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PCR-based investigation of enterotoxin profile among Staphylococcus Aureus isolated from women with vaginosis

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Abstract

Bacterial vaginosis (BV) is the communal cause of foul-smelling vaginal secretions among females at sexual age. BV is called vaginosis and not vaginitis because it is associated with alteration in normal vaginal flora rather than due to specific infection. In particular, Staphylococcus spp. has been well-known as one of the causative agent of BV. Staphylococcal-related infections is supposed to part of causes in unreceptive pregnancy outcomes and female sterility. This study aimed to investigate some of enterotoxin profile among Staphylococcus aureus isolated from women with BV. All isolates inoculated on mannitol salt, blood and UTI chromogenic agar for primary screening of Staphylococcus aureus and then confirmed by amplification of species specific 16S rDNA gene. Sets of enterotoxins were investigated using specific primer pairs. Twenty five (29.09%) isolates were used in this study and recovered from 86 high vaginal swap during a period of 3 months. PCR results showed that (48%, 28%, 40%, 36% and 12%) of isolates have sea gene, seb gene, sec gene, sed gene and see gene respectively. The results revealed no difference in sensitivity could be found for the Staphylococcus aureus detection between culture and PCR and enterotoxins existence is varied.

Keywords: *S. aureus*, enterotoxin, bacterial vaginosis

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INTRODUCTION

The balances between vaginal bacterial flora is very important for health vagina and once unsettled will leads to Bacterial vaginosis (BV). Equilibrium of lactobacilli and pathogenic bacteria is imperative and thus when the two counterparts disequilibria will result BV. The sequelae of BV ranged from late miscarriage, preterm labor, rupture of the membranes, tubal factor infertility and endometritis (Ghiasi et al. 2014). Lactobacilli afford a continual acid pH rate and uphold the suitable hydrogen peroxide amounts in the genital. Upon BV occurrence, the lactobacilli communities will reduced and thus the vaginal pH is troubled (Mastromarino et al. 2013). Bacterial vaginosis may be unimicrobial of polymicrobial with rate of incidence ranged from 20-60%. Staphylococcus spp. can be implicated as prominent pathogenic causative agent of BV with aid of cocktail of virulence factors including enterotoxins (Allsworth et al. 2007, Becker et al. 2003, Butt 2001, Koumans et al. 2007, Veeh et al. 2003). Staphylococcal enterotoxins (SEs) belong to a large family of staphylococcal pyrogenic exotoxins which cause toxic shock-like syndromes. It is includes SEA, SEB, SEC, SED and SEE. They are also act as superantigen and can induce high fever, lethal shock and immune status upregulation (Tseng et al. 2004). This study was aimed to investigate of enterotoxin profile among S. aureus isolated from women with bacterial vaginosis via PCR technique.

MATERIALS AND METHODS

Isolates

Eighty six high vaginal swap [from women with vaginosis and with age between (25-40) year] were collected in this study. Twenty fife S. aureus isolates were recovered from these sample during a period of 3 months. All isolates inoculated on manitol salt agar, blood agar for primary screening of Staphylococcus spp. and then confirmed by chromogenic agar & PCR using specific primer for 16S rDNA gene of Staphylococcus spp.

Genomic DNA Extraction

All isolates were inoculated to Lauria Bretani broth (LB broth) as a first step of genomic DNA extraction.

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Table 1. Primer Pair Sequences and Amplicon Size

Primer	Primer sequence (5-3)	Amplicon (bp)	Reference
Stap 16SF	CCTATAAGACTGGGATAACTTCGGG	791	This study
Stap 16SR	CTTTGAGTTTCAACCTTGCGGTCG		
SA-Sea-F	GGTTATCAATGTGCGGGTGG	102	(Mehrotra et al. 2000)
SA-Sea-R	CGGCACTTTTTTCTCTTCGG		
SA-Seb-F	GTATGGTGGTGTAACTGAGC	164	
SA-Seb-R	CCAAATAGTGACGAGTTAGG		
SA-Sec-F	AGATGAAGTAGTTGATGTGTATGG	451	
SA-Sec-R	CACACTTTTAGAATCAACCG		
SA-Sed-F	-CCAATAATAGGAGAAAATAAAAG-	278	
SA-Sed-R	ATTGGTATTTTTTTTCGTTC		
SA-See-F	AGGTTTTTTCACAGGTCATCC	209	
SA-See-R	CTTTTTTTTCTTCGGTCAATC		

