PROCEEDINGS

STSS 2008

BROADENING HORIZONS THROUGH RESEARCH

3 - 4 June 2008

M.S. Garden Hotel
Kuantan, Pahang

SCIENCE & TECHNOLOGY

PROCEEDINGS OF THE NATIONAL SEMINAR ON SCIENCE, TECHNOLOGY AND SOCIAL SCIENCES 2008

Science and Technology : Volume 1

© Proceedings of the National Seminar on Science, Technology and Social Sciences 2008 (ISBN : 978-983-2607-17-5) is published by the Universiti Teknologi, MARA, Pahang.

Cover Design by:
NurMazwan Ramli
Unit Perkhidmatan & Teknologi Maklumat (UPTM)
Universiti Teknologi MARA

Antimicrobial Resistance of *Staphylococcus aureus* in Dairy Products from Kedah

S. Sasidharan
B. Prema

ABSTRACT

This research focused on the isolation and characterization of S. aureus from dairy products using conventional methods and also on their antimicrobial resistance pattern. A total of 50 dairy samples was used for the isolation of the S. aureus before subjected to an antibiotic susceptibility test. Of 50 samples examined, 5 (10 %) were contaminated with S. aureus. Sample 29 showed resistance to methicillin and vancomycin. Sample 18 showed intermediate response to tetracycline. The other samples were susceptible to all the antibiotics tested. Broth microdilution test was conducted on all the 5 samples using ampicillin and tetracycline to measure the Minimum Inhibitory Concentration (MIC). Samples 2, 10, 14, 18 and 29 that were tested with tetracycline showed MIC of 25.00 µg/mL, 6.25 µg/mL, 6.25 µg/mL, 3.13 µg/mL and 12.50 µg/mL respectively. The understanding of antimicrobial resistance of S. aureus enables the development of preventive strategies to limit the existing resistance and to avoid the emergence of new strains of resistant S. aureus. This calls for the better control of sources of food contamination.

Keywords: *Antimicrobial drug resistance, milk, Staphylococcus aureus*

Introduction

Staphylococcus aureus is a versatile pathogen in humans and animals which is responsible for a diverse spectrum of diseases ranging from minor skin infections to life threatening diseases such as pneumonia and meningitis (Lowy, 1998). It is also known as one of the most important agents of food poisoning globally (Genigeorgis, 1989). It has been believed that given a vast array of effective antimicrobial agents, all bacterial infections were treatable. However, the emergence of resistance to multiple antibiotics among *Staphylococcus aureus* has created breaking news for health practitioners and researchers (Swartz, 1997). It has been reported that shortly after the introduction of penicillin in 1940s, resistance developed in *S. aureus* followed by resistance to methicillin and more recently to glycopeptides such as vancomycin (Monroe & Polk, 2000).

The objective of this study was to isolate *Staphylococcus aureus* from dairy products which includes fresh cow milk, pasteurized milk, goat milk, yogurt and cheese, to characterize *S. aureus* using conventional methods like Gram staining, coagulase test and catalase test and also to determine the prevalence of antimicrobial resistance among *S. aureus* isolated from dairy products.

Material and Methods

Study Population and Sample Collection

A total of 50 dairy samples (12 fresh cow milk, 13 pasteurized milk, 13 goat milk, 6 yogurt and 6 cheese) from various places in Kedah, Malaysia were analyzed from September 2007 to November 2007. The samples were collected at dairy farms, groceries and hyper markets. The samples were refrigerated at 4 °C before subjected to microbiological analyses.

Bacteriological Examinations

Isolation and identification of *Staph. aureus* were performed according to the National Mastitis Council (1992) recommendations on examination of quarter-milk samples. Briefly, immediately after delivery, the milk samples were inoculated on blood agar plates (Difco, Detroit, MI) and followed by on mannitol salt agar (Difco, USA) plates, which were divided into 4 sections. A 10-μL loop was used to streak the milk sample, and 6 to 8 lines were made in one agar section. Samples were incubated for 24 to 72 h at 37°C and examined for bacterial growth. Pure cultures were further examined for morphological (convex elevation and smooth margin), staining, and cultural characteristics, and for biochemical reactions according to standard keys. Staphylococci were studied in particular for hemolysis and coagulase production. Coagulase testing was performed according to a tube method using oxalated rabbit plasma in a 1:10 dilution in a nutrient broth (Arbeit, 1988). Only typical colonies identified as *Staph. aureus* were stored in cryogenic vials containing 1 mL of trypticase soy broth containing 15% glycerin at −80°C.

Antibiotic Susceptibility Testing

Before antibiotic susceptibility testing (NCCLS, 2002), the isolates were revived by subculturing on blood agar base (Difco) at 35°C for 24 h. The isolates were tested for their susceptibility to 5 different antimicrobial drugs: methicillin (Met), vancomycin (Van), kanamycin (Kan), chloramphenicol (Chl) and tetracycline (Tet). The antibiotic disks (Oxoid, Amsterdam, The Netherlands) were gently pressed to ensure contact with the inoculated Mueller-Hinton agar surface, and the plates were incubated at 35°C. The plates were examined after 18 h and the zones of inhibition were measured to the nearest millimeter. The interpretive breakpoints for resistance were determined according to the standard table provided by the manufacturer of the antibiotic disks, and the isolates were reported as susceptible, intermediate, or resistant. For quality control, *Staph. aureus* ATCC 25923 was used as control strain. It should be pointed out that the interpretive breakpoints used were originally developed for human infections.

