Egypt. J. Rad. Sci. Applic., Vol. **24**, No. 1, pp. 101-115 (2011)

Molecular Studies on Methicillin-Resistant *Staphylococcus aureus*

Z. S. Tawfik, H. A. Mohamed, H. M. Abu Shady* and M. R. Abu Shady*

*Microbiological Radiation Research Dept., National centre for Radiation Research and Technology (NCRRT), P. O. Box; 29 Nasr City, Cairo, Egypt and *Microbiology Dept., Faculty of Science, Ain Shams University, Cairo, Egypt.*

THE PRESENT STUDY of the MecA gene in our clinical isolates has been detected and verified by antibiotic disc diffusion test & nested Polymerase Chain Reaction (PCR). Part of the product of the second PCR was also sequenced. The results indicated 97.7% similarity between the sequences of the mecA gene isolated from an Egyptian *Staphylococcus aureus* strain and that compared from *Staphylococcus aureus* strain no. GI46628 cited at the European Molecular Biology Laboratory (EMBL) database under accession number Y00688 in the region from nucleotide 467 to 875. The effect of γ-radiation on these isolates was observed by plotting the dose survival curves of these isolates and determining their D_{10} values. Their D_{10} values were found to be ranged from 0.44 to 0.66 kGy. Antibiotic sensitivity tests were also carried out after exposure of Oxacillin-susceptible isolate to sub –lethal doses of γ-radiation.

Results indicated that *Staphylococcus aureus* isolates which were sensitive to oxacillin discs were found by PCR to harbor the mecA gene in their genomes. Also, exposure of a sensitive isolate to sublethal doses of gamma radiation led to the emergence of a oxacillin-resistant variant which could be a serious problem in case of using sub-lethal doses of radiation for the sterilization of medical products.

Keywords: *Staphylococcus aureus, γ-rays, mecA gene, antimicrobial agaent, resistance.*

Bacterial resistance to antibiotics is a serious global problem which includes strains of β-lactam resistant *Staphylococcus aureus*, in general and methicillin-resistant *Staphylococcus aureus* (MRSA) in particular. MRSA has emerged as a major nosocomial pathogen, causing therapeutic and infection control problems in many hospitals worldwide. Serious infections caused by Gram-positive pathogens are increasingly difficult to treat because of pathogens such as MRSA, vancomycin-resistant enterococci (VRE) and penicillin-resistant

Streptococcus pneumoniae. The more recent emergence of vancomycin-intermediate and -resistant MRSA (VISA and VRSA) has further compromised treatment options. Reports of resistance and clinical failures with newer antimicrobial agents such as linezolid and quinupristin/ dalfopristin are also emerging. Consequently, there is a clinical need for new antimicrobial agents that have suitable pharmacokinetic properties and safety profiles, with activity against these Gram-positive pathogens (Pastagia *et al.*, 2011).

Classical MRSA is transmitted nosocomially, is enhanced by prior antibiotic use (especially cephalosporins) and is prominent in particular surgical specialties and intensive care units. In some specialties the readmission of patients for repeated procedures has also been a cause for persistence (Zetola *et al.*, 2005). Genetic mutation and gene transfer are thought to be of great impact on the emergence of new born strains. Environmental conditions, through physical and chemical factors, may dramatically affect this process, promoting gene transfer and recombination between different bacterial strains. Besides the desired effect of ionizing radiation on the materials being irradiated, the radiation bears a continuous influence on the surrounding environment. In the course of routine operation of industrial irradiation facilities, the microflora in the surrounding environment is exposed to several cycle of radiation stress. Such practice might pose a risk of producing and preferentially selecting microbial mutant having enhanced radiation resistance as well as changes in other properties, such as sensitivities to antibiotics (Tawifik, 1990). The present study aims at accurate detection of oxacillin-resistant *Staphylococcus aureus* clinical isolates and studying the role played by radiation in the development of oxacillin resistance.

Materials and Methods

Samples collection

This study was performed on clinical bacterial strains. All the samples (28) in this study were collected from different general hospitals in Egypt. They were isolated from patients during their periods of hospitalization. The samples were collected from the culture of different specimens, such as blood, urine, sputum, pus and vaginal swabs.

