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การตรวจหาความสัมพันธ์ของเชื้อสแตฟฟิโลคอคัส ออเรียส ที่สร้างสารพิษในอาหารกับที่พบในผู้ประกอบอาหาร

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Study on the correlation between the enterotoxin-producing *Staphylococcus aureus* isolated from prepared food and the cooks

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บทคัดย่อ

เชื้อสแตฟฟิโลคอกคัส ออเรียส ได้รับการศึกษาอย่างกว้างขวาง เนื่องจากมีคุณสมบัติที่สามารถสร้างสารพิษ ซึ่งทนความร้อนได้ดีในอาหาร(1) และเนื่องจากอาหารสำเร็จรูปได้รับความนิยมในหมู่ประชาชนทั่วไป การศึกษาครั้งนี้จึงต้องการศึกษาอาหารที่วางขายในเขตจังหวัดขอนแก่น เพื่อตรวจหาสารพิษที่พบในอาหารและในจมูกและมือผู้ประกอบอาหารว่าเป็นสารพิษชนิดใดและมีความสัมพันธ์กันเพียงใด และอาหารประเภทใดมีอัตราการเสี่ยงต่อโรคอาหารเป็นพิษจากเชื้อนี้ได้มาก เพื่อเป็นข้อมูลทางระบาดวิทยาและเป็นแนวทางในการควบคุมโรคอาหารเป็นพิษได้ดียิ่งขึ้น

จากการศึกษาอาหารที่วางขายในจังหวัดขอนแก่นจำนวน 193 ตัวอย่าง ส่วนใหญ่พบว่าจำนวนแบคทีเรียทั้งหมดพบอยู่ระหว่าง 10^6 - 10^7 เซลล์ต่อกรัมอาหาร เชื้อ Staphylococcus พบอยู่ระหว่าง 10^5 - 10^6 เซลล์ต่อกรัมอาหาร เชื้อ *S.aureus* พบอยู่ระหว่าง 10^3 - 10^4 เซลล์ต่อกรัมอาหาร เชื้อ Staphylococcus coagulase positive พบอยู่ระหว่าง 10^2 - 10^4 เซลล์ต่อกรัมอาหาร

ตัวอย่างอาหารที่พบเชื้อสร้างสารพิษส่วนใหญ่พบว่าจำนวนแบคทีเรียทั้งหมด, จำนวนเชื้อ staphylococcus และ *S. aureus,* พบว่ามีปริมาณค่อนข้างสูง ยกเว้นเชื้อ Staphylococcus coagulase positive พบเชื้อสร้างสารพิษมากที่สุดเมื่อเชื้อจำนวน 10^2– 10^3 เซลล์ต่อกรัมอาหาร อาหารที่พบสารพิษและมีเชื้อ Staphylococcus coagulase positive มากกว่า 10^5 เซลล์ต่อกรัมอาหารพบอยู่ 4 ตัวอย่าง (11.76%) ของอาหารที่พบสารพิษหรือ 2.07% ของตัวอย่างอาหารทั้งหมด

จากจำนวนตัวอย่างอาหาร 193 ตัวอย่างพบอาหารที่มีเชื้อ Staphylococcus coagulase positive อยู่ 97 ตัวอย่าง (50.26%) พบอาหารที่มีเชื้อสร้างสารพิษอยู่ 34 ตัวอย่าง (17.62%) ชนิดของสารพิษที่พบมากที่สุดคือชนิด ABCD (25.64%) รองลงไปคือชนิด A (23.08%) ชนิด C (20.5%) ชนิด ABD (15.31%) ชนิด D,ACD (5.13%) และชนิด AD,BD (2.56%) ตามลำดับ อาหารพื้นเมืองอีสานประเภทแหนมและย่ำลาบ พบจำนวนตัวอย่างที่พบเชื้อสร้างสารพิษอยู่จำนวนมากและร้านอาหารที่พบเชื้อสร้างสารพิษในอาหารพบจำนวน 28 ร้านใน 98 ร้าน (28.57%)