Table 2. Thermocycler Conditions

Gene	Temp (°C) Time (sec.)	Ini. Dent.	Denat.	Anneal.	Ext.	Final Ext.	Reference
Staph16S rDNA	Temp (°C)	95	95	56	72	72	This study
	Time (sec.)	120	30	30	70	300	
	Cycle#	1		30		1	
Sea	Temp (°C)	95	95	58.3	72	72	This study
	Time (sec.)	120	30	30	20	300	
	Cycle#	1		29		1	
Seb	Temp (°C)	95	95	56.2	72	72	This study
	Time (sec.)	120	30	30	20	300	
	Cycle#	1		30		1	
Sec	Temp (°C)	95	94	57	72	72	(Mehrotra et al. 2000)
	Time (sec.)	300	120	120	60	420	
	Cycle#	1		35		1	
Sed	Temp (°C)	95	94	57	72	72	
	Time (sec.)	300	120	120	60	420	
	Cycle#	1		35		1	
See	Temp (°C)	95	94	57	72	72	
	Time (sec.)	300	120	120	60	420	
	Cycle#	1		35		1	

After 18 hrs. at 37 °C centrifugation at 14000 rpm for 5 min were done to harvest the bacterial cells. Supernatant were disposed and decontaminated by alcohol and the pellet of bacterial cells were resuspended in phosphate buffer saline for washing, centrifugation and this step can be repeated three times. The rest of steps were performed according to the manufacturer company (Favorgen/Tiwan) (Oyediran et al. 2017).

Primer Pair Preparation

The primer pairs for 16S rDNA, sea, seb, sec, sed and see were purchased from Realgene (Realgene/China). Stock primer of 100pmole/µl were prepared and then working primer of 10pmole/µl used directly in PCR. The reaction mix of 25µl were prepared using 12.5µ of master mix (New England Biolabs/UK), 2µl of forward working primer, 2µl of reverse working primer, 4µl of DNA and 4.5µl of nuclease free water (New England Biolabs/UK). The sequence of primer pair, amplicon size were mentioned in **Table 1**.

PCR Conditions

The PCR tubes with complete mix were place in thermocycler (Techno/UK) and optimization were performed in this study to get the best conditions. The conditions for each primer were mentioned in **Table 2**.

1Biosafety and Hazard Material Disposing

Biosafety aspects followed during the work include disposing of all swabs, petri dishes and all contaminated supplies by autoclaving and then incineration. All benches cleaned with alcohol before and after the work. Simply safe stain were used instead of ethidium bromide.

RESULTS AND DISCUSSION

The results of Staphylococcus spp. isolation reveal no difference between results of identification by culturing on [manitol salt agar (yellow colony), blood agar (colony with β hemolysis) and UTI chromogenic agar (white colony)]; gram stain (Gram positive cocci in grape-like clusters) and results of amplification of 16S rDNA of Staphylococcus spp. (in which all twenty fife isolates give +ve result for this gene) (**Fig. 1** and **2**).

S. aureus has been stated to settle the vagina in 4-22% of pregnant female (Creech et al., 2010) Presence of S. aureus in the pregnant woman's vagina may subsidize to the development of various life-threatening infections (Reichman and Sobel 2009, Tang et al. 2010). From our results we found the percentage of S. aureus was (29.06%) (**Table 3**) also we found no difference in sensitivity could be found for the detection of Staphylococcus aureus between culture and PCR and this agree with Karmakar et al. (2016). Our results were also agreed with Ghiasi et al. (2014) who found that the S.aureus is the furthermost predominant vaginal patho-bacteria followed by E. coli.