Culture media for isolation and antimicrobial agent (Oxoid, England) used

Nutrient Agar: Peptone 5, Sodium Chloride 5, Beef extract 1, Yeast extract 3 and agar 15 (g/L); Baird Parker Media: Peptozimatic 10, Beef Extract 5, Yeast Extract. Sodium Pyruvate 10, Glycine 12, Lithium Chloride 5, Agar 15 (g/L);

Thioglycolate Liquid Media: Peptone 5, Sodium Chloride 5, Beef Extract 1, Yeast Extract 2, Dextrose 5, Thioglycolate 1.1, Methylene Blue (2%) (g/L). The antibiotic discs used in the study for antibiotic sensitivity tests were Ampicillin (10µg/disc), Oxacillin (Methicillin, Prostaphlin A) (1µg/disc), Tobramycin (10µg/disc), Chloramphenicol (30µg/disc), Rifampicin (30µg/disc). All of the collected samples were first cultivated on nutrient agar slants, and then grown on plates of nutrient agar medium using the streak plate method. The plates were incubated at 37˚C for 24 to 48 h. The shape of colonies was described for identification. Identification was carried out as recommended by (Ryffel *et al.*, 1992). From each tested isolate inoculums of 10^8 c.f.u./ml were prepared in sterile isotonic saline. Antibiotic sensitivity tests were performed by using the disc diffusion method (Tokue *et al.*, 1992). The ready made antibiotic discs were distributed on the surface of the inoculated agar plates. Inhibition zones were measured in mm after 24 h incubation period at 33-35˚C. Ranges of resistance and susceptibility were determined according to Bannerman *et al.* (2003), Table 1.

TABLE 1. Interpretive criteria in (mm) for antimicrobis disk diffusion test.

Antibiotic	Abb.	Disc Content (µg)	Inhibition Zone Diameter in mm.		
			Resistant	Moderately Susceptible	Susceptible
Tobramycin	Tob	10	12	13-14	15
Chloramphenicol	C	30	12	13-17	18
Ampicillin	AM	30 10	13 11	14-16 11-12	17 12
Rifampin	RA	30	16	17-19	20
Oxacillin	Ox	30	10	11-12	13

Irradiation facility

The test pieces were irradiated in their final package to different absorbed dose values using Gamma-cell 220 ^{60}Co source with dose rate of 19.8kGy/ h at NCRRT. The doses delivered to the collected samples were 1, 2, 4 and 6kGy, respectively.

Extraction of genomic DNA

The bacterial isolates were cultured on nutrient agar plates and incubated at 37˚C for 24-48 h. Up to 100 colonies from each agar plate were suspended in 3ml TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH is adjusted at 8) and then incubated in a dry heating block at 95˚C for 15 min. The cell suspensions were sonicated in a Vibro Cell (Sonics & Materials) sonicator for a period of 15 min with repeated cycles of 10 seconds sonication followed by 5 seconds pause for a total of 60 cycles.

One hundred and eighty microlitres of a high salt solution TKM (pH 8, 10mM Tris-HCl, 10 mM $MgCl_2$, 10 mM KCl, 0.4 mM NaCl and 2 mM EDTA) and 10µl of 20% SDS solution were added to 200µl of the sonicated culture. The mixture was then incubated at 65˚ C for 10 min. One hundred micro liters of NaCl (5M) were added and mixed well by vortex, followed by centrifugation at maximum speed for 10 min to precipitate proteins. A tight pellet of proteins and cell debris is formed. The supernatant is removed and released into a clean eppendorf tube containing double the volume absolute isopropanol. Centrifugation at maximum speed precipitates the DNA pellet. Isopropanol is decanted and the pellet is washed with 70% ethanol. The pellet is air dried and the DNA is resuspended in 50 µl TE buffer (Singer, 1998). The concentration of the DNA was determined by measuring the absorbance of DNA at a wavelength equal to 260 nm by a Spectro UV-VIS RS digital spectrophotometer (Labomed Inc.).

Detection of mec-A gene in staphylococcus aureus by PCR

For the initial PCR two oligonucleotide primers were synthesised of according to the DNA from *Staphylococcus aureus* TK784 (EMBL database accession number Y00688). Outer primer 1A nucleotide 37 to 57 of mec-A gene. Outer primer 2A (the reverse complement of nucleotides 1827 to 1854). Nested primer 1B (nucleotides 466 to 489) and, Nested primer 2B the reverse complement of nucleotide 1549 to 1573 (Unal *et al.,* 1992). They were performed with primers 1A and 2A covering the nucleotides from 37 to 1854 of the mecA gene, Table 2. The nested PCRs were performed on 5 µl aliquots of initial PCR products as templates, with primers 1B and 2B covering nucleotides from 466 to 1573 of the gene under study (Table 2).

TABLE. 2. The sequences of the primers used in the amplification by PCR.