จากการตรวจเชื้อจากจมูกและมือผู้ประกอบอาหารจำนวน 182 คน พบว่าจากจมูกพบเชื้อ Staphylococcus coagulase positive อยู่ 47.3% พบเชื้อที่สร้างสารพิษอยู่ 14.5 % ชนิดของสารพิษที่พบมากที่สุดคือ A (29.6%) และจากมือพบเชื้อ Staphylococcus coagulase positive อยู่ 18.7% พบเชื้อที่สร้างสารพิษอยู่ 4.4 % ชนิดของสารพิษที่พบมากที่สุดคือ A และ ABCD (25%) แต่ไม่พบความสัมพันธ์ของสารพิษจากจมูกและมือของผู้ประกอบอาหารคนเดียวกัน จากอาหารและมือ/หรือจากอาหารและจมูกของผู้ประกอบอาหารในร้านเดียวกันพบเชื้อที่สร้างสารพิษชนิดเดียวกันเพียง 2.04% จากผลการทดลองนี้ชี้ให้เห็นว่าการแพร่กระจายของเชื้อ *Staphylococcus aureus* ในอาหาร น่าจะมาจากปัจจัยอื่นมากกว่าที่จะมาจากผู้ประกอบอาหารโดยตรง ซึ่งอาจได้แก่วัตถุดิบตั้งแต่เริ่มต้น, ภาชนะที่บรรจุอาหาร โดยเฉพาะอาหารที่ปรุงไม่สุกหรือขบวนการขายที่ตั้งอาหารทิ้งไว้เป็นเวลานานโดยมิได้ปกปิด รวมถึงการส่งต่อของอาหาร สิ่งเหล่านี้อาจจะทำให้เชื้อจากผู้ซื้อหรือบุคคลอื่นลงไปรวมถึงเชื้อจากสิ่งแวดล้อมด้วย

Abstract

Staphylococcus aureus was widely studied because it is capable of producing heat-stable enterotoxin in the prepared food which is now popular in the general public (1). This study was designed to experimentally survey the prepared foods in Khon Kaen, to identify the toxins therein and microorganisms in the nasal cavities and hands of the cooks, and to find their correlations. The informations to obtain would be beneficial for the prevention of staphylococcal food poisoning and the management aspect of epidemiology.

Our experimental studies on 193 prepared food samples collected in Khon Kaen and on KKU campus revealed that the majority of samples contained 10^6 - 10^7 bacterial cells per gram of food. The staphylococci were $10^{5\text{-}106}$ cells/gm., *S. aureus* 10^3-10^4 cells/gm., and staphylococcus coagulase positive 10^2-10^4 cells/gm. of food.

The food samples containing the enterotoxin-producing *S. aureus* were such that the total bacteria, staphylococci, and *S. aureus* were quite high in number except the Staphylococcus coagulase positive bacteria where only 10^2 - 10^3 cells/gm. of food could be found the highest the enterotoxin-producing *S. aureus* .

Four food samples were found to have enterotoxins and staphylococcus coagulase postitive more than 10^5 cells/gm. of food (11.76% of enterotoxin-producing *S. aureus* food samples or 2.07% of the total food samples)

We have found 97 food samples containing staphylococcus coagulase positive bacteria out of 193 (50.26%). The food samples accommodating enterotoxin-producing *S. aureus* were 34 in number (17.62%). The most frequently found enterotoxin types were ABCD (25.64%); and A (23.08%), C(20.5%), ABD

(15.31%), D,ACD(5.13%), AD,BD(2.56%) were found in the less frequency in that order. The northeast of Thailand prepared foods (i,e. preserved raw pork-- nam, papaya salad-- som-tom, minced beff salad-- larb) were found to have predominated the number of positive samples. Twenty-eight out of 98 prepared food shops (28.57%) were found to be positive for the enterotoxin-producing *S. aureus*

The swab samples from 182 cooks indicated that the nasal cavity samples yielded 47.3% of the staphylococcus coagulase positive ; and there were 14.48% of them that produced the enterotoxin. The most prevalent toxin was type A (29.63%). The hand swab samples showed 18.7% of the staphylococcus coagulase positive and 4.4% out of this produced enterotoxins. The enterotoxins A and ABCD were most frequently indentified (25%). We have found no significant correlation of enterotoxins between the nasal cavity origin and the hand origin of the same cook. We can conclude from our experiment that the origin of this bacteria may be not from the cook, but rather from the raw material, from the environment while waiting for the buyers especially the food sample without the lids, and while delivering from the kitchen including the people involved.

