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Detection of *eap* Gene in Clinical Isolates of *Staphylococcus aureus* Before and After *In vitro* X-Irradiation and its Association with Antimicrobial Susceptibility

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Abstract: Extracellular adherence protein (Eap) constitutes an important virulence factor enhancing binding and internalization of *Staphylococcus aureus* into eukaryotic cells. In the present study 63 *S. aureu*s isolates from cancer patients were examined for the existence of the Eap-encoding gene (*eap*) before and after *in vitro* X-irradiation using PCR amplification to determine whether this gene could be used as a molecular diagnostic target for sensitive detection and identification of *S. aureus* infections in X-ray treated cancer patients. Also, to investigate the relations to their antimicrobial sensitivities. The prevalence of the *eap* gene was (100%) before irradiation however, only (55.56%) were shown to harbor this gene after irradiation ($P<0.001$). The incidence of linezolid and vancomycin susceptibility was high (100%), while, it was (97.14%) & (94.29%) for Eap-bearing isolates after irradiation, respectively. Interestingly, relations between *eap* presence and change in the antimicrobial sensitivity pattern before and after irradiation were highly significant for amoxicillin/clavulanic acid ($P = 0.000$), chloramphenicol ($P = 0.006$), levofloxacin ($P= 0.003$), meropenem ($P = 0.000$), minocycline ($P = 0.000$), oxacillin($P = 0.000$) and penicillin ($P=0.002$) and significant for quinupristin/dalfopristin ($P= 0.038$) indicating the association between missing the encoding gene after irradiation and shifting to antibiotic susceptibility.

Key words: *Staphylococcus aureus* · Extracellular adherence protein (Eap) · Antimicrobial susceptibility pattern · *In vitro* X-irradiation

INTRODUCTION

Staphylococcus aureus is a versatile pathogenic bacterium recognized as a worldwide human health problem. *S. aureus* is responsible for a wide spectrum of infections, ranging from minor skin and wound infections to severe disseminated diseases, such as osteomyelitis, endocarditis and sepsis, particularly in the immunocompromised [1]. The capacity of *S. aureus* to cause disease depends on the adaptation to the host and the secretion of many virulence factors acting in concert [2]. Among a high number of virulence factors, *S. aureus* produces a wide range of extracellular matrix binding proteins (ECMBPs), which are proposed to contribute to successful colonization and persistence at various sites in the host [3]. ECMBPs are also known as microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) and receptions [4]. The anchorless extracellular adherence protein (Eap), also designated Map (major histocompatibility [MHC] class II analogous protein), is 45–70 kDa molecule of the group of secreted expanded repertoire adhesive molecules (SERAM), with a broad spectrum of interactions to host extracellular matrix components (ECM) [5, 6]. Eap shows structural homology to the C-terminal domain of bacterial superantigens but lacks superantigen activity [7]. It has also been shown that Eap binds to many plasma proteins, including fibrinogen, fibronectin and prothrombin, enhances the binding and internalization of the microorganism into eukaryotic cells, inhibits wound healing, plays a role as an imunomodulating protein by interfering with the T-cell function and also functions

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as a potent angiostatic agent [8-11]. In addition, Chavakis *et al.* [12] demonstrated direct interaction of Eap with the host adhesive proteins intercellular adhesion molecule 1 (ICAM-1) *in vitro*. The Eap-encoding gene (*eap)* is highly conserved among *S. aureus* clinical isolates and appears to be particularly suitable for molecular diagnostics of *S. aureus*. [13]. Medical electron linear accelerator is important equipment used in radiotherapy departments worldwide. In radiation therapy, high-energy radiation from X- ray is directed at a person's body to kill cancer cells and keep them from growing and multiplying [14]. Cancer and its treatments lead to profound immunosuppression. As cancer therapies have become more aggressive the risk for infection has increased. Despite improvements in long-term survival, bacterial infections remain a common complication of cancer therapy and accounts for the majority of chemotherapy and radiotherapy-associated deaths. The superimposition of the compromised host defenses and critical illness makes the detection and management of infections in such patients more difficult, but crucial toward salvaging patient outcome [15-18]. To this end and due to the polymorphism of the *eap* gene as a result of different numbers of repeats resulting in size variability of the gene product [19], this study was aimed to use a previously designed [20] PCR primer pair referred as EAP-CON1 and EAP-CON2 targeting the *eap* gene of *S. aureus* with sensitivity and specificity values were 100% [20] to determine the prevalence of such gene in *S. aureus* isolates after *in vitro* X-irradiation, in order to demonstrate that whether this gene is suitable for molecular diagnostics, sensitive detection & identification of *S. aureus* infections in cancer patients receiving X-ray therapy. Also, to review the antimicrobial sensitivity spectrum of those EAP adhesin – bearing isolates in such patients with relation to *in vitro* X- irradiation.

MATERIALS AND METHODS

Bacterial Isolates: A total of 63 *S. aureus* isolates from clinical specimens submitted to the microbiology laboratory at the National Cancer Institute (NCI), Cairo, Egypt, were included in the present study. None of these cancer patients were on prophylactic antimicrobial chemotherapy prior the time of samples collection. Only one isolate per patient was included in this study. All isolates were identified on the species level by conventional methods (Gram-positive cocci, beta hemolytic on blood agar, catalase positive, coagulase positive and mannitol fermenting) [21] and then confirmed by using MicroScan WalkAway-96 SI System (Dade Behring , Germany) at the NCI. The strains were stored in brain heart infusion (BHI) broth (Oxoid) plus 20% glycerol at -70°C until studied.

Irradiation Source: Linear accelerator X-ray production 6 MV Siemens (LA, Siemens) - PRIMUS2 treatment machine located at the NCI (Cairo, Egypt) was the irradiation source used. The field size (F.S) was 15 X 15 cm^2, depth = 1.5 cm + 1 cm Perspex, source skin distance (SSD) was 100 cm, total dose = 2441 cGy/1 fraction (single shot S.S), total monitor units (MU) = 2374, monitor units / fraction = 2374, time = 2374 MU. This total single dose is biologically equivalent to the fractionated multiple therapeutic doses of 70 Gy/35 fractions used in the treatment of cancer patients and was calculated by using the linear quadratic (LQ) formula described by Barton [22].

PCR for the Detection of the *eap* Gene in *S. aureus* Isolates Before and After *In vitro* X- Irradiation: Bacterial DNA was isolated using PrepMan® Ultra Sample Preparation Reagent (Applied Biosystems) according to the manufacturer's instructions. For the detection of the *eap* gene, a previously designed [20] *eap*- targeting oligonucleotide primers, referred as EAP-CON1 (5'- TAC TAA CGA AGC ATC TGC C-3') and EAP-CON2 (5'-TTA AAT CGA TAT CAC TