

# Molecular detection of MSRA Erythromycin-Resistant gene in Staphylococcus spp

Osamah M. Hassan \* MSc

## Abstract:

**Background:** The resistance to antimicrobial factors is an increasingly global problem worldwide, especially among nosocomial bacteria. Staphylococci have become one of the common causes of nosocomial infections. Multi drug-resistant staphylococci pose a growing problem for human health.

**Objective:** This study was carried out to evaluate the association between the antibiotic susceptibility patterns and the msrA Erythromycin-Resistant gene in staphylococci isolates obtained from various clinical samples of patients.

**Methods:** A total of 25 staphylococci clinical isolates were collected from the central Health Lab. DNA from each sample was extracted and The gene implicated in resistance to erythromycin (msrA) was amplified using PCR method.

**Results:** The PCR of msr A gene showed positive 16 out of 25 for isolated staphylococci (gave 163 sized amplicons), which results may be considered as an important criterion to treating Staphylococcal infections.

**Conclusion:** The PCR is a useful and practical device for the routine diagnosis of macrolide resistance staphylococci use to be microbiology laboratory work flow. The detection of resistance genes by PCR was more reliable, accurate and specific than other methods of detection.

**Key words :** PCR, msrA, staphylococci, erythromycin

*J Fac Med Baghdad*  
2017; Vol.59, No .1  
Receive Nov. 2016  
Accepted Jan 2017

## Introduction:

The resistance to antimicrobial factors is an increasingly global problem worldwide, especially among nosocomial bacteria. Staphylococci have become one of the common causes of nosocomial infections. Multi drug-resistant staphylococci pose a growing problem for human health. 1,2. Macrolides are antibiotics widely used for the treatment of human and animal infections. The use of these antibiotics has been accompanied by selection of resistant bacteria, e.g. Staphylococci (3). Resistant bacteria, or genetic determinants of resistance, can be transmitted from animals to humans by food stuffs (4). The msrA gene encodes macrolide efflux pump which is mostly present in *S. aureus*, belongs to the ABC transporter family, and stimulate resistance to 14- and 15-membered macrolides (M phenotype) and streptogramins B (5). The structural changes in ribosomal RNA (rRNA) which prevent the connection of macrolides are the other important resistance mechanism conferring high-level resistance. (6) The methylation of rRNA leads to cross-resistance to macrolides, lincosamides and streptogramin B (MLS<sub>B</sub>-resistance), which can be either constitutive (cMLS<sub>B</sub>) or inducible (iMLS<sub>B</sub>) (7). It is very important clinically to find the difference between isolates with iMLS<sub>B</sub> resistance encoded by (erm genes) and isolates with efflux-mediated resistance due to the (msrA gene) (8). These genes, erm(A), erm(C) and msr(A) were found frequently in Staphylococcus, alone or in combination, more than other genes (9,10). The resistant genes detected can be lead to find the best treatment of the infection. combination of molecular and blood culture analyzes would

significantly increase the rate for pathogen detection. This molecular method (PCR) which detects pathogens rapidly complements conventional Timely detection of the active pathogens (11).

## Materials and methods:

**Sample collection,** a total of 25 clinical isolates of staphylococci were isolated from blood, wounds, urine and pus samples. The samples were sent immediately to the microbiology laboratory at genetic engineer institute university of Baghdad. The samples were inoculated onto 5% sheep blood agar plates and incubated at 37°C for 48 h. The isolates were cultured on mannitol salt agar (MSA), which is considered a selective and differential growth medium that is used for encouraging the growth of staphylococci. The selection of staphylococci was on the basis of colony morphology, Gram staining, biochemical tests which are catalase test, DNase test and coagulase tests. Several biochemical tests were carried out to identify the staphylococci. All Gram-positive isolates gave positive results in the Catalase tests. In order to support the previous biochemical test, DNase tests and tube coagulase tests were carried out. The combination of all the biochemical tests increased the sensitivity to identify the staphylococci among the bacterial isolates. (25) isolates showed a positive coagulase test. DNase results were positive in 20 (83.33%) and the rest of (5) isolates (16.5%) were negative for DNase test. DNase production was detected by culturing the isolates on DNase agar, DNase is an extracellular enzyme that cleaved DNA into subunits composed of nucleotides (Oligonucleotides). The appearance of clear zone around bacterial growth was

\*Dept. of Basic Medical Science, Dentistry College, University Al-Mustansiriyah University.  
Osamagenetic@gmail.com

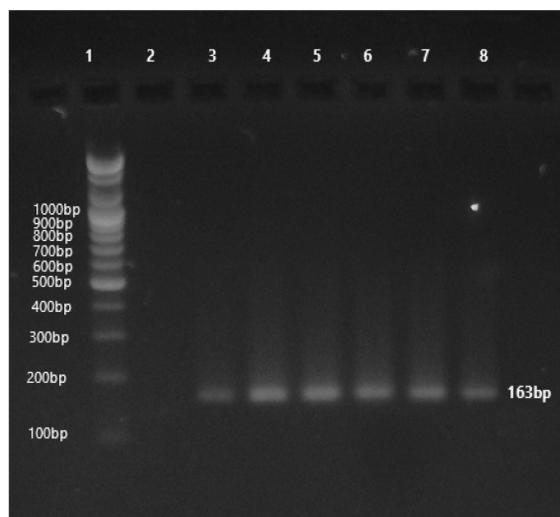
considered as the positive activity that indicated the presence of Deoxyribonuclease enzyme hydrolyses DNA (12).

**Bacterial Genotyping:** The phenol/chloroform extraction method was used for nucleic acid extraction and DNA was precipitated in 1 ml 70 per cent ethanol. The DNA precipitate was dissolved in 50 µl of TE buffer (10 mM Tris chloride-1 mM EDTA(13). PCR cycles consisted of an initial denaturation step (94°C for 5 min) followed by 30 amplification cycles (denaturation at 94°C for 60 seconds, annealing at 55°C for 60 seconds, and extension at 72°C for 60 seconds) with a final extension at 72°C for 5 min. The sequences of the primers (forward 5'-TCCAATCATTGCACAAAATC-3', reverse 5'-AATCCCTCTATTTGGTGGT-3'). PCR product was resolved on a 2% agarose gel and stained with ethidium bromide and visualized under UV transillumination.

### Results:

The presence of the *msrA* gene responsible for erythromycin resistance was confirmed by PCR analysis using primers *msrA* gene.

This study have been revealed the Prevalence of *msrA* gene (56%) in 14 out of 25 staphylococci isolates. Positive strains for *msrA* gene showed a band with 163bp in 2% agarose gel (Figure 1).



**Figure 1:** Agarose gel electrophoresis (2% agarose, for 60 min 5V/cm,) for *msrA* gene (amplified size 163 bp) compared with (100 bp) DNA ladder, line 1 DNA Ladder; lines 3,4,5,6,7 and 8 positive results of bands; lines 2 negative results.

### Discussion

Staphylococcal strains resistant to macrolides and frequently harbour *msrA* gene, which encodes an ATP-dependent efflux pump (14). In this study, 56% of staphylococci isolates were *msrA* gene positive. It was found that *msrA* was detected among the resistant isolates, which further supported the

evidence that efflux mechanism exist in the resistant Isolates. These findings are in contrast with the study by Zmantar et al. (2013) conducted on staphylococci in Tunisia in which an incidence of (*msrA*) 36% positive (15). This finding was also shown by Ding, Z.F et al (2012). It was found that neither *mefA* nor *msrA* was detected among the resistant isolates (16). Franz-Josef et al (2000) also found that Macrolide resistance by efflux due to the *msrA* gene was only 13% in staphylococci isolates (17). On the other hand, our results are in agreement with Almer et al. (2002) (18) and Duran et al. (2012) (19). They found resistance to erythromycin in staphylococci was 55.4 percent due to *msrA* gene that detected by PCR method. None of 44% our staphylococci isolates harboring with *msrA* gene. The resistance of these isolates presented due to the role of other resistance genes. In *S. aureus* isolates can be resistant to macrolides by two mechanisms, ATP-dependent efflux pump which is encoded by the *mrsA* gene as mentioned previously, and also effected by the role of membrane protein that coded by *mef* gene (20). Other mechanisms of resistance done by *erm* genes family that have about forty types of *erm* genes, only one type of *erm* gene expression can lead to resistance against antibiotics (21). In conclusion, Utilization of the conventional PCR technology in the clinical laboratory enable physicians to prescribe suitable antibiotic therapy. The PCR is a useful and practical device for the routine diagnosis of macrolide resistance staphylococci use to the microbiology laboratory work flow. The detection of resistance genes in PCR was more reliable, accurate and specific than other method of detection.

### References

- 1-Livermore DM. Antibiotic resistance in staphylococci. 1. *Int J Antimicrob Agents* 2000; 16 : S3-S10.
- 2-Zapun A., Contreras-Martel C., Vernet T., Penicillin-binding. 2. proteins and beta-lactam resistance. *FEMS Microbiol Rev* 2008; 32 : 361-85.
- 3-Schlegelova J., Babak V., Klimova E., Lukasova J., Navratilov P., Sustackova A., Sediva I., Rysanek D. Prevalence of and resistance to anti-microbial drugs in selected microbial species isolated from bulk milk samples. *J. of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health* 2000; 49:216-225.
- 4-Schlegelova J., Napravnikova E., Dendis M., Horvath RBenedik J., Babak V., Klimova E., Navratilova P., Sustackova A. Beef carcass contamination in a slaughterhouse and prevalence of resistance to antimicrobial drugs in isolates of selected microbial species *Meat Science*, 2004; 66: 557-565.
- 5-Roberts, M. C., Sutcliffe, J., Courvalin, P., Jensen, L. B., Rood, J. Seppala, H. Nomenclature for macrolide and macrolide-lincosamide streptogramin B resistance determinants. *Antimicrob Agents. Chemother.* 43: 2823-2830. 1999.;
- 6-Peche're, J. C. Macrolide resistance mechanisms in Gram-positive cocci. *Int. J. Antimicrob. Agents.* 2001.; 18: 25-28.
- 7-Weisblum B. Inducible resistance to macrolides, lincosamides and streptogramin type B antibiotics—the resistance phenotype,

- its biological diversity, and structural elements that regulate expression – A Review. *J. of Antimicrobial Chemotherapy*. 1985; 16:63–90.
- 8-Leclercq R. Mechanisms of resistance to macrolides and lincosamides Nature of the resistance elements and their clinical implications. *Oxford J. Clinical Infectious Diseases*. 2002; 34: 482–492.
- 9-Luthje P., Schwarz S. Antimicrobial resistance of coagulase-negative staphylococci from bovine subclinical mastitis with particular reference to macrolide lincosamide resistance phenotypes and genotypes. *J. of Antimicrobial Chemotherapy*. 2002; 57: 966–969.
- 10-Bagcigil F.A., Moodley A., Baptiste K.E., Jensen V.F., Guardabassi L.: Occurrence, species distribution, antimicrobial resistance and clonality of methicillin- and erythromycin-resistant staphylococci in the nasal cavity of domestic animals. *Journal of Veterinary Microbiology*. 2007; 121: 307–315.
- 11-Sanlı O., Özdemir M., Feyzioğlu B., Baykan M.: Research on Diagnostic Value of Real-Time PCR in comparison with culture method to detect agents for sepsis. *Advances in Clinical and Medical Microbiology*. 2016 ; 2 : 1-6
- 12-Gaasbeek, E.J., Wagenaar, J.A., Guilhabert, M.R., Woosten, M.M.S., van Putten, M.J.P., van der Graaf-van Bloois, M.L., Parker, C.T., van der Wal, F.J. A DNase encoded by integrated element CJIE1 inhibits natural transformation of *Campylobacter jejuni*. *J. Bacteriol.* 2009; 191: 2296–2306.
- 13-Sambrook J, Fritsch EF and Maniatis T (1989) *Molecular cloning: a laboratory manual*, Cold Spring Harbor Laboratory Press, New York.
- 14-Eady EA., Ross JI, Tipper JL., Walters CE., Cove JH., Noble WC .  
Distribution of genes encoding erythromycin ribosomal methylases and an erythromycin efflux pump in epidemiologically distinct groups of staphylococci. *J. Antimicrob. Chemother.* 1993; 31:211-217
- 15- Zmantar, T., Bekir, K., Elgarsad, S.I., Hadad, O., Bakhrouf, A. Molecular investigation of antibiotic resistance genes in methicillin resistant *Staphylococcus aureus* isolated from nasal cavity in pediatric service . *African J. of Microbiology Research*. 2013; 7: 4414-4421.
- 16-Ding, Z.F., Zhang, H., Tang, W., Tong, C.Y., Li, R.T., Chen, L.X., Pu, L.J., Zhu, Z.B. 2 and Cui, Y.D . Methylase Genes-Mediated Erythromycin Resistance in *Staphylococcus aureus* from Bovine Mastitis in China. 2012. *Israel J. of Veterinary Medicine*. 2012; 67:170-179 .
- 17- Schmitza ,F.J., Sadurskia,R., Kraya,A., Boosa ,M., Geisela,R., Köhrera,K., Verhoeft,J., Fluitb,AD. Prevalence of macrolide-resistance genes in *Staphylococcus aureus* and *Enterococcus faecium* isolates from 24 European university hospitals. *Journal of Antimicrobial Chemotherapy* 2000; 45:891-894.
- 18-Almer, LS., Shortridge, VD., Nilius, AM., Beyer, JM., Soni, NB., Bui, MH., Stone, GG., Flamm, RK. Antimicrobial susceptibility and molecular characterization of community-acquired methicillin-resistant *Staphylococcus aureus*. *Diagn. Microbiol. Infect. Dis.* 2002; 43:225-232.
- 19-Duran N., Ozer B., Duran GG., Onlen Y., Demir C. Antibiotic resistance genes and susceptibility patterns in staphylococci. *Indian J. Med. Res.* 2013; 135:389-396.
- 20-Zmantar, T., Kouidhi, B., Miladi, H., Bakhrouf, A. Detection of macrolide and disinfectant resistance genes in clinical *Staphylococcus aureus* and coagulase-negative staphylococci. *BMC Res Notes*. 2011; 4:453.
- 21-Stefanie, He., Gallert, C. Demonstration of staphylococci with inducible macrolide lincosamide streptogramin B (MLSB) resistance in sewage and river water and of the capacity of anhydroerythromycin to induce MLSB. *FEMS Microbiol Ecol.* 2014; 88:48- 59.