Introduction

Staphylococcus aureus is one of the main causative agents for food poisoning in different parts of the globe (1). It is so because of its capability to produce 7 types of heat stable-enterotoxins namely A,B,C_1,C_2,C_3,D and E. They can cause food poisoning only 2-6 hrs. after ingestion (1-2).

The *S. aureus* epidemic broke out many times a year in Thailand. Eight out of 12 food poisoning outbreaks were reported in 1991. Regional observation indicates that the northeast of Thailand has the greatest incidence (3). It is interesting to study and find out what kind of enterotoxins are in various dishes. How much relation would there be between enterotoxins from the nose and hands of the cook with that found in prepared food? As is generally known, the nose, hand, wound, and skin are the most frequent sources of contamination (1). We planned to search for the answers to, 1) what kinds of prepared food are most likely to spread this bacteria? 2) what type of enterotoxins are found? These will be leading to the safer practices in the prevention of food poisoning.

Enterotoxin type A of *S. aureus* were found in most food samples studied elsewhere, the next less frequently found were type D, AD; type B,C, and E were found in very less frequently (4,5). In Thailand, Sumonta et al (6) studied enterotoxins of *S. aureus* from nine samples of food and the patients and identified enterotoxin type A 33.5% (food sold in the market and food known to cause food poisoning). The enterotoxin type D was found 27.2% (frozen meat and the strains isolated from the patients). This group of researchers reported the finding of *S. aureus* coagulase positive in food poisoning samples amounted to 4.0 x 105 - 4.55 x 105 cells/gm of food.

The survey of the *S. aureus* in the nasal cavities of the hospital cooks in Barzil revealed the enterotoxin types B,C,AE and ADE (8).

The purpose of this study would be to 1) isolate *S. aureus* in the food sample, Staphylococcus coagulase positive in the food samples, nasal cavities and hands of the cooks, 2) do the typing of enterotoxins, and 3) to find the relationships of enterotoxins and the risky food samples caused by this bacteria including the quantity of the restaurants possessing enterotoxin-producing *S. aureus* bacteria.

Materials and Methods

1. Selection of Prepared Food Venders for Sample Collection

The prepared food samples were fetched from the prepared food venders on KKU campus and in the city of Khon Kaen.

2. Type of Prepared Foods

The types of prepared food collected were curry, noodle soup, stir-fried, papaya salad, minced-beef half-cook salad (larb), preserved raw pork (nam), preserved raw pork with rice (nam-cook), bamboo-shoot salad, desserts, ice-cream, pepper and soil-sauce mixes and others.

3. Sample Collection

The food samples were kept in the ice cylinders while carrying to the KKU Dept. Microbiol. Laboratory.

4. Collection of the Samples from the Cooks

The dry cotton swabs were applied to collect the samples from the nasal cavity and the wet swabs were also used to collect the samples from hand of the cooks in the same persons. The dry and wet samples were put in the transport media and hurried to the laboratory.

5. Laboratory Experiment

The standard procedures as published by references 9-13 were followed

5.1 Added 450 ml. of phosphate buffer salt (PBS) pH 7.0 to 50 gm of food sample and diluted the sample to 1:105 Performed the total bacterial count by pour plate method .

5.2 *S. aureus* were cultured on Mannitol salt agar by the spread plate method then examined and counted Staphylococci and *S. aureus.*

5.3 Cultured the sample on Baird Parker medium to isolate the staph. coagulase positive bacteria by the spread plate method. The positive colonies would be shiny black in color surrounding the clear or opague colonies. The shiny black ones were collected to test for the coagulase enzyme. This was done by the coagulation test with human serum, the presence of the enzyme (coagulase) would cause the coagulation,

5.4 The swabs from the nasal cavity and the hand of the cooks were done as 5.3

5.5 Identification of the enterotoxin was carried out after the published techniques (6,7,12). The staphylococcus coagulase positive were cultured in the brain heart infusion broth and subsequently transferred to the cellophane over agar medium. This was incubated at 37°c for 24 hrs. The cells were then washed by 25 ml. PBS. (0.02M sodium phosphate, pH 7.4, in 0.9% NaCl and 1:104 merthiolate); and then were centrifuged at 4,000 rpm. for 15 minutes. The enterotoxins were in the suspension after centrifugation.