Fig. 1. Staphylococcus aureus on: A-manitol salt agar (yellow colony) and B- UTI chromogenic agar (white colony)

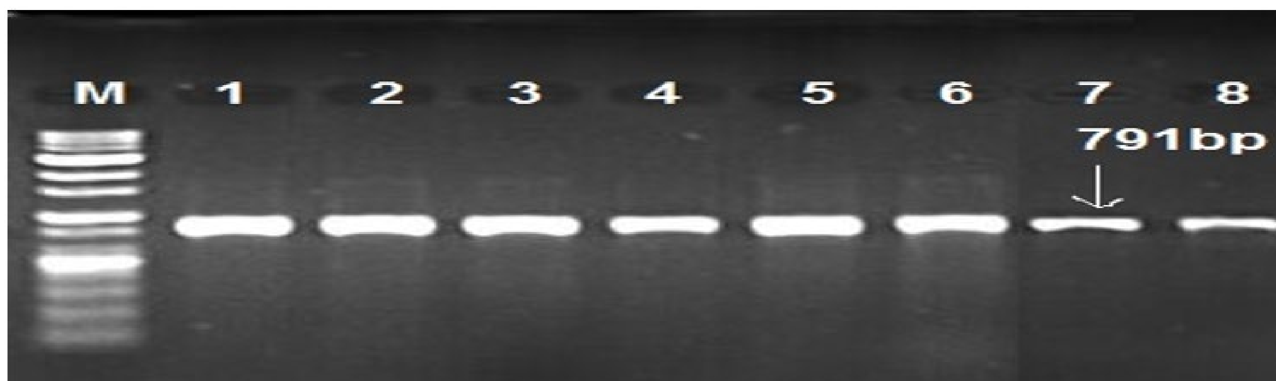

Fig. 2. 1.5% agarose in TBE of PCR products (791 bp) after amplification of 16S rDNA gene of Staphylococcus spp. M lane represent 100 bp DNA Marker while the rest lanes represents positive samples for gene

Table 3. Percentage (%) of *S. aureus isolates*

No. of sample	Pure isolates of S. aureus		Mix or other species Isolates	
	No.	%	No.	%
86	25	29.06	61	70.94

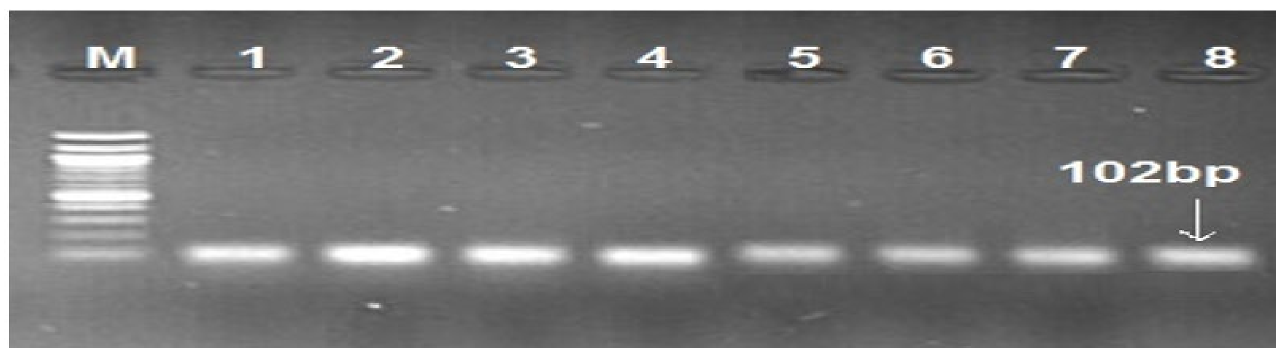

Fig. 3. 1.5% agarose in TBE of PCR products (102 bp) after amplification of sea gene of Staphylococcus spp. M lane represent 100 bp DNA Marker while the rest lanes represents some of positive samples for this gene