Primer	**Position 5'— 3'**	**Length** (base)	**Sequence**
1A	37 to 57	20	5-GTTGTAGTTGTCGGGTTTGG-3.
2A	1827 to 1854	27	5-CCACCCAATTTGTCTGCCAGTTTCTCC-3.
1B	466 to 489	23	5-GACCGAAACAATGTGGAATTGGCC-3.
2B	1549 to 1573	24	5-CACCTTGTCCGTAACCTGAATCAGC-3.

Fifteen micro litters of template was amplified in a 50 µl reaction mixture containing final concentrations of 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 2 mM deoxyribonucleotides, 25 pM of each primer and 1 U Taq DNA polymerase (Unal. *et al.,* 1992). The PCR was performed on a Biometra

thermocycler (UNO-thermoblock RS232). A thermal step program recommended by (Unal. *et al.,* 1992), denaturation at 94˚C for 1 min, annealing at 55˚C for 1 min and primer extension at 72˚C for 2 min, for a total of 30 cycles was used. The PCR products were loaded to 0.8% agarose gel (molecular biology grade) which previously dissolved in 100 ml TAE buffer and 10⁒ ethidium bromide was added. The samples are mixed with 10 X loading buffer which is composed of 20% Ficoll., 0.1 M Na_2 EDTA (pH 8), 1 % SDS, 0.25% bromophenol blue and 0.25% xylene cyanol and loaded in the wells with micropipette along side AΦX 174 DNA-Hae III DNA digest marker and -A lamda Hind III digest DNA-high molecular weight The markarers were kindely provided by Tripharma Egypt Co. The power supply Biometra programmable high voltage power pack [pp 3000], is connected and adjusted at 100 volts. The gel is visualized over an ultraviolet transilluminator with wavelength (UVP dual-intensity transilliuminator model TM-20E) and photographs are captured with an instant polaroid gel camera.

Analysis of the PCR products by the use of the gel documentation software

The bands produced by the initial and second PCRs were analysed with the use of the Alpha Ease Fc (Digital documentation 1000) analysis software on an alpha innctech Co. computer system in finger printing lab in NCRRT.

Sequencing of DNA fragment

PCR purification was performed by using a Qiagen purification kit (Qiaquick gel extraction kit). The qiaquick gel extraction kit is suitable for gel extraction or cleanup of DNA (70 bp to 10 kb) from enzymatic reactions.

Automated DNA sequencing system uses a single or multiple primers that are fluorescently labelled. The PRISM sequenase fluorescent dye-labeled dideoxy nucleotide kit (AB Applied Biosystem Inc.) is optimized for use with PRISM 310. The following mixture was placed in an 0.5 ml microcentrifuge tube: seven µl double stranded DNA template, 1-2 µl Primers dye-labeled primer (1p mole/µl), 5µl 5x Excel buffer, 2.5µl 10x sequencing buffer and 1µl Sequitherm™ DNA polymerase. The temperature profile used was 95° C for 30 sec, 56°Cfor 15 sec and 70°C for 30 sec for a total of thirty cycles.The DNA sequencing was carried out at Agriculture Genetic Engeineering Research Institute, Agriculture Research Centre.

Determination of the dose response curves of the isolates under study

The method used was that described by (Christen and Kristensen, 1981) for the preparation of test pieces. Test pieces of *Staphylococcu aureus* tested strains were prepared by spotting 20 µl droplets containing 10^7cfu/ml on a sterile polyethylene sheet. They were left to dry at room temperature in a laminar air-flow cabinet. The dried droplets were then covered and heat sealed with another layer of sterile polyethylene sheet. The test pieces were irradiated in their final package to different absorbed dose values, ranging from 1 to 6 kGy. Viable count assay was carried out for plotting the logarithm of the number of surviving cells against the radiation doses. D_{10} values were calculated by using the relation:

$$D_{10} = \text{Radiation dose} / \log N_o - \log N_1$$

where, N_o= initial count and N_1 = count at specific dose.

Antibiotic sensitivity test of irradiated Staphylococcus aureus isolates at 6kGy

The irradiated test pieces at 6 kGy were opened as mentioned before and distinct colonies were selected and tested for their sensitivity to oxacillin discs. Non irradiated isolate was used for comparison.