S. aureus species producing A,D,E, enterotoxins were kindly supplied by professor Dr. Merlin S. Bergdoll. Strain producing B enteroxin was obtained from Thailand National Board of Research. Enterotoxin C producing bacteria was obtained from NCTC of the United Kingdom .

5.6 The Reversed Passive Latex Agglutination (RPLA) after Park and Szabo 1986 (14) was employed to indentify the types of enterotoxin.

Result

We found from 193 prepared food samples that most of the samples contained the bacteria in the range of 10^6 to 10^7 cells/gm of food. The staphylococci were as densely populated as 10^5-10^6 cells/gm. of food; *S. aureus* were 10^3 - 10^4 and Staph. coagulase positive were 10^2-10^4 (Data not shown)

Table 1 Correlation between the bacterial number, type of bacteria and the enterotoxin-producing staphyloccus.

Number of bacteria cells/gm. of food	Positive Samples $							
	Total bacteria		Staphylococcus		*S. aureus*		S. coag +*	
	Number of samples	Percent	Number of samples	Percent	Number of samples	Percent	Number of samples	Percent
$>10^1$-10^2	0	0	0	0	1	2.94	1	2.94
$>10^2$-10^3	0	0	3	8.82	3	8.82	13	38.24
$>10^3$-10^4	3	8.82	2	5.88	7	20.59	9	26.47
$>10^4$-10^5	2	5.88	9	26.47	10	29.41	7	20.59
$>10^5$-10^6	6	17.65	10	29.41	11	32.35	3	8.82
$>10^6$-10^7	7	20.59	9	26.47	2	5.88	1	2.94
$>10^7$	16	47.06	1	2.94	–	–	–	–
Positive Samples	34	17.62	34	17.62	34	17.62	34	17.62

*S. coag. + = Staphylococcus coagulase positive
– = no sample possesses bacteria to the specified number (10^7) level.
Total Samples = 193
$ = producing enterotoxin

Table one shows that the prepared foods readily available to the public have toxin- producing stalphyloccoci about 17.62% (34 out of 193 samples). The samples with enterotoxins usually have total bacterial number greater than 107 cells/gm of food. The staphylococcus coagulase positive sample with the bacterial number of greater than 105 cells/gm. could cause food poisoning (6,7), which we found 4 samples (2.07% of total sample) in our series.

Table 2 Food samples studied, number of Staphylococcus coagulase positive samples, toxin-positive samples and types of enterotoxin.

Types of food sample	Number of Samples	Coag. positive sample	Toxin positive samples	Types of enterotoxin							
				A	C	D	AD	BD	ABD	ACD	ABCD
1. O-liang (sweetened black coffee)	1	1(100.00)	1(100.00)	-	-	-	-	-	1	-	-
2. Gaeng (Curry, vegetable soup)	38	12(31.51)	2(5.26)	2	-	-	-	-	-	-	-
3. Noodle soup	14	5(35.71)	3(21.43)	-	1	-	-	-	-	-	2
4. Stir-fried dishes	43	17(39.53)	4(9.3)	1	1	1	-	-	-	-	1
5. Papaya salad	29	20(68.97)	5(17.24)	1	1	-	-	1	-	1	2
6. Larb (minced meat salad)	18	13(72.22)	6(33.33)	-	3	-	1	-	2	-	3
7. Nam (raw pork salad)	18	12(66.66)	7(38.89)	3	1	1	-	-	1	1	-
8. Pla-ra (preserved raw fish)	8	3(37.50)	0(0)	-	-	-	-	-	-	-	-
9. Bamboo-shoot salad and egg plant salad	4	2(50.00)	1(25.00)	1	-	-	-	-	1	-	1
10. Icecream	7	5(71.43)	2(28.57)	-	1	-	-	-	-	-	1
11. Dessert	7	4(57.14)	2(28.57)	1	-	-	-	-	-	-	-
12. Green papper source	4	1(25.00)	0(0)	-	-	-	-	-	-	-	-
13. Mee-ka-ti (noodle mixed with coconut milk)	2	2(100.00)	1(50.00)	-	-	-	-	-	1	-	-
Total	193	97(50.26)	34(17.62)	9	8	2	1	1	6	2	10
Percent				23.08	20.5	5.13	2.56	2.56	15.38	5.13	25.64

