http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 8 (2): 278-280, 2005
ISSN 1028-8880
© 2005 Asian Network for Scientific Information

Coagulase Gene Polymorphism of *Staphylococcus Aureus* Strains Isolated from Human, Animals and Environment

[1]M.E. Wisal, [2]M.A. Hamid, [1]E.A. Hadia, [2]I.M. El Jalii and [2]A.S. Ali
[1]Department of Bacteriology, Central Veterinary Research Laboratory, Sudan
[2]Department of Preventive Medicine and Public Health, Faculty of Veterinary Medicine, University of Khartoum, Khartoum North, P.O. Box 32, Sudan

Abstract: A total of 29 *Staphylococcus aureus* strains isolated from human, animals and their environment were subjected to genotypic analysis on the basis of coagulase gene polymorphism. The coagulase gene was amplified using a pair of oligonucleotide nested primers. Presumptive phenotypic identification of the strains showed production of free and bound coagulases, production of acetion, anaerobic utilization of mannitol with acid production. PCR-amplified coagulase genes of *S. aureus* revealed different pattern in base pair lengths and number of amplified bands. There were no obvious specific PCR pattern for all types of isolates thus, genotypic clustering correlated to human, animal and environmental isolates was not passable. Given the specificity of the coagulase gene, the isolates were thus confirmed to be belong to the coagulase positive *S. aurueus.* Out of 29 PCR-amplification isolates, 21 produced a single band while 8 isolates produced two bands. The length of the amplicon ranged from 430 to 1000 bp. Amplicons of the 21 isolates were thus categorized as 670, 930, 950 and 1000 bp. In conclusion, Amplification of coagulase gene is useful in confirmation of coagulase gene positive *S. aurueus.*

Key words: *Staphylococcus aureus,* PCR, coagulase gene, polymorphism

INTRODUCTION

Staphylococcus aureus is Gram-positive cocci characterized by formation of clusters. For may years, *S. aureus* has been known as one of the major organism associated with many animal and human conditions such as abscesses, clinical and subclinical mastitis. It causes mastitis in cattle with acute form showing marked toxin symptoms[1]. Harrey and Gilmour[2] isolated the organism from raw milk. In Sudan, *S. aureus* was regarded as the most important cause of mastitis[3-6]. On the other hand, milk and milk products contaminated with *S. aureus* are common cause of food poisoning[7].

The DNA analysis of *S. aureus* coagulase gene has been widely investigated. Goh *et al.*[8] reported that staphylocoagulase, is a major phenotypic determinant of *S. aureus*, exists in multiple allelic forms, in part because of the existence of gene variants within the 3-end coding region and this region contains a series of repeating 81 bp DNA sequences which differ both in the number of tandem repeats and the location of restriction sites. The objective of the present study was to type some *S. aureus* isolates based on polymerase chain reaction amplification of the variable region of the coagulase gene.

MATERIALS AND METHODS

Isolates: Ninety eight isolates of *S. aureus* were used in this study. The isolates were isolated from both healthy and different pathological conditions of man and animal as well as from their environment.

DNA extraction: Extraction of genomic DNA of isolates was performed as described by Yetes *et al.*[9] by boiling the isolates at 80°C for one hour in an equal volume of chloroform and distilled water.

PCR specific primer: The sequences of primers used were as follow: 5-AGA CCA AGA TTC AAC AAG-3 and 5- AAA GAA AAC CAC TCA CAT CA-3 which hybridize to sites 1632 to 1651 and 2589 to 2608, respectively[8-10].

PCR: The PCR reaction mixture consisted of 5 μL of template DNA, 1.5 U of Tag DNA polymerase, 5 μ 10x PCR

Corresponding Author: Dr. Isam Mohamed Ali El Jalii, Department of Preventive Medicine and Public Health, Faculty of Veterinary Medicine, Shambat, P.O. Box 32, Khartoum North, Sudan
Tel: +249 192 924029 Fax: +249 185 312638 E-mail eljalii@yahoo.com

amplification buffer, 20 pmol μL^{-1} each primer, 200 mM deoxynucleoside triphosphate (dNTPs), 3 mM of $MgCl_2$ and double-distilled water to a final volume of 50 μL. The reaction mixture was covered with 30 of mineral oil.

The thermal cycle device (Techne) was programmed to give DNA denaturation at 95°C for 3 min, primers annealing at 51.2°C for 45 sec, extension at 72°C for 1 min and final extension at 72°C for 10 min. The total cycles were 40.

Gel electrophoresis of amplification products: The PCR products were electrophoresed after being stained with ethidium bromide at 90 V for half an hour. A 100 bp DNA Ladder and negative control were applied in each run. The amplicons were visualized under ultraviolet illuminations and photographed.

RESULTS AND DISCUSSION

The coagulase gene polymorphism had been authenticated among *S. aureus* strains isolated in Sudan. Successful PCR amplification was revealed by the 29 isolates. The amplified bands were approximately of 670, 930, 950 and 1000 bp in sizes. The reference *S. aureus* strain showed a band of 930 bp.

Different base pair sizes were produced by human and animal isolates (Fig. 1).

Utilizing the optimized PCR assay, the primers described[8,10] have been found to amplify *S. aureus* of human and animal and their environmental isolates successfully. This may refer to the optimization and of the concentration of Mg^{+2} inos in addition to stability of the Tag polymerase which they found to enhance the sensitivity and specificity of the PCR[11-13].