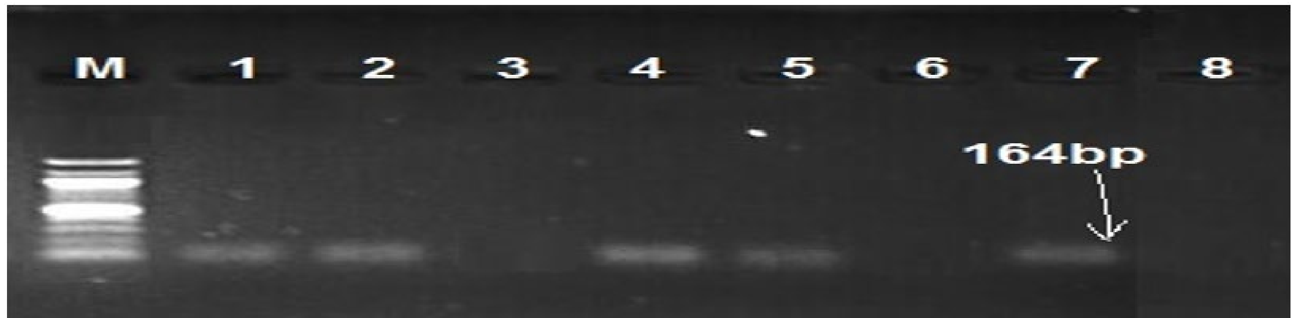

Fig. 4. 1.5% agarose in TBE of PCR products (164 bp) after amplification of seb gene of Staphylococcus spp. M lane represent 100 bp DNA Marker, the (3-6-8) represents some of negative samples for this gene. while the rest lanes represents some of positive samples for this gene

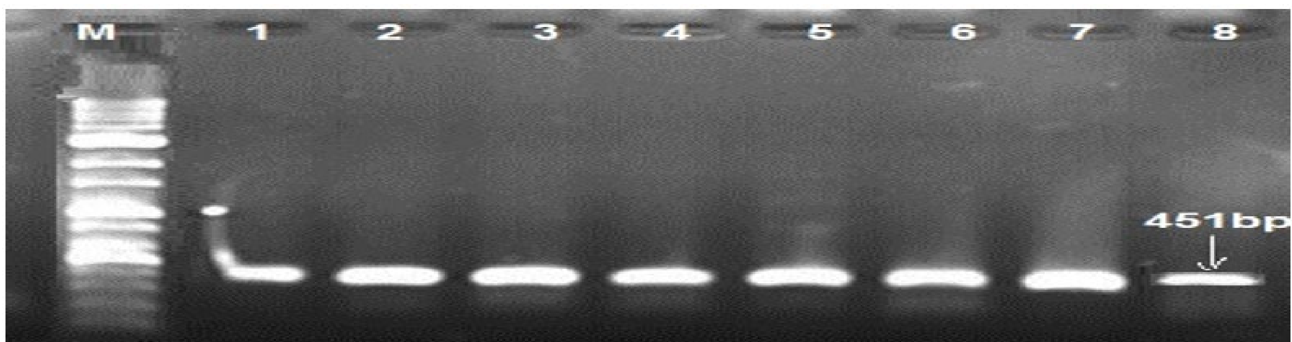

Fig. 5. 1.5% agarose in TBE of PCR products (451 bp) after amplification of sec gene of Staphylococcus spp. M lane represent 100 bp DNA Marker while the rest lanes represents some of positive samples for this gene