Results

In the present study *Staphylococcus aureus* has been isolated from several hospital environments and from various types of clinical samples A total of 28 isolates were identified, seven of them were confirmed by biochemical tests and growth on Baird Parker medium to be *Staphylococcus aureus* (no. 1-7) Therefore these isolates were chosen to be subjected to further study .The isolate no 8 to 28 were identified as *Staphylococcus* species other than *Staphylococcus aureus*. The phenotypic behaviour of the *Staphylococcus aureus* tested strains towards oxacilin was determined by using the antibiotic disc diffusion method. Accordingly, two of the isolates, 1 and 5 were found to be oxacillin-resistant, while, the others showed an inhibition zones ranged from 11 to 13 mm in diameter around oxacillin discs (oxacillin is a surrogate compound for methicillin). Isolate no.1 was resistant to oxacillin, ampicillin, and it was susceptible to the other antibiotics. Isolate 5 was resistant to oxacillin only and it was susceptible to the other antibiotics, while isolates 2,3,4,6 and 7 were found to be susceptible to all of the antibiotics used in this test with the exception of oxacillin which showed moderate and susceptible reaction. Also, in this study, genotypic behaviour of all staphylococcus strains was studied by using the PCR technique. The isolates in this study were further tested for the

presence of the mecA gene by using the PCR technique. The extracted DNA was loaded on 0.8% agarose gel, and the concentration of each DNA sample was measured. The concentrations ranged from 0.8 to 1.7µg/µl. In the initial PCRs performed on the resistant isolates 1 and 5, band at 1.8 kbp amplicons were produced (Fig.1). These bands, 1.8 kilo bp amplicons, represent the mecA gene. The specific primers 1A and 2A used in the initial PCR amplify the region from the nucleotide 37 to 1854 of the mecA gene. In the case concerning these two isolates (1 & 5) the phenotype and genotype coincided.

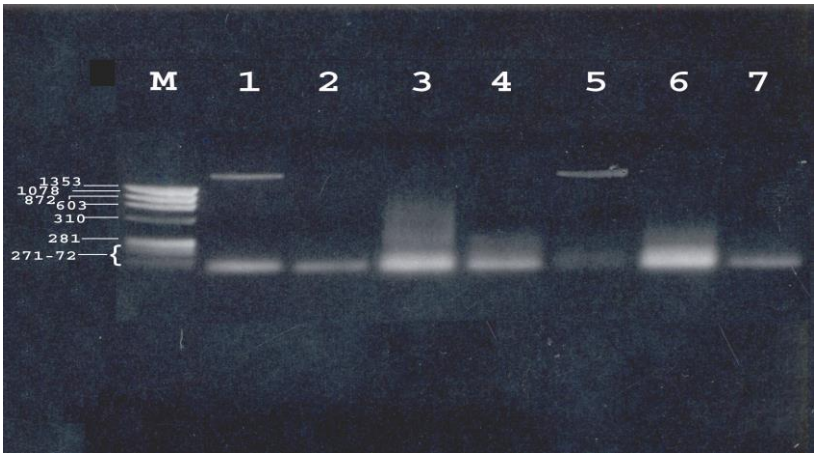

Fig. 1 Agarose gel electrophoresis of specific first PCR product 1818 bp resulted from ampilification of genomic DNA of Staphylococcus aureus using 1A and 2A primers flanking the gen.

A nested PCR is used to detect the presence of mecA gene which confers resistance to oxacillin in *Staphylococcus aureus*. While primers B1 and B2which amplify the region from nucleotide 466 to 1573 of the mecA gene were used in the second PCR to verify its presence by producing 1.1 kb fragments of this gene (Fig.2). The first PCR product was used as a template for the second PCR. The first and second PCR products were loaded on 0.8% agarose gels for electrophoresis. The lengths of the products were measured by a ϕ X 174 DNA marker. Isolates 1 and 5 gave positive results for the detection of the mecA gene in the first PCR. Isolates 1, 2, 5 and 6 gave positive results for the detection of the mecA gene in the second PCR. The *Staphylococcus aureus* isolates 2 and 6 which were found to be susceptible to oxacillin disc did not produce the 1.8 kbp mecA amplicons in the initial PCRs but, gave positive results in the second PCR by showing 1.1 kilo bp fragments of the mecA gene.

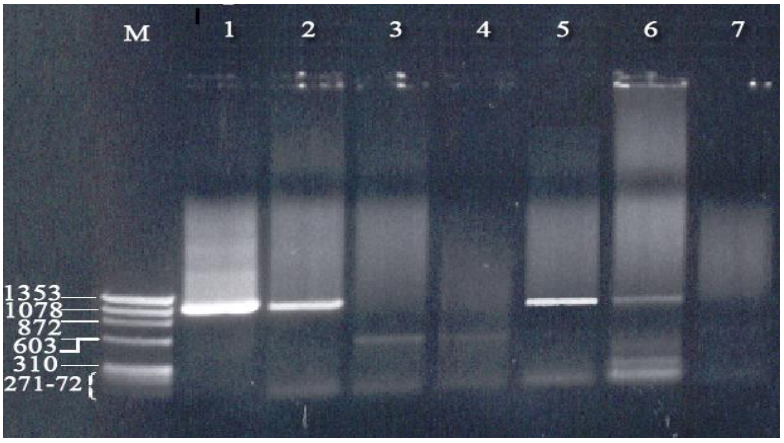

Fig. 2 Agarose gel electrophoresis of specific second PCR product (1084 bp) resulted from amplification of initial PCR product of *Staphylococcus aureus* using1B and 2B primers flanking an inner fragment of mec A gene.