The coagulase gene has been found polymorphic and genotypically variable among *S. aureus* strains isolated in this study and the polymorphism obtained was clearly revealed due to multi allelic forms at the 3- end of the gene (tandem repeats) which differ in their sequences and restriction sites. Phenotypic variations were demonstrated clearly in the production of Stahpylocagulases among human and animal and environmental isolates which may be due to polymorphism of the gene.

The polymorphism of the coagulase gene which was obtained in the present study was not of a wide range as recognized earlier[8,10]. This may be due to utilization of the outer primers used by those authors and the PCR products obtained was pretended as a template DNA for other nested PCR assay in a comparison with the present investigation which utilized directly the nested primers and the genomic DNA of the isolates. It concluded that

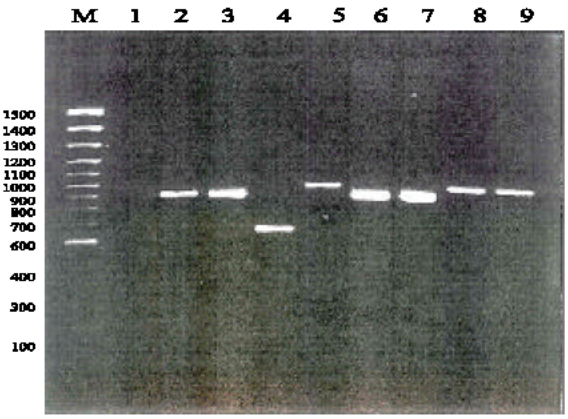

Fig. 1: Gel electrophoreses of PCR products obtained from Polymorphism of the coagulase gene of *S. aureus* strains. Lanes 1, negative control (*S. epidermidis*). 2, standard *S. aureus* of approximately 930 bp; 3, *S. aureus* of 930 bp; 4, *S. aureus* of 670 bp; 5, *S. aureus* of 1000 bp; 6,7, *S. aureus* of 930 bp.; 8, *S. aureus* of 950 bp; 9, *S. aureus* of 930 bp; M, 100 bp ladder serving as a size marker. *Strains presented in the figure represented the four groups according to the band size produced

polymorphism based on PCR amplified coagulase gene was revealed without obvious specific PCR pattern for all types of isolates.

ACKNOWLEDGMENTS

The research received a generous donation of PCR kits from Professor Alfred Wittenhofer of the Max Plank Institute (Dortmund, Germany). Mr. Adil Mahgoub is thanked for technical assistance.

REFERENCES

1. Brunner, D.W. and J.H. Gillespie, 1973. Hagan Infectious Disease of Domestic Animals. 6th Edn., Comell University Press, London.
2. Harvey, J. and A. Gilmour, 1988. Isolation and characterization of *Staphylococci* from goat's milk product produced in Northern Island. Lett. Applied Microbiol., 7: 79-82.
3. Adlan, A., A.M. Shommein and E.D.M. El Amin, 1980. A survey of bovine mastitis in four diary farms. Sud. J. Vet. Res., 2: 37-39.
4. Farah, 1.O., 1991. Host specific biotypes and host specificity of pathogenic staphylococci isolated from humans, cattle, sheep, goats and camels in the Sudan. Sud. J. Vet. Sci. and Anim. Husb., 30: 32-44.

5. Mamoun, I.E. and M.R. Bakhiet, 1992. Bovine Staphylococcal mastitis in the Sudan. Sud. J. Vet. Sci. and Anim. Husb., 31: 62-64.
6. Mamoun, I.E., 1981. Aerobic bacteria of bovine milk in the Sudan. M.Sc Thesis, University of Khartoum.
7. Bakhiet, M.R., Hadya, J. Ahmed and E.D.M. El Amin, 1991. Isolation of *Staphylococcus aureus* from food poisoning of cheese. Bull. Anim. Him. Prod. Afr., 39: 459.
8. Goh, S.H., S.K. Byrne, J.L. Zhang and A.W. Chow, 1992. Molecular typing of *Staphylococcus aureus* on the basis of coagulase gene polymorphism. J. Clin. Microbiol., 30: 1642-1645.
9. Yates, M.D., F.A. Drobniewski and S.M. Wilson, 2002. Evaluation of a rapid PCR-based epidemiological typing method for routine studies of *Mycobacterium tuberculosis*. J. Clin. Microbiol., 40: 712-714.
10. Nimmo, G.R., J. Schooneveldt, G. O'Kane, B. McCall and A. Vickery, 2000. Community acquisition of gentamycin-sensitive methicillin resistant *Staphylococcus aureus* in Southeast Queensland, Australia. J. Din. Microbiol., 38: 3926-3931.
11. Newton, C.R. and A. Graham, 1997. PCR, 2nd Edn., Bios Sci. Publ. Oxford, UK. Cited by: Elmagboul, S.B., 2001, Polymerase Chain Reaction (PCR) for detection of Leptospira (Ph.D Thesis, Univ. Vet. Med., Vienna, Austria), pp: 192.
12. Wilson, I.G., 1997. Inhibition and facilitation of nucleic acid amplification. Applied Environ. Microbiol., 63: 3741-3751.
13. Chou, Q., M. Russel, D.E. Birch, J. Raymond and W. Bloch, 1992. Prevention of pre-PCR mis-priming and primer dimerization improves low-copy number, amplifications. Nucleic Acids Res., 16: 1717-1723.