Table 2 indicates that 97 food samples (50.26%) were found to harbour staphylococcus coagulase positive. Thirty-four samples (17.26%) possessed enterotoxin and the most frequently found was ABCD type (25.64%) . The next frequently detected were A (23.08%),C (20.5%) while the others were found in the succesive smaller number. The uncooked samples (nam and salad) were greater in number of containing enterotoxins (38.89%). The minced meat salad (larb) was also found in the next quantity (33.33%), samples of icecream and desserts with entrotoxins made up 28.57% which was also great. O-liang and Mee-kati were so little in number that we can not conclude.

Table 3 Location of contaminated enterotoxin in food shops and types of enterotoxin

Location	Number (shop)	Number of contamination		Types and number of enterotoxin		
		Number	Percent	one type	two types	three types
on KKU Campus	24	6	(25)	6	0	0
outside KKU. Campus	74	22	(29.73)	16	4	2
Total Number (percent)	98	28	(28.57)	22 (78.57)	4 (14.29)	2 (7.14)

As shown in table 3 there are 28.57% of the shops possessing contaminated enterotoxin producing *S. aureus* in food, and the majority (78.57%) of the contamination has one type of enterotoxin.

Table 4 Staphylococcus aureus from the nasal cavities and hands of the 182 cooks

Type of *S. aureus*	Number of Samples			
	Nasal cavity		Hand	
	Number	Percent	Number	Percent
Negative sample	8	(4.4)	12	(6.6)
Coagulase positive	86	(47.3)	34	(18.7)
Coagulase negative	88	(48.4)	136	(74.7)
Total	182	(100.00)	182	(100.00)

Table 5 Contaminated cooks and enterotoxin production

Enterotoxin production	Number of samples			
	Nasal cavity		Hand	
	Number	Percent	Number	Percent
Enterotoxin production	27	(14.84)	8	(4.4)
No enterotoxin production	155	(85.16)	174	(95.6)
Total	182	(100.00)	182	(100.00)

We can see in tables 4 and 5 that the staphylococcus coagulase positive could be found in 47.3%

Table 6 Types of enterotoxin isolated from the cooks

Type of enterotoxin	Number of positive and negative isolates			
	Nasal Cavity		Hand	
	Number	Percent	Number	Percent
S. aureus coagulase + but no-toxin production	59	68.6	26	76.5
S. aureus coagulase + toxin production	27	31.4	8	23.5
A	8	9.3(29.63)	2	5.9(25.0)
B	1	1.2(3.7)	-	--
C	3	3.5(11.11)	1	2.9(12.5)
AB	3	3.5(11.11)	-	--
AC	1	1.2(3.7)	-	--
AD	1	1.2(3.7)	-	--
BC	2	2.3(7.41)	-	--
BD	1	1.2(3.7)	-	--
ABC	-	--	1	2.9(12.5)
ABD	4	4.7(14.81)	1	2.9(12.5)
ACD	1	1.2(3.7)	1	2.9(12.5)
ABCD	2	2.3(7.4)	2	5.9(25.0)
Total isolates	86	100.0	34	100.0

Note : *The figures in the parentheses are the percentage of the total contaminated isolates.*

of the samples from the nasal cavities whereas 18.7% were from the hand samples. And only 14.84% of nasal cavity samples and 4.4% of hand samples produced enterotoxin.

