Detection of Erythromycin Resistance Genes erm(A), erm(B), erm (C) and msr(A) in Staphylococcus Nasal carriers in Khartoum State

Makarem Ahmed Salih[1], Alsadig Mohammed Abdalla[2] and Mai Abdul Rahman Masri[1]

[1] Department of Microbiology, Faculty of Science, University of Khartoum, Sudan
[2] Department of Microbiology, Faculty of Medicine, Sebha University, Libya
E-mail: alsadig58@yahoo.com

Abstract: *Staphylococcus aureus* continues to be one of the most difficult pathogens to treat because of its resistance to antibiotics. The significant prevalence of nosocomial infections caused by multi-resistant *S. aureus* and coagulase-negative staphylococci (CoNS) has been documented. Resistance to erythromycin in staphylococci is usually associated with resistance to other macrolides. The aim of this study was to determine the susceptibility of S. aureus strains isolated from nostrils of patients to erythromycin antibiotic by disc diffusion method. And detection of S. aureus species specific gene (sau gene) and Erythromycin resistance genes (ermA, ermB, ermC and msrA) by using PCR technique. A total of 50 nasal swabs samples collected from outpatients at admission to Academic Charity Hospital (Khartoum). The organisms isolated were 50 Staphylococci and they were classified as, *Staphylococcus aureus* 12, *Staphylococcus epidermidis* 26, *Staphylococcus hyicus* 12. All *S.aureus* isolates were positive for the presence of (*S. aureus* specific gene) and all the CoNS isolates were negative. All the *S. aureus* strains were sensitive to erthromycin antibiotic by using disc diffusion method. Ten out of the twelves *S.aureus* isolates were found negative for the presence of Erythromycin resistance genes (ermAb, ermB, ermC and msrA), when ermAb gene was found positive in 2 isolates out of 12.
[Makarem Ahmed Salih, Alsadig Mohammed Abdalla and Mai Abdul Rahman Masri. **Detection of Erythromycin Resistance Genes erm(A), erm(B), erm (C) and msr(A) in Staphylococcus Nasal carriers in Khartoum State.** *Nat Sci* 2014;12(4):106-110]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 14

Keywords: Molecular detection, antibiotic resistance, Staphylococcus bacteria

1. Introduction

Staphylococci ('staph') are a common type of bacteria that live on the skin and mucous membranes (eg. in nose) of humans. Other staphylococci, including *S. epidermidis*, are considered commensals, or normal inhabitants of the skin surface (Ryan and Ray, 2004). The anterior nares are considered to be the primary colonization site, and approximately 30% of healthy people carry this bacterium (Aída et al, 2010). *Staphylococcus aureus* is a ubiquitous human pathogen and a common cause of invasive and life threatening infections. It is the most common cause of community-associated cellulitis and endocarditis, and is a common cause of bacteremia (Brian et al, 2010). ***S****. aureus* has a formidable ability to adapt to varying environmental conditions and an extraordinary capacity to rapidly become resistant to virtually all antibiotics (McCallum et al, 2010). Resistance to antibiotics is often acquired by the horizontal transfer of genes from outside sources, although chromosomal mutation and antibiotic selection are also important (Gorwitz et al, 2008).

Staphylococcus aureus strains were once nearly uniformly susceptible to semi-synthetic penicillinase-resistant β-lactams (e.g. methicillin, oxacillin), the most commonly used class of antibiotics for skin infection. Widespread resistance to methicillin eventually developed and, by 1996, about one-third of *S. aureus* strains were no longer susceptible to this drug. Methicillin resistant *Staphylococcus aureus,* (MRSA) implied cross-resistance to all β-lactams including all penicillins and cephalosporins (Brian et al, 2010). Several new classes of antibiotics have been recently approved for use to treat multi–drug-resistant *S. aureus*. Based on past history, however, it is likely to be only a matter of time before significant resistance to these drugs develops (Patrick et al, 2007).

Erythromycin was introduced in 1952 as the first macrolide antibiotic. Unfortunately, within a year, erythromycin-resistant (Emr) staphylococci from the United States, Europe, and Japan were described (Zhang et al, 1992). Resistance to erythromycin in staphylococci is usually associated with resistance to other macrolides. Three genes (ermA, ermB, and ermC) encoding methyltransferases responsible of resistance to macrolides, lincosamides and type B streptogramins (MLSB phenotype) by modification of the ribosomal target site have been found in staphylococci. The msrA gene displays another mechanism of inducible resistance to erythromycin by encoding an ATP - dependent efflux pump. On the other hand, macrolide efflux is affected by a membrane protein encoded by the mef gene (Tarek et al, 2011). The recent emergence of a highly virulent community-associate

MRSA (CA-MRSA) and vancomycin-resistant, intermediate-resistant, or heteroresistant *S. aureus* further heightens public health concerns (Gillet et al, 2002). Prevention of *S. aureus* infection and reduction of the spread of virulent and resistant strains are therefore of great importance. In this study, we examined the susceptibility of *S. aureus* strains isolated from nostrils of patients to erythromycin by disc diffusion method. Erythromycin resistance genes (ermA, ermB, and ermC), macrolide efflux genes (msrA) and *S. aureus* species specific gene (sau gene) were detected by PCR technique to compare conventional phenotypic methods in routine laboratory practice with an established molecular method.

2. Materials and methods

Isolation and identification of bacteria:

Samples were obtained for culture from anterior nares using swabs from 50 individual patients at admission to Academic Charity Hospital (Khartoum). Isolation attempts were made on all samples on the same day of collection. At the laboratory each sample was direct-plated onto blood agar and mannitol salt agar, incubated at 35°C for 24 h.

Staphylococcus aureus was identified by Gram stain, catalase, slide and tube coagulase test and DNase test. According to procedures described by Cowan & Steels (Barrows & Feltham, 2003).

Susceptibility test of isolated bacteria to erythromycin Antibiotic:

Sensitivity of the different isolates to erythromycin antibiotic was studied by the Standard disc diffusion method according to criteria of the National Committee for Clinical Laboratory Standard (NCCLS, **2004**).

Molecular analysis by using PCR method:

PCR technique used to detect Erythromycin resistance genes (ermA, ermB, ermC and msrA) in *Staphylococcus aureus* isolates and *S. aureus* species specific gene (sau gene).

DNA extraction:

About 3-5 bacterial colonies were collected from blood agar plate and resuspended in 300µl D.W in 1.5 ml Eppendorf tube using micropipettes. The samples were heated at 100ºC for 15 minutes, cooled and then centrifuged at 13000(rpm) for 10 minutes the supernatant was used as a template in a 25 µl PCR mixture (Zhang *et al,* 2004).

Oligonucleotide primers:

The oligonucleotide primers used in this study were synthesized and purchased from Vivantis Malaysia

Table (I) Primers used for detection of genes encoding antibiotics resistance.

Primer pair	Sequence (5---→3)	Ampilcon Size bp
ermB –for	5´-CCG TTT ACG AAA TTG GAA CAG GTA AAG GGC-3´	335
ermB-rev	5´-GAA TCG AGA CTT GAG TGT GC-3´	335
ermAb-for	5´-GTT CAA GAA CAA TCA ATA CAG AG-3´	420
ermAb-rev	5´-GGA TCA GGA AAA GGA CAT TTT AC-3´	420
msrA-for	5´-GGC ACA ATA AGA GTG TTT AAA GG-3´	913
msrA-rev	5´-AAG TTA TAT CAT GAA TAG ATT GTC CTG TT-3´	913
Sau1-for	5´-AAT CTT TGT CGG TAC ACG ATA TTC TTC ACG -3´	107
Sau2-rev	5´-CGT AAT GAG ATT TCA GTA GAT AAT ACA ACA-3´	107

PCR amplification:

A single PCR assay targeting *Staphylococcus aureus* species specific gene (Sau), and macrolide resistance genes (ermA, ermB, ermC and msrA) was used in our study. Amplification was performed by using the thermal cycler. It was carried out as follows: A PCR mixture (25 µl) contained 1µl forward and reverse primers (25 pmol/L), dNTP mix (100 µm mol/L of each), 1 U of GO *Taq* DNA polymerase (Promega, France), 5 µl green Go *Taq* buffer (5×), and 3µ L of DNA template (50 ng). PCR conditions included initial denaturation (5 min 94 °C), 30 cycles of denaturation (94 °C 1 min), annealing (55 °C 1 min), and extension (2 min 72 °C), and final extension (10 min72 °C). PCR products (5 µ L) were analyzed on 2 % agarose gel stained with ethidium bromide, visualized under UV trans illumination and photographed using Gel Doc XR apparatus (*BioRad*, USA) (Martineau et al, 2000) and **(**Lim et al, 2002).

Detection of PCR products: The PCR products were analyzed on a 2% agarose gel. The gel electrophoresis separation method was used. A 2% agarose gel stained with 1µl ethidium bromide was prepared. 5µl of the PCR product is mixed with 3µl bromophenol blue dye. 1µl of a 100bp DNA molecular weight marker was transferred into the first well. In the following wells DNA samples products were transferred followed by –ve template. Gel electrophoresis was performed at 85 v for 45 min. and the analysis was done by using an automated gel photo documentation system.

3. Results

Bacteriological findings: The total samples collected were 50 samples. The total number of Gram positive bacteria isolated was 50. The organisms isolated were Gram-positive, they were made of the genus *Staphylococcus* and they were classified as. *Staphylococcus aureus* 12, *Staphylococcus epidermidis* 26, *Staphylococcus hyicus* 12 (Table 2)

Antibiotic sensitivity testing:

All the *S. aureus* strains were sensitive to erthromycin antibiotic.

Results of PCR Amplification Test:

All S. aureus isoates (100%) were positive for the presence of (*S. aureus* specific gene) and all the CoNS isolates were negative. Ten out of the twelve *S.aureus* isolates were found negative for the presence of Erythromycin resistance genes (ermAb, ermB, ermC and msrA), when ermAb gene was found positive in 2 isolates out of 12 which was detected by PCR assay (table 3).

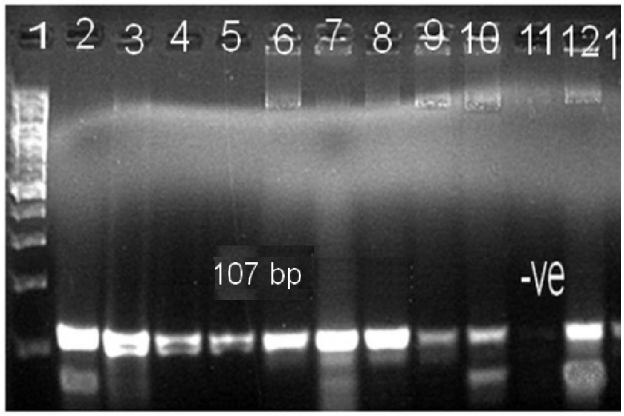
Table (2) Results of biochemical reaction for different bacterial isoiates

Organ. Test & No.	Co	Ca	La	Ma	Mn	Xy	DNase	NV
S.aureus 12	+ve	+ve	+ve	+ve	+ve	-ve	+ve	S
S.epidermidis 26	-ve	+ve	+ve	+ve	-ve	-ve	-ve	S
S.hyicus 12	-ve	+ve	+ve	-ve	-ve	-ve	-ve	S

Co = coaglase Ca = Catalase, NV = Novobiocin, La = Lactose
Ma = Maltose -ve = Negative, +ve = Positive, S = Sensitive
Mn = Mannitol Xy = Xylose DNase

Table (3) interpretation of PCR result for all tested genes

No of samples	*S.aureus* gene specific	msrA gene	ermAb gene	ermB gene	erm C b gene
12	12	0	2	0	0



PCR product of *S. aureus* species specific gene (107 bp), lane1: DNA 100bp ladder, lane 11: –ve control.

4. Discussions

In general, *S. aureus* is present in the noses of 20% to 30% of healthy people (Wertheim et al, 2005) and in higher frequencies in crowded populations (Ellis et al, 2004).

Recently, the increasing numbers of device-related infections associated with methicillin-resistant staphylococci have raised awareness toward the need for alternative agents to prevent these infections.

In this study the total *Staphylococcus aureus* isolated from the nasal swabs were 12 of 50 samples (24%), this in agreement with Fen QU *et al*., (2010) who found that there is a 16.1% to 24.6% *S. aureus* nasal carriage rate in military camps in the Beijing area, which is similar to rates found in general community-based nasal carriage studies in the United States, Turkey, Australia, and Malaysia (Munckhof et al, 2009). This indicate that numerous studies of *S. aureus* nasal carriage have been carried out in various geographic regions so prevention of *S. aureus* infection and reduction of the spread of virulent and resistant strains are therefore of great importance.

Investigating the spread of drug resistance genes in staphylococci is important for controling its dissemination (Sekiguchi et al, 2004). Several studies had been done using PCR technique that identified antibiotic resistance genes in staphylococci (Strommenger et al, 2003). For appropriate therapy decision making, accurate susceptibility data are important. However, no any published articles are available about the prevalence of erythromycin (MLS phenotype) resistance of *S. aureus* in sudan.

In this study we found that all the12 samples appear as *S. aureus* by ordinary bacteriological tests gave positive result under PCR for *S. aureus* species specific gene, this result similar to that obtained by Francis et al (2000), who found 100% correlation for species identification by PCR and classical methods.

