, 1987).

Another bacterium, *S.aureus*, has been known for decades as a bacterium that contaminated milk and causes food poisoning (Farzana*et al.* 2004). *Staphylococcus aureus*, a ubiquitous human pathogen and a common cause of invasive life threatening infections, is the most common cause of community-associated cellulitis (Brook and Frazier, 1995; Diekema *et al.,* 2001) and endocarditis (Hoen *et al.,* 2002). and urinary track disturbance (Onanuga, 2012). In regards to these pathogenic bacteria, it is important to identify whether the milk consumed by people in South Sulawesi is contaminated by these bacteria and whether these bacteria, once identified, are still sensitive to antibiotics or not. The emergence of antibiotic-resistant bacteria poses a severe challenge to both veterinary and health professions because they have negative impact on therapy (Daka *et al*., 2012). Based on these issues we conducted a study to determine the presence of *E. coli* and *S. aureus* in the milk consumed by the people of South Sulawesi. Once these bacteria are detected, we performed antibiotics sensitivity testing to identify the susceptibility status of these bacteria. By knowing the susceptibility status of these bacteria, we will be able to provide more information to the public regarding the hazard likelihood that will be present when the milk is consumed.

RESEARCH METHODS

The study was conducted at the Medical Microbiology Laboratory, Department of Microbiology, Faculty of Medicine, Hasanuddin University, Makassar, South Sulawesi, Indonesia. A cross-sectional study design was used to determine the bacteriological analysis of cow milk in four farms located in Sinjai district (South Sulawesi, Indonesia) during 2012.

Collection of Samples

A total of 60 milk samples (250 mL each sample) were collected aseptically in sterilised glass bottles with ice pack. All milk samples were immediately transported to the Microbiology Laboratory of Hasanuddin University for analysis. Upon arrival in the Laboratory, samples were immediately analysed. The serial decimal dilutions of these milk samples were then prepared in 0.1% peptone, which was supplemented with 0.05% (w/v) of Tween-80 and 0.1% (w/v) $MgCl_2.6H_2O$ (Lachica, 1984).These dilutions were prepared in duplication and then transferred to Plate Count Agar (Oxoid, UK), Baird Parker Agar (Oxoid, UK), and Mac ConkeyAgar (Oxoid, UK). All plates were incubated at 37°C for 48 hours.

Morphological and Biochemical Characterization of the Isolates

Identification, morphological, and biochemical characterization of bacterial strains were tested in the laboratory. The presence of colonies was confirmed by several tests such as Gram staining (Tortora *et al.,* 1995), the catalase and coagulase tests (Pelczar*et al.,* 1999), Eosin Methylene Blue agar (Oxoid, UK), Mannitol Salt Agar (Oxoid, UK), and MacConkey Agar (Oxoid, UK).The plates were incubated aerobically at 37°C for 18-24 hours. Positive colonies from each plate were further purified by sub-culturing onto specific mediums and were retained for further identification.

Gram-positive cocci occurred in clusters under the microscope were subjected to preliminary biochemical tests (the catalase and oxidasetests). The identities of the isolates were confirmed based on positive results for Indole, Methyl Red,Voges Proskauer, Triple Sugar Iron Agar, Citrate and Carbohydrate Metabolism Test.

Antibiotic Susceptibility Test

Antibiotic susceptibility tests were performed on all isolates to determine their antibiotic-resistance profiles. Fresh overnight cultures were prepared and used for antibiotic sensitivity tests. An aliquot (100µL) of each isolate suspension equivalent to a 0.5% Mc

Farland Standard was spread plated on Mueller Hinton Agar (Oxoid, UK). Susceptibilities of the isolates to a panel of several different antibiotic discs (Oxoid, UK) were determined. Antibiotic discs were gently pressed onto the inoculated Mueller Hinton Agar to ensure intimate contact with the surface and the plates were incubated aerobically at 37°C for 18-24 h (NCCLS, 2004). Inhibition zone diameters measured and values obtained from the National Committee on Clinical Laboratory Standards (2004) were used to interpret the results obtained. Bacterial isolates were then classified as resistant, intermediate resistant or susceptible to a particular antibiotic.

RESULTS AND DISCUSSION

A total of 60 samples,pasteurised (P) and non-pasteurised(NP)milk (36) samples, the presence of *S.aureus* (2P and 2 NP) and *E.coli* (8P and 6 NP) was biochemically identified. These isolates were subjected to antibiotic sensitivity tests. Twenty seven agents, from different antibiotic classes were used. Some were selected because several studies have shown an increasing trends of bacterial resistance against these antibiotics (Ateba and Bezuidenhout, 2008; Moneoang and Bezuidenhout, 2009). Antibiotics of veterinary and human health relevance were also considered. A summary of the percentage of *S. aureus* and *E. coli* that were resistant to these antibiotics is provided in Table 1.

Table 1. Antibiotic resistance profiles of *S. aureus* and *E.coli* isolated from milk originating from Sinjai district, South Sulawesi.

No.	Antibiotics	Mechanisms	Zone Inhibition Against Pathogen (mm)	
			S.aureus	*E.coli*
1.	Ampicillin	Inhibit bacterial cell wall synthesis	42.3 (S)	0 (R)
2.	Oxacilline	Inhibit bacterial cell wall synthesis	20.02 (S)	0 (R)
3.	Cefepime	Inhibit bacterial cell wall synthesis	26.85 (S)	8.8 (R)
4.	Cefotaxime	Inhibit bacterial cell wall synthesis	28.6 (S)	0 (R)
5.	Cefoperazone	Inhibit bacterial cell wall synthesis	33.35 (S)	5.5 (R)
6.	Ceftriaxone	Inhibit bacterial cell wall synthesis	15 (I)	15.3 (I)
7.	Ceftazidime	Inhibit bacterial cell wall synthesis	26.3 (S)	0 (R)
8.	Cefazolin	Inhibit bacterial cell wall synthesis	41.6 (S)	0 (R)
9.	Bacitracin	Inhibit bacterial cell wall synthesis	23.8 (S)	0 (R)
10.	Ertapenem	Inhibit bacterial cell wall synthesis	28.9 (S)	29.5 (S)
11.	Meropenem	Inhibit bacterial cell wall synthesis	33.25 (S)	25.35 (S)
12.	Imipenem	Inhibit bacterial cell wall synthesis	54.1 (S)	30.1 (S)
13.	Vancomycin	Inhibit bacterial cell wall synthesis	18.6 (S)	0 (R)
14.	Amikacin	Inhibit bacterial protein synthesis	26.8 (S)	13.7 (R)
15.	Erythromycin	Inhibit bacterial protein synthesis	30.07 (S)	0 (R)
16.	Gentamicin	Inhibit bacterial protein synthesis	21 (S)	11.4 (R)
17.	Kanamycin	Inhibit bacterial protein synthesis	27.8 (S)	20.15 (S)
18.	Neomycin	Inhibit bacterial protein synthesis	21.7 (S)	7.5 (R)
19.	Tetracycline	Inhibit bacterial protein synthesis	34.55 (S)	0 (R)
20.	Doxycycline	Inhibit bacterial protein synthesis	41.15 (S)	5 (R)
21.	Chloramphenicol	Inhibit bacterial protein synthesis	32.05 (S)	24.3 (S)
22.	Nalidixic Acid	Inhibit bacterial nucleic acid synthesis	18.9 (S)	0 (R)
23.	Ciprofloxacin	Inhibit bacterial nucleic acid synthesis	34.8 (S)	0 (R)
24.	Levofloxacine	Inhibit bacterial nucleic acid synthesis	32.6 (S)	0 (R)
25.	Norfloxacine	Inhibit bacterial nucleic acid synthesis	32.8 (S)	0 (R)
26.	Ofloxacine	Inhibit bacterial nucleic acid synthesis	30.5 (S)	0 (R)
27.	Novobiocin	Inhibit bacterial nucleic acid synthesis	42.3 (S)	0 (R)