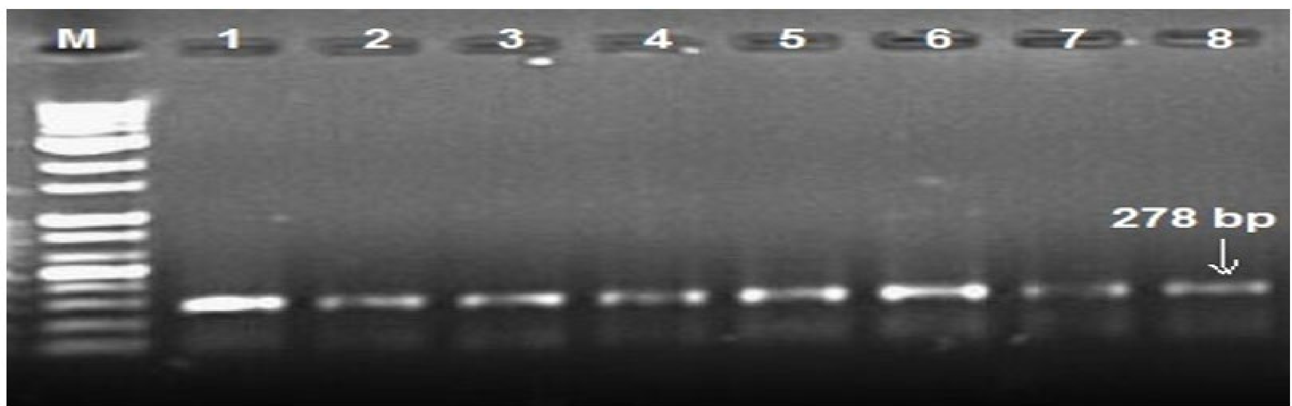

Fig. 6. 1.5% agarose in TBE of PCR products (278bp) after amplification of sed gene of Staphylococcus spp. M lane represent 100 bp DNA Marker while the rest lanes represents some of positive samples for this gene

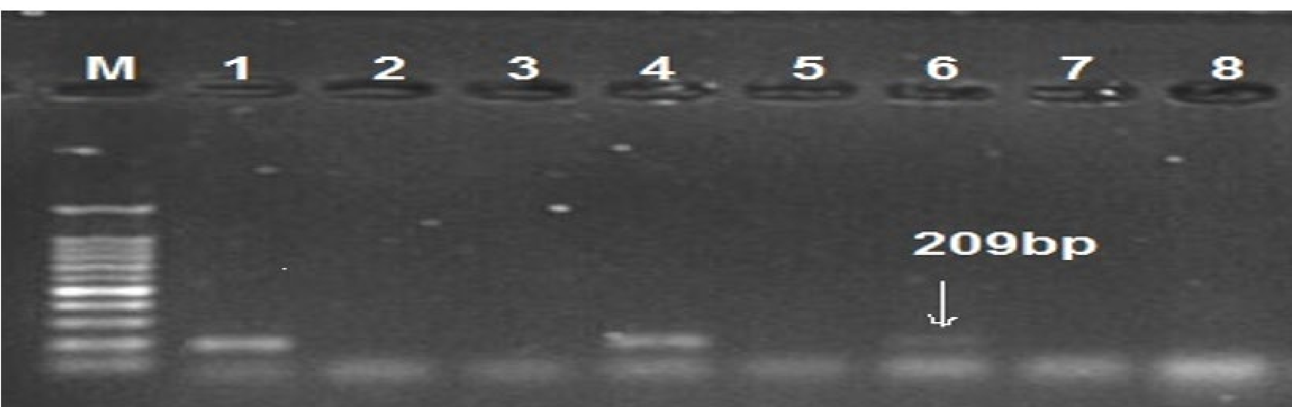

Fig. 7. 1.5% agarose in TBE of PCR products (209 bp) after amplification of see gene of staphylococcus. M lane represent 100 bp DNA Marker while the (1-4-6) represents some of positive samples for this gene. while the rest lanes represents some of negative samples for this gene

Table 4. Sample Table (one column) to put into column

Enterotoxin type	No. of + ve isolates (%)
Sea	12 (48)
Seb	7 (28)
Sec	10 (40)
Sed	9 (36)
See	3 (12)