Table 3. shows the gel documentation analysis of the initial PCR products of isolates 1 and 5. It is clear from the results that isolates 1 and 5 gave positive results in the initial PCR by producing 1818bp bands which represent the mecA gene.

TABLE 3. The gel documentation analysis of the initial pcr product for isolate 1 & 5.

Isolates No.	Gel Documentation Analysis			
	Band No.	Mwt	Band %	RF
1	1	1818	17.5	0.15
5	1	1818	28.5	0.15

The results of the analysis of the second PCR product of the isolates 1,2,5,6 indicating positive results in the second PCR by producing 1084bp bands, Table 4. Accordingly these isolates are confirmed to harbor the mecA gene which confers resistance to methicillin/amoxicillin.

TABLE. 4. The gel documentation analysis of the second PCR product for isolate No. 1, 2, 5 and 6.

Isolates No.	Gel Documentation Analysis			
	Band No.	Mwt	Band %	RF
1	1	1084	21.8	0.53
2	1	1084	17.5	0.53
5	1	1084	16.4	0.53
6	1	1084	6.34	0.53

Comparison was made between the sequences of the mecA gene isolated from an Egyptian *Staphylococcus aureus* strain (isolate No. 1) (Fig 3) and *Staphylococcus aureus* strain no. GI46628 cited at the EMBL database under

accession number Y00688 in the region from nucleotide 467 to 875. From Table 5. and Fig 3. the degree of similarity between the 2 sequences is calculated to be 97.7%.

TABLE 5. Comparison of the sequences of the mecA gene isolated from an Egyptian *Staphylococcus aureus* strain (isolate 1) and that isolated from *Staphylococcus aureus* strain no. GI46628 cited at the EMBL database.

Nucleotide No.	Mutation
34	***A*** inverted to ***T***
89	***C*** inverted to ***G***
94	***G*** inverted to ***C***
135	***A*** inverted to ***T***
309	***C*** inverted to ***G***
311	***AAA*** deleted
391	***C*** inverted to ***G***

Two types of mutations were detected: Inversions at nucleotides 34, 89, 94, 135, 309 and 391. While there was one deletion mutation found at nucleotide 301 to 330 since AAA sequence was found to be deleted in the Egyptian *Staphylococcus aureus* isolate under study (Table 5).

1 GACCGAAACA ATGTGGAATT GGCCAATACA 30
31 GGATCACATA TGAGATTAGG CATCGTTCCA 60
61 AAGAATGTAT CTAAAANAGA TTATAAAGGA 90
91 ATNCCTAAAG AACTAAGTAT TTCTGAAGAC 120
121 TATATCAACA ANAANTGGAT CAAAATTGGG 150
151 TACAAGNTGA TACCTTCGNN CCACTTTAAA 180
181 ACCGTTAAAA AAATGGATGN ATATTTAAGT 210
211 GATTTCGCAA AAAAATTTCA TCTTACAACT 240
241 AATGAAACAG AAAGTCGTAA. CTATCCTCTA 270
271 GAAAAAGCGA CTTCACATCT ATTNGGTTAT 300
301 GTTGGTCCGA TTAACTCTGA NGAATTAAAA 330
331 CAAAAAGAAT ATAAAGGCTA TAAAGATGAT 360
361 GCAGTTATTG GTAANAAGGG ACTCGAAAAA 390
391 GTTTACGANA AAAGCTC 420

Fig. 3. Nucleotide sequence of part of the mecA gene of *Staphylococcus aureus*.

The dose survival curves of gamma irradiated tested strains to dose level ranged from 1-6 KGy were drown and their D_{10}-values were determine (is the dose required to inactivate microbial population by 90%).

The D_{10} – values of the isolates of all tested isolates ranged from 0.44 to 0.66 kGy. The dose response curves were also plotted to investigate the radiation resistance of each isolate (Fig. 4). The test pieces of isolate No. 6 were exposed to sublethal doses of gamma radiation (kGy) to investigate the role of gamma radiation in the induction of changes in antibiotic sensitivity. This isolate was selected since it showed susceptibility to oxacillin in the antibiotic sensitivity test but was found by PCR to harbor the mecA gene. Each colony represents a single cell of the original strain which survived the radiation treatment. Several colonies were selected for antibiotic sensitivity test against oxacillin discs. One of the tested colonies was found to be oxacillin-resistant. This strain is referred to be as "variant strain".

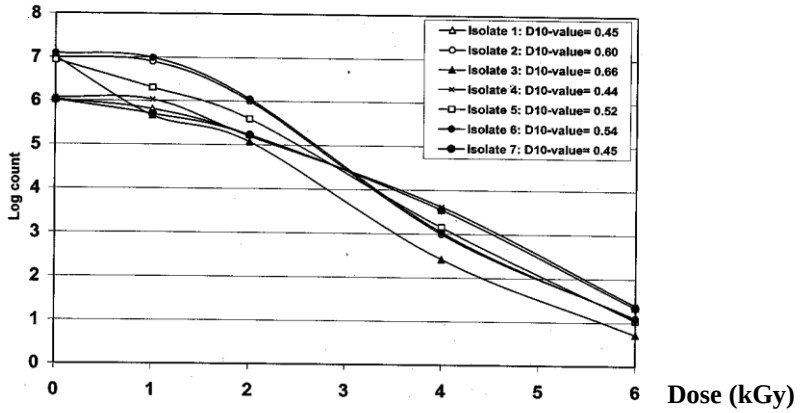

Fig. 4. The D_{10}- values of the Staphylococcus oureus strains ranged from 0.44 to 0.66 kGy.

Discussion

MRSA is an established nosocomial pathogen, but has recently begun to appear in the community. The clones in the community may not have originated in the hospital setting, and are referred to as community-acquired MRSA (CA-MRSA) (Hall *et al.*, 1988). Community-acquired MRSA is becoming an important public-health problem (Hisato *et al.*, 2011). The fact that isolates 2 and 6, did not give positive results in the initial PCR but, gave positive results in the second PCR may be attributed to one of the reasons mentioned by (Unal *et al.*, 1992). Amplification of the mecA gene in the first PCR was not efficient enough to give visible products on the agarose gel. The first PCR amplification could have been hindered by mutations found in the sites complementary to the

primers used in this reaction. As in case of primer A2 which is complementary to the region from nucleotide 1827–1854. This region harbors mutations at the position 1834 of the mecA gene. Therefore, these isolates (2 & 6) also harbor the mecA gene and accordingly, should be considered as methicillin-resistant *Staphylococcus* aureus. In the studies carried out by (Wayne, 2005), strains classified as methicillin-susceptible by susceptibility tests were found by PCR to harbor the mecA gene and accordingly should be reclassified as methicillin-resistant. Ryffel *et al.,* 1992) Observed that when the mecA gene is under the control of mecR1 (the strong mecA repressor), its derepression can be very slow. As a consequence, detection of such methicillin-resistant staphylococcal strains would be difficult and unpredictable with standard susceptibility tests.

Another theory which could explain the masking of methicillin resistance of *Staphylococcus aureus* is that reported by both (Bannerman, 2003 and Wayne, 2005) which states that: accurate detection of oxacillin/methicillin resistance can be difficult due to the presence of two subpopulations (one susceptible and the other resistant) that may coexist within a culture of staphylococci. All cells in a culture may carry the genetic information for resistance, but only a small number may express resistance in vitro. This phenomenon is termed heteroresistance and occurs in staphylococci resistant to penicillinase-stable penicillins, such as oxacillin. Cells expressing heteroresistance grow more slowly than the oxacillin-susceptible population and may be missed at temperatures above 35°C (Wayne, 2005).

From the results obtained in our study, disc diffusion method is not sufficient enough for accurate detection of antibiotic resistance. This method is solely relied on by physicians to prescribe the antibiotics used in the treatment of all kinds of bacterial infections. PCR detection of mecA gene is independent of its level of expression and therefore overcomes the shortcoming of conventional susceptibility tests (Ryffel *et al.,* 1992). PCR can be used as a technique which detects antimicrobial resistance in cases where the expression of the resistance genes is delayed. It can also detect the presence of genes responsible for antimicrobial resistance in mixed populations where either resistant and susceptible strains or subpopulations coexist. To avoid missing the growth of MRSA in a heteroresistant population the CLSI (Clinical Laboratory Standard Institute) recommends incubating isolates being tested against

oxacillin, methicillin, or nafcillin at 33-35°C (maximum of 35°C) for a full 24 hours before reading. Farrag (2001) stated that physical factors, such as radiation may also affect the development of drug resistance and slime production as on important virulence factor. An important virulence factor is slime production. Slime-producing strains of MRSA and *Pseudomonas aeruginosa* show higher antibiotic resistance than non-producing strains since slime acts as a mechanical barrier against antimicrobial agents. The production of slime is also associated with more severe infections since it can prevent phagocytosis and interfere with host immunity (Takahashi *et al.*, 1997).

Farrag and Saleh (1997) and Farrag (2001) reported that the low doses of radiation had an effect on the antimicrobial activity, DNA content, ploidy pattern growth and adherence ability of strains of *Pseudomonas aerugi nosa* and *Staphylococcus epidermidis.* The dose survival curves of the isolates were drawn by plotting the number of viable cells against gamma radiation dose. Simple exponential-relation dose survival curves of type A were obtained in case of isolates 3 and 5. This type of relation shows a constant slope over the entire dose range. While the dose survival curves of the other isolates were characterized at the beginning with a small shoulder where n=1 and followed by a linear relationship.D_{10} values are in direct proportionality with the radiation resistance of the microorganism. The action of radiation on a microorganism is influenced by species, concentration, chemical composition of the media, physical state of the media and post irradiation storage condition (Lowrence, 1971 and Ito and Tamura, 1993).

The exposure of oxacillin-susceptible isolate 6 to sublethal dose of gamma radiation (at 6 kGy), lead to the emergence of a new resistant variant from among the surviving fraction of cells which were tested for oxacillin-sensitivity after exposure to radiation. This finding is in accordance with that of Abshire *et al.* (1980) who reported that exposure of *Staphylococcus aureus* strains to sublethal doses of gamma radiation resulted in modifications in the biochemical as well as other properties of the culture. This result is in agreement with (Tawfik, 1990), the study indicated the, development of resistant mutants was due to exposure to 15 kGy. The resistant isolate appeared as separate colonies distributed inside the inhibition zone formed against cephaloridine. This inhibition zone disappeared completely after 24 h, indicating the development

of resistant mutants over an extended period of time.

Conclusion

The PCR technique is recommended to be used for the accurate detection and monitoring of drug resistance in pathogenic bacteria. In cases where such a technique is not available it is recommended to incubate the isolates being tested against oxacillin, at 33-35˚C (maximum of 35˚C) for a full 24 h before reading the results. Delivery of substerilizing radiation doses could lead to the emergence of resistant strains in the contaminated items. So, caution should be taken while determining the dose setting protocol for each item being sterilized or decontaminated by radiation.

Acknowledgment

Special thanks are due to our colleagues at the finger-printing lab, and the department of microbiology at NCRRT, Atomic Energy for their help and support during this work.

References

Abshire, R. L., Bain, B. and Williams, T. (1980) Resistance and recovery studies on ultraviolet-irradiated spores of *Bacillus pumilus. Appl. Environ. Microbiol.*, **39**, 695.

Bannerman, T. L. (2003) *Staphylococcus, Micrococcus* and other catalase-positive cocci that grow aerobically. In P.R. Murray, E.J. Baron, J.H. Jorgensen, M.A. Pfaller, R.H. Yolken (eds.), Manual of Clinical Microbiology 8 th ed. ASM Press, Washington, D.C., pp. 45-52.

Farrag, A. H. (2001) In vitro effect of low radiation doses on some pathogenic bacteria. Its relation to surface hydrophobicity adherence and viability on plastic catheter materials. *J. Biolog. Sci.*, **1**, 966. .

Farrag, A. H. and Saleh, M. A. (1996) Changes in DNA content, ploidy pattern and radiosensitivity before and after test dose radiation in some microorganisms. isolated from urinary transitional cell carcinoma. *Egyp. J. Nat. Cancer Inst.*, **8**, 213.

Hall. E. J., Astor, M., Bedford, J., Borek, C. and Curtis, S. B. (1988) Basic Radioboilogy. *Am. J. Clin. Oncol.*, **11**, 220.

Hisato, K., Ito, T., Matsunago, N. and Komatsu, M. (2011) Dissemenation of Multiple MRSA clones among community-associated methicillin-resistant *Staphyloccous aureus* infections from Japanese Children with impetigo. *J. Inf. Chemother.*, Under press.

Ito, H. and Tamura, T. (1993) Comparative sensitivity of *E. coli* between gamma rays

and ultraviolet rays. *Shokuhin-Shosha*, **28**, 11.

Christensen,E.A. and K.ristensen,H. (1981) Radiation Resistant Microorganisms Isolated from Textiles. *Acta. Path. Microbiol. SC and Sect. B.*, **89**, 302.