Several genes are implicated in erythromycin resistance, especially in staphylococci and streptococci. Simultaneous resistance to macrolides, lincosamides, and type-B streptogramins (MLS resistance) in clinical isolates is a form of acquired resistance due to several evolutionary variants of *erm*

genes, which encodes a 23S rRNA methylase (Francis et al, 2000).

In this study we compared erythromycin susceptibility testing obtained by conventional method (disc diffusion) with those obtained by PCR assay for the detection of antibiotic resistance genes. All the twelve *S. aureus* isolates were sensitive to erythromycine by disc diffusion method, the results obtained by PCR method showed that two out of twelve isolates were positive for ermAb gene but negative for all the other genes. This is in agreement with Zmanter *et al* who reported that there is no correlation between genotypic and phenotypic methods for the determination of ERM resistance (Zmantar et al, 2008).

Francis *et al*., (2000). in their study found that there was 98.5% correlation between classical and PCR methods for erythromycin resistance.

This type of discrepancy between the genotype and the phenotype results may be explained by the heterogeneous nature of erythromycin resistance suggests that numerous factors could explain the sensitive phenotype in these strains such factors include the regulation of the expression and the absence of host factors associated with the phenotypic expression of resistance (Archer et al**,** 1994). We can extract the fact that we were able to select resistant cells from originally susceptible strains demonstrates that upon in vitro selection in the presence of increasing gradients of the antimicrobial agent, it is possible to select for resistance. Furthermore, once induced, the resistance phenotype was shown to be stable (Kolbert et al, 1998). Therefore, discrepant results between susceptibility methods should alert microbiologists

Based upon the results of this study, we can state that, in the Sudan, the occurrence of *Staphylococcus* isolates resistant to erythromycin was low in *S.aureus* nasal colonized people although the findings are not alarming, preventions and precautions must be taken to control dissemination and spreading of *S. aureus* (colonization) in the community especially those harboring the drug resistance gene.

Acknowledgements

We would like to thank all the technical staff of the microbiology research laboratory of the University of Khartoum, Alneelain and medical staff at Academic Charity Hospital (Khartoum). Sudan, and our dear patients who took part in the study.

Corresponding Author:
Dr. Alsadig Mohammed Abdalla
Department of Microbiology
Faculty of Medicine, Sebha University
Sebha, Libya
E-mail: alsadig58@yahoo.com

References:

1. Ryan KJ, Ray CG. *Sherris Medical Microbiology*. An introduction to infectious disease (4th ed.) 2004.
2. Aída Hamdan-Partida, Teresita Sainz-Espun˜es, and Jaime Bustos-Martínez. Characterization and Persistence of *Staphylococcus aureus* Strains Isolated from the Anterior Nares and Throats of Healthy Carriers in a Mexican Community. Journal of clinical Microbiology 2010, p. 1701–1705 Vol. 48,
3. Brian W.J. Mahy Atlanta, Ga, J. Todd Weber. Antimicrobial Resistance Beyond the Breakpoint. Stockholm Basel. Issues in Infectious Diseases Vol. 6. 2010
4. McCallum N, Berger-Bächi B, Senn MM. Regulation of antibiotic resistance in *Staphylococcus aureus*. Int J Med Microbiol. 2010;300 (2-3):118-29.5.
5. Gorwitz, R. J. et al. Changes in the prevalence of nasal colonization with *Staphylococcus aureus* in the United States, 2001–2004. *J. Infect. Dis*. 2008. 197, 12261234
6. Patrick G. Guilfoile, I. Edward Alcamo, David Heymann. antibiotic resistant bacteria pages 76-77copyright © by infobase publishing. 2007
7. Zhang, H.-Z., H. Schmidt, and W. Piepersberg. Molecular cloning and characterization of two lincomycin-resistance genes, *lmrA* and *lmrB*, from *Streptomyces lincolnensis* 78-11. Mol. Microbiol., 1992, 6:2147–2157.
8. Tarek Zmantar, Bochra Kouidhi, Hanene Miladi and Amina Bakhrouf. Detection of macrolide and disinfectant resistance genes in clinical Staphylococcus aureus and coagulase-negative staphylococci BMC Research Notes, 2011, 4:453 http://www.biomedcentral.com/1756-0500/4/453.
9. Gillet, Y., B. Issartel, P. Vanhems, J. C. Fournet, G. Lina, M. Bes, F. Vandenesch, Y. Piemont, N. Brousse, D. Floret, and J. Etienne,. Association Between *Staphylococcus aureus* strains carrying gene for Panton- Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. Lancet. 2002, 359:753–759.
10. Barrow, G.I and Feltham, R.K.A. Cowan and Steels. Manual for identification of Medical bacteria. Third edition. Cambridge University Press 2003
11. NCCLS. Performance standards for antimicrobial susceptibility testing: 14th

informational supplement. NCCLS document. 2004, M100-S14.Wayne, PA:.