Table 6 indicates that the majority of samples from the nasal cavity contain type A toxin (29.63%) and type ABD is next (14.81%). The samples from the hands contain types A and ABCD at the highest percentage of 25%

There is no correlation between the toxins from the nesal cavity and hand of the same cook, not the same type of toxin were produced.

Table 7 Relations of enterotoxins isolated from foods and the cooks

Enterotoxin production	Toxin isolated from food & nasal cavity of the cook		Toxin isolated from food and hands of the cook	
	Number of shops	Percent	Number of shops	Percent
Same type	2	(2.04)	2	(2.04)
Different type	44	(44.91)	30	(30.61)
No toxin production	52	(53.06)	66	(67.35)
Total	98	(100.00)	98	(100.00)

Table 7 shows that there is so little correlation between the toxins isolated from the food samples and the nasal cavities and hands of the cook because only 2.04% of them produced the same type.

Discussion and Conclusion

We have found Staphylococcus coagulase positive in 97 samples out of 193 (50.26%) and 34 samples containing the enterotoxin producing *S. aureus* (17.62%). The uncooked, prepared food samples were found to have high quantities of the enterotoxin producing *S. aureus* (Nam, Salad, 38.89%). The next is the uncooked meat salad (Larb) amounting to 33.33%. The third rank is icecream and desserts (28.57%). The noodles also contained the enterotoxin-producing bacteria in great quantities. This may be because of the original contamination prior to cooking and the cooking is not long enough to kill these germs. We have discovered the following enterotoxins in the order of great to small, ABCD, A,C, ABD. We found D, ACD,AD and BD occasionally. The food samples with staphylococcus coagulase positive of 10^5 cells/gram are capable of producing food poisoning (4,5). This was found in 4 samples (11.76%) out of the toxin positive samples or 2% of 193. Almost of these positive samples were the traditional northeast dishes.

Our findings are different from that of Sumontha et al (6,7) who had done similar studies in Bangkok. The reasons are many, firstly the technique (Reverse passive latex agglutination) is rather senstitive (only 0.75 nanogram/ml of enterotoxin could be detected which allowed us to detect more types of toxins), secondly the food samples were collected from different location and environment which could mean different strains of bacteria. The enterotoxin type A, however, were detected in a high quantity just like in Bangkok. And lastly the difference of prepared foods, the origin of raw material, and the cooking procedure all of which could contribute to the different findings from that of the Bangkok results.

Comparing the findings in the food samples collected on KKU. campus and outside, we discovered no significant difference. Concerning the origins of Staph. coagulase positive, we detected in more samples from the nasal cavities than from the hands. The toxins originated from the cooks were more of type A. The relationship of enterotoxin-producing *S. aureus.* and those from the hand was as little as 2.04%. This finding suggests that the bacteria in the uncooked samples could be in the raw materials rather than from the hand and nasal cavity. The time lapse between cooking and serving could also contribute to the problem. This could also make the air-borne route of contamination possibble because many of the containers had no lids. The source of contamination might as well be from the customers themselves. Considering such variety of sources, one would have only little doubt that the enterotoxins could be so many types. We were not able to identity the cooks who exactly involved in the cooking processes. Many times the cooks and the persons who dished out the foods were not the same. The finding on correlation, therefore, are not satisfactory.

It is concluded that the half-cooked foods are most likely to cause food poisoning due to *S. aureus.* The correlation between the enterotoxins and the cooks could not be confidently stated because we were not able to pinpoint the cooks because many

people lent helping hands as already mentioned. It is ,therefore, not possible to infer from this study that the contamination originated from the raw materials, transportation, the cooking process, the handling of the prepared food or from the cooks themselves.

Most methods for epidemiological surveillance of Staphylococcal enterotoxins have been based on immunological procedures for the measurement of toxin protein in the culture fluids of suspect strains or in extracts of food contaminated with staphylococcal enterotoxins. These methods are dependent on sufficient amounts of toxin being produced in the absence of interfering bacterial products. That is why we followed this technique in conducting this experniment.

DNA techniques such as DNA hybridizalion, PCR were introduced to employ as an additional means of analyzing strains that carried the toxin gene. These techniques may be applied for the more accurate study in Epidemiology of *S. aureus*

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