Lawrence, D.E. (1971) Cellular radiobiology. Lawrence, D.E. Ed. Williams Sons, Ltd, London, pp. 367-392.

Pastagia, M., Euler, C., Chahales, P., Fuentes-Duculan, J., Krueger, J. G. and Fischetti, V. A. (2011) A novel chimeric lysin shows superiority to mupirocin for skin decolonization of methicillin-resistant and -sensitive Staphylococcus aureus strains. *Antimicrob Agents Chemother.,* **55,** 738.

Ryffel, C., Kayser, F.H. and Berger, B. (1992) Correlation between regulation of mecA transcription and expression of methicillin resistance in staphylococci. *Antimicrob. Agents Chemother.,* **36**, 25.

Singe, H. W. (1998) DNA isolation from Staphylococcus aureus. *J. Bacterial.*, **216**, 771.

Takahashi, A., Yomoda, S., Kanda, T., Fuku mura, Y., Ohkubo, T., Inoue, M. and Kobayashi, I. (1997) Slime formation as a marker of serious infection with methicillin-resistant *Staphylococcus aureus*. *J. Med.*, **28**, 87.

Tawfik, S. Z. (1990) Antibiotic sensitivity of the microflora of high-level radiation environment. *Nuclear Sci. J.*, **27**, 5648.

Tokue, Y., Shoji, S., Satoh, K., Watanabe, A. and Motomiya, M. (1992) Comparison of a polymerase chain reaction assay and a conventional microbiologic method for detection of methicillin-resistant *Staphylococcus aureus. Antimicrob. Agents Chemother.*, **36**, 6.

Unal, S., Hoskins, J., Flokowitsch, J. E., Wu, C. Y., Preston, D. A. and Skatrud, P. L. (1992) Detection of methicillin-resistant staphylococci by using the polymerase chain reaction. *J. Clin.Microbiol.*, **30**, 1685.

Wayne, P. A., (2005) Clinical and Laboratory Standards Institute, Performance standards for antimicrobial susceptibility testing.CLSI approved standards. pp. 100-115.

Zetola, N., Francis, J. S., Nuermberger, E. L. and Bishai, W. R. (2005) Community-acquired meticillin-resistant *Staphylococcus aureus*: an emerging threat. *Infect.Dis.*,**5**, 275.

(Received: 22/06/2011;

accepted: 25/08/2011)

دراسات جزيئية على ستافيلوكوكس أوريوس المقاومة للميثيسللين

زهيرة سيد توفيق ، و هبه عبد الحميد محمد ، و هاله محمد أبو شادى* ، و محمد رمضان أبو شادى*

قسم الميكروبيولوجيا الإشعاعية ، المركز القومى لبحوث وتكنولوجيا الإشعاع* ، ص.ب. ٢٩ مدينة نصر و *قسم الميكروبيولوجى ، كلية العلوم ، جامعة عين شمس.

تهدف هذه الدراسة إلى التعرف على العامل الوراثى المسئول عن المقاومة الميثيليسللين فى ستافيلوكوكس أوريوس المعزولة من عينات مرضى أثناء تواجدهم فى مستشفيات عامة مصرية.

وقد تم كشف وجود جين الـ mecA لبعض المضادات الحيوية منها اوكسيسلين فى السلالات المعزولة عن طريق كل من إختبارات الحساسية بالأقراص وتقنية تفاعل البلمرة المتسلسل وتم عمل تحليل لتتابع القواعد النيرتوجينية لجزء من هذا الجين وقد تمت دراسة تأثير الإشعاع الجامى على هذه العزلات عن طريق رسم منحنيات البقاء وتحديد قيم D_{10} لهذه السلالات كما تم عمل إختبار الحساسية ضد المضاد الحيوى بعد التشعيع للتعرف على تأثير إحدى العوامل الفيزيائية مثل أشعة جاما والتى تستخدم فى تعقيم المستحضرات الصيدلية ومعالجة.

وقد وجد أن عزلات الستافيلوكوكس أوريوس التى أظهرت حساسية للأوكسيسلين إنما تحتوى الجين المسئول عن مقاومة هذا المضاد الحيوى وذلك عن طريق تفاعل البلمرة المتسلسل لذلك ينصح بعدم الإكتفاء بإجراء إختبار الحساسية بالأقراص فقط فى تحديد مقاومة السلالات البكتيرية للمضاد الحيوى كما أن تعرض السلالات لجرعات إشعاعية أقل من الجرعة الكافية للتعقيم يساعد على ظهور أنواع جديدة مقاومة تضر بعمليات التعقيم بالإشعاع.