12. Zhang, K., Sparling, J., Chow, B. L., Elsayed, S., Hussain, Z., Church, D.L., Greson, D.B., Louie, T., and Conly, J.M. New Quadriplex PCR Assay for detection ofmethicillin and mupirocin resistance in simultaneous discrimination of *Staphylococcus aureus* from Coagulase-negative Staphylococci. Journal of clinical Microbiology November 2004, p.4947-4955, vol.42, No.11.
13. Martineau F., Picard J.F., Lansac N., Menard C., Roy H.P., Ouellette M., Bergeron G. M. Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic susceptibility patterns of *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Antimicrob.Agents Chemother*. 2000, 44, 231–238
14. Lim J.A., Kwon A.R., Kim S.K., Chong Y., Lee K., Choi E.C.: Prevalence of resistance to macrolide, lincosamide and streptogramin antibiotics in Gram-positive cocci isolated in a Korean hospital. J. Antimicrob. Chemother. 2002, 49, 489–495.
15. Wertheim, H. F., D. C. Melles, M. C. Vos, W. van Leeuwen, A. van Belkum, H. A. Verbrugh, and J. L. Nouwen. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect. Dis. 2005, 5:751–762.
16. Ellis, M. W., D. R. Hospenthal, D. P. Dooley, P. J. Gray, and C. K. Murray. Natural history of community-acquired methicillin-resistant *Staphylococcus aureus* colonization and infection in soldiers. Clin. Infect. Dis. 2004. 39: 971–979.
17. Fen Qu, Enbo Cui, Tongsheng Guo, Haijing Li, Suming Chen, Liming Liu, Wang Han, Chunmei Bao, Yuanli Mao, and Yi-Wei Tang. Nasal Colonization of and Clonal Transmission of Methicillin-Susceptible *Staphylococcus aureus* among Chinese Military Volunteers. journal of clinical microbiology, 2010, vol. 48, p. 64–69,.
18. Munckhof, W. J., G. R. Nimmo, J. M. Schooneveldt, S. Schlebusch, A. J. Stephens, G. Williams, F. Huygens, and P. Giffard. Nasal carriage of *Staphylococcus aureus*, including community-associated methicillin-resistant strains, in Queensland adults. Clin. Microbiol. Infect. 2009. 15:149–155.
19. Sekiguchi J., Fujlno T., Konosaki H., Nishimura H., Kawana A., Kudo K., Kondo T., Yazaki Y., Kuratsuji T., Yoshikura H., Kirikae T.: Prevalence of erythromycin-, tetracycline-, and aminoglycoside-resistance genes in methicillin-resistant *Staphylococcus aureus* in hospitals in Tokyo and Kumamoto. *Japan J.Infect.Dis*. 2004, 57, 74–77.
20. Strommenger B., Kettlitz C., Werner G., Witte W.: Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in *Staphylococcus aureus*. J. Clin. Microbiol. 2003, 41, 4089–4094.
21. Francis Martineau, Franc¸Ois J. Picard, Nicolas Lansac, Christian Menard, Paulh. Roy, MARC Ouellette, and Michel G. Bergeroni. Correlation between the Resistance Genotype Determined by Multiplex PCR Assays and the Antibiotic Susceptibility Patterns of *Staphylococcus aureus* and *Staphylococcus epidermidis* Antimicrobial Agents and Chemotherapy. 2000, Vol. 44, No. 2,, p. 231–238.
22. Zmantar K, Chaieb F, Ben Abdallah A, Benkahla-Nakb A, Ben Hassen K, Mahdouan A, Bakhrouf. Multiplex PCR Detection of the Antibiotic Resistance Genes in *Staphylococcus aureus* Strains Isolated from Auricular Infections. Folia Microbiol. 2008, 53 (4), 357–362
23. Archer, G. L., D. M. Niemeyer, J. A. Thanassi, and M. J. Pucci. Dissemination among staphylococci of DNA sequences associated with methicillin resistance. Antimicrob. Agents Chemother. 1994, 38:447–454.
24. Kolbert, C. P., J. Arruda, P. Varga-Delmore, X. Zheng, M. Lewis, J. Kolberg, and D. H. Persing. Branched-DNA assay for detection of the *mecA* gene in oxacillin-resistant and oxacillin-sensitive staphylococci. J. Clin. Microbiol. 1998. 36:2640–2644.

4/8/2014