I = Intermediate; R = Resistant; S = Sensitive

Fresh milk obtained from the farm was positive for *S.aureus* and *E.coli* and both bacteria were resistant to most antibiotics tested. According to the results of antibiotic sensitivity testing, *S.aureus* isolate was susceptible to all antibiotics tested but ceftriaxone. On the other hand, *E.coli* isolate, obtained from bovine milk,was found to be resistant to 21 out of 29 antibiotics tested and moderately susceptible to ceftriaxone (Table 1). Those 21 antibiotics were fallen under three main mechanisms; interrupting the bacterial cell wall formation, inhibiting bacterial protein synthesis, and inhibiting bacterial nucleic acid synthesis.The best antibiotics for *S. aureus* were *imipenem* (54.1 mm), *ampicillin* (42.3 mm), *cefazolin* (41.6 mm), *doxycycline* (41.15 mm), and for *E.coli* were *imipenem* (30.1 mm), *ertapenem* (29.5 mm), and *meropenem* (25.35 mm).

In Indonesia, other developing countries, epizootics of bacterial diseases occur frequently in poultry farms. In the present study, we described the isolation and antibiotic susceptibility pattern of *S.aureus* and *E.coli* from milk obtained from healthy animals. Our results indicate that these bacteria were present and positively identified in the milk samples.

Twenty nine antibiotics were used in the antibiotics susceptibility testing of this study. In regards to their mechanism of action, these antibiotics were divided intothree main classes; inhibitor of bacterial cell wall synthesis, inhibitor of bacterial protein synthesis, and inhibitor of nucleic acid synthesis (Table 1). Most antibiotics used in this study were classified as the inhibitor of bacterial cell wall synthesis which is including penicillin, cephalosporin, and carbapenems group.

According to Garrod *et al.* (1981), *penicillin* is still an effective antibiotic against *S.aureus*. In this study we found that *S.aureus* isolate is susceptible to all β-lactam antibiotic of the penicillin class. It is in agreement with Garrod *et al.* (1981)despite of the fact that some strains of *S.aureus* that is resistant to penicillin-derived antibiotics have been documented elsewhere (Crago *et al.*,2012*;* Sampimon *et al*., 2011; Tavakol *et al.,* 2012).

In addition, it has also been identified that *S.aureus* isolated in this study was susceptible to all antibiotics but ceftriaxone. We found that erythromycin and tetracycline were effective against *S.aureus*, in agreement with the result of Hassan *et al.* (1978), and the *S. aureus* isolate was susceptible to all quinolones-antibiotics. This has implied that the *S.aureus* isolate has no mutation in genes responsible to the expression of DNA gyrase and topoisomerase IV.

Among all 27 antibiotics were used, a case of reduced-susceptibility of *S.aureus* against ceftriaxone was identified. It is an interesting finding considering that the *S.aureus* isolated in this study was susceptible to other cephalosphorins. It is tempting to speculate that the identification of beta-lactamases from this isolate will help to reveal the mechanisms by which this bacterium have a reduced susceptibility to ceftriaxone.

In contrast to *S.aureus* isolate, *E.coli* isolate obtained in this study illustrates a high degree of resistance against several main antibiotics. Twenty one out of 29 antibiotics that were found to be ineffective in killing and/or inhibiting the growth of *E.coli* isolate are fallen under cephalosphorins, aminoglycosides, tetracyclines, and quinolones (and derivatives) antibiotics. In agreement with our finding, White *et al*. (2002) mentioned in their review that multi-drug resistant strain of *E.coli*, type O157:H7, has been documented in some areas. However, based on our biochemical test, the *E.coli* isolate that we obtained from the milk was not O157:H7 type due to the presence of negative result in sorbitol Mac Conkey agar test.

CONCLUSION

The bovine fresh milk examined was contaminated by *S.aureus*and *E.coli* and to some extent, were resistant to most antibiotics tested.*S. aureus*and *E.coli*is normally resident in humans. Therefore, the presence of these bacteria in the bovine's milk may have resulted from transmission from humans, which raises questions regarding the hygiene practices followed.

SUGGESTION

The presence of E-coli isolates and S.aureus isolates in the milk suggests that the milk must be sterilized before being consumed. It also highlights the importance of maintaining good management of the dairy farm and cattle health checks continuosly.

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