Several methods have been used for detection and typing of the S.aureus Enterotoxin genes. It is potential to identify gene produces immunologically using a range of enzyme-linked immune-sorbent assays and radio-immunoassays (Omoe et al. 2002, Olowa and Olowa 2015) but these methods have some disadvantages. For example, misidentification by immunologic methods could easily occur because toxigenic S. aureus have low levels of expelled toxin(s) or cross-reactive antigens. Also ELISA kits are commercially available but time-consuming and expensive, in addition to limitations in antigen detection and cross-reactions with other types of enterotoxins. Other methods for gene identification, such as DNA hybridization, have been used to analyze strains for the presence of staphylococcal toxin genes. PCR has an benefit over DNA hybridization in that the sensitivity is adequate to let finding of microbial DNA directly in pathological specimens (Chen et al. 2004, Kwon et al. 2004). Many researches have exposed the really high capability of PCR for exactly detecting bacteria and genes of interest. Numerous authors have previously publicized the practicability of the PCR methodology for the documentation of S. aureus strains (Letertre et al. 2003). Different studies have also presented the validity of PCR to the recognition of staphylococcal enterotoxin genes.

Current present study stated that, the sea and sec gene conquered over the other gene. Comparable results have been stated in Jordan (Randa et al. 2006), Germany (Becker et al. 2003) and Canada (Mehrotra et al. 2000) amongst isolates of human origin. Nevertheless, other detectives have described that the sec gene is the furthermost among bovine S. aureus strains in Europe and the USA (Larsen et al. 2002, Sharma et al. 2000, Valle et al. 1990). Also we found that there was some isolates have more than one of these gene. So we conclude that it was highly virulent strains like strain which have seb gene made it Methicillin resistance because there was a significant correlation between presence of seb gene and Methicillin resistance (Imani Fouladi et al. 2011).

REFERENCES

Allsworth JE, Peipert JF (2007) Prevalence of bacterial vaginosis: 2000–2004 National Health and Nutrition Examination Survey data. Obstet Gynecol., 109: 114-120. https://doi.org/10.1097/01.AOG.0000247627.84791.91

Barati B, Saadati M, Bahmani MK (2006) Isolation and Detection of enterotoxigenic Staphylococcus aureus type A by multiplex PCR. J Military Medicine, 8: 119-128.

Becker K, Friedrich A, Lubritz G, Weilert M, Peters G, von Eiff C (2003) Prevalence of genes encoding pyrogenic toxin superantigens and exofoliative toxins among strains of Staphylococcus aureus isolated from blood and nasal specimens. J Clin Microbiol., 41: 1434-1439. https://doi.org/10.1128/JCM.41.4.1434-1439.2003

Butt T (2001) The inexorable march of Staphylococcus aureus. Pak J Pathol., 12 (2): 1-3.

Chen TR, Chiou CS, Tsen HY (2004) Use of novel PCR primers specific to the genes of staphylococcal enterotoxin G, H, I for the survey of Staphylococcus aureus strains isolated from food-poisoning cases and food samples in Taiwan. Int J Food Microbiol., 92: 189-197. https://doi.org/10.1016/j.ijfoodmicro.2003.10.002

Creech CB, Litzner B, Talbot TR, et al. (2010) Frequency of detection of methicillin-resistant Staphylococcus aureus from rectovaginal swabs in pregnant women. Am. J. Infect. Control., 38(1): 72-74. https://doi.org/10.1016/j.ajic.2009.06.015

Ghiasi M, Fazaeli H, Kalhor N, Sheykh-Hasan M, Tabatabaei-Qomi R (2014) Assessing the prevalence of bacterial vaginosis among infertile women of Qom city. IJM, 6(6): 404-408.

Imani Fouladi AA, Choupani A, Fallah Mehrabadi J (2011) Study of prevalence of Enterotoxin type B gene in Meticillin Resistant Staphylococcus aureus (MRSA) isolated from wound. Kowsar Medical Journal, 16(1): 21-25.

Karmakar A, Dua P, Ghosh C (2016) Biochemical and Molecular Analysis of Staphylococcus aureus Clinical Isolates from Hospitalized Patients. Canadian Journal of Infectious Diseases and Medical Microbiology: Article ID 9041636. https://doi.org/10.1155/2016/9041636

Koumans EH, Sternberg M, Bruce C, McQuillan G, Kendrick J, Sutto M, Markowitz LE (2007) The prevalence of bacterial vaginosis in the United States, 2001–2004; associations with symptoms, sexual behaviors, and reproductive. Sex Transm Dis., 34: 864-869. https://doi.org/10.1097/OLQ.0b013e318074e565