Results

A total of 50 milk samples from various sources were cultured for *S. aureus*; 24.0% of the samples tested were from fresh cow milk samples, 26.0% were from pasteurized milk samples, 26.0% were from goat milk samples, 12.0% were from yogurt samples and 12.0% were from cheese samples. *S. aureus* was isolated from a total of 5 (10.0%) of the 50 samples (Table 1). From all the 50 samples, 35 samples had growth on tryptic blood agar. When those colonies were subcultured, 21 samples had growth on the mannitol salt agar which is a selective media for *Staphylococcus aureus*. When those colonies subjected to biochemical characterization which involves Gram staining, hemolysis coagulase and catalase test, only 5 samples showed positive result for all the three tests. The samples that were positive for *S. aureus* include 4 fresh cow milks and 1 pasteurized milk (Table 1).

Overall, 6.0% of the *S. aureus* were susceptible to all of the antimicrobial agents tested. All the *S. aureus* isolates from sample 2, 10 and 14 were susceptible to methicillin, vancomycin, kanamycin, chloramphenicol and tetracycline. Of the 5 *S. aureus* isolates, isolate from sample 29 was resistant to 2 antimicrobials drugs (methicillin and vancomycin) and isolate from sample 18 showed intermediate response to tetracycline (Figure 1) .

Table 1. Occurrence Of *Staphylococcus aureus* In Dairy Products

Source	Isolate	Number of samples positive for	
	No	%	*Staphylococcus aureus*
Fresh cow milk	12	24	4
Pasteurized milk	13	26	1
Goat milk	13	26	-
Yogurt	6	12	-
Cheese	6	12	-
Total	50	100	5 (10 %)

- Negative

Discussion

Food borne diseases are an important public health problem as its does not only affects human health, but also has a significant impact on economic and trade issues. The global changes affecting population growth, lifestyle, international food trade, food production and processing, agricultural and animal husbandry practices and antimicrobial resistance have posed a threat to the emergence of food borne diseases. As most of the food borne diseases is not reported, the true dimension of the problem is unknown (FAO/WHO, 1997).

In Malaysia the incidence of notifiable food borne diseases, namely cholera, typhoid, food poisoning, hepatitis A and dysentery is less than 5/100,000 population, sporadic in nature and outbreaks are confined to certain areas only (FAO/WHO, 1997). The increased incidence of food borne diseases with widespread outbreaks, the emergence of new food borne pathogens and the development of antimicrobial resistance can be regarded as threats to the food safety.

Susceptible populations of bacteria may become resistant to antimicrobial agents through mutation and selection or by the acquisition of new genetic material from other resistant organisms through transformation, transduction and conjugation. In sample 29 (Table 3.3), *S. aureus* has become resistant to multiple classes of antibiotics (methicillin and vancomycin) which can cause serious health problems (Tenover, 2006). However, the assessment of cheeses and yogurt indicated that they did not contain satisfactory levels of *S. aureus* at the time of consumption. *Staphylococcal* food poisoning due to cheese and yogurt has not been reported to any great extent to the authorities (Lindqvist et al., 2001).

Surveillance of food borne diseases is very important to reduce the number of *Staphylococcus aureus* occurrences in any food, especially dairy products. An enhanced supervision provides valuable information in the estimation of the burden of food borne diseases and in the rapid detection and response to outbreaks. For it to be effective, surveillance of food borne diseases has to be integrated with food monitoring data along the entire food chain from farm to table, thus improving the ability to link the pathogen in food to the disease in humans (FAO/WHO, 1997).

References

Arbeit, D. (1988). Laboratory procedures for the epidemiological analysis of *Staphylococci*. In G. Archer & T. Crossley (Eds.), *Staphylococci and Staphylococci diseases*. New York: Churchill Livingstone.

FAO/WHO (Food and Agriculture Organization / World Health Organization). (1997). *Risk management and food safety. A report of Joint FAO/WHO Expert Consultation. FAO, Rome.*

Genigeorgis, C.A. (1989). Present state of knowledge on *Staphylococcal* intoxication. *Int J. Food Microbiol., 9*, 327-360.

Lindqvist, R., Andersson, Y., Lindbäck, J., Wegscheider, M., Eriksson, Y., Tideström, L., Lagerqvist-Widh, A., Hedlund, K.O., Löfdahl, S., Svensson, L. & Norinder, A. (2001). A one year study of foodborne illnesses in the municipality of Uppsala, Sweden. Emerg. *Infect. Dis., 7*, 588–592.

Lowy, F.D. (1998). *Staphylococcus aureus* infection. *N Engl J Med, 339*, 520- 532.

Monroe, S. & Polk, R. (2000). Antimicrobial use and bacterial resistance. *Curr. Opin. Microbiol., 3*, 496-501.

NCCLS (National Committe for Clinical Laboratory Standards). (2002). Performance standards for antimicrobial susceptibility testing. *12th Informational Supplement, Vol 21, No 1, Pennsylvania PA: NCCLS, M100-S12.*

National Mastitis Council. (1992). *Laboratory handbook on bovine mastitis*. Madison WI: National Mastitis Council, Inc.

Swartz, M.N. (1997). Use of antimicrobial agents and drug resistance. *N. Engl. J. Med., 337*, 491-492.

Tenover, F.C. (2006). Mechanisms of antimicrobial resistance in bacteria. *Am. J. Med., 119*, 3-10.

S. SASIDHARAN & B. PREMA, Department of Biotechnology, Faculty of Applied Sciences, AIMST University Malaysia. srisasidharan@yahoo.com