Kwon NH, Kim SH, Park KT, Bae WK, Kim JY, Lim JY, et al. (2004) Application of extended single-reaction multiplex polymerase chain reaction for toxin typing of Staphylococcus aureus isolates in South Korea. Int J Food Microbiol., 2: 137-145. https://doi.org/10.1016/j.ijfoodmicro.2004.04.014

Letertre C, Perelle S, Dilasser F, Fach P (2003) Detection and genotyping by real-time PCR of the staphylococcal enterotoxin genes sea to sej Molecular and Cellular Probes. Int J Food Microbiol., 17: 139-147. https://doi.org/10.1016/S0890-8508(03)00045-8

Loir YL, Baron F, Gautier M (2003) Review. Staphylococcus aureus and food poisoning. GMR, 2: 63-76.

Mastromarino P, Vitali B, Mosca L (2013) Bacterial vaginosis: a review on clinical trials with probiotics. New Microbiol., 36: 229-238.

McLauchlin J, Narayanan GL, Mithani V, O'Neill G (2000) The detection of enterotoxins and toxic shock syndrome toxin genes in Staphylococcus aureus by polymerase chain reaction. J Food Prot., 63: 479-488. https://doi.org/10.4315/0362-028X-63.4.479

Medina MB (2006) Development of a fluorescent latex microparticle immunoassay for the detection of staphylococcal enterotoxin B (SEB). J Agric Food Chem., 54: 4937-4942. https://doi.org/10.1021/jf053253q

Mehrotra M, Wang G, Johnson WM (2000) Multiplex PCR for detection of genes for Staphylococcus aureus enterotoxins, exofoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. J Clin Microbiol., 38: 1032-1035.

Naffa RG, Bdour SM, Migdadi HM (2006) Enterotoxicity and genetic variation among clinical Staphylococcus aureus isolates in Jordan. Journal of Medical Microbiology, 55: 183-187. https://doi.org/10.1099/jmm.0.46183-0

Olowa OA, Olowa OW (2015) Gender Issues of Labour Participation in Vegetable Production in Ikorodu Local Government Area of Lagos State. Current Research in Agricultural Sciences, 2(4): 114-122. https://doi.org/10.18488/journal.68/2015.2.4/68.4.114.122

Omoe K, Ishikawa M, Shimoda Y, Hu D, Ueda S, Shinagawa K (2002) Detection of seg, seh, and sei genes in Staphylococcus aureus isolates and determination of the enterotoxin productivities of S. aureus isolates harboring seg, seh, or sei genes. J Clin Microbiol., 40: 857-862. https://doi.org/10.1128/JCM.40.3.857-862.2002

Oyediran WO, Omoare AM, Osinowo OA (2017) Contributive Roles of Sorghum Production to Food Security and Economic Empowerment of Rural Farming Households in Katsina State, Nigeria. Canadian Journal of Agriculture and Crops, 2(1): 42-49. https://doi.org/10.20448/803.2.1.42.49

Reichman O, Sobel JD (2009) MRSA infection of buttocks, vulva, and genital tract in women. Curr. Infect. Dis. Rep., 11(6): 465-470. https://doi.org/10.1007/s11908-009-0067-6

Tang YW, Himmelfarb E, Wills M, et al. (2010) Characterization of three Staphylococcus aureus isolates from a 17-year-old female who died of tampon-related toxic shock syndrome. J. Clin. Microbiol., 48(5): 1974-1977. https://doi.org/10.1128/JCM.00224-10

Tseng CW, Zhang S, Stewart GC (2004) Accessory gene regulator control of staphyloccocal enterotoxin d gene expression. Journal of bacteriology, 186(6): 1793-1801. https://doi.org/10.1128/JB.186.6.1793-1801.2004

Veeh RH, Shirtliff ME, Petik JR, Flood JA, Davis CC, Seymour JL, et al. (2003) Detection of S. aureus biofilm on tempons and menses components. J Infect Dis., 188: 519-530. https://doi.org/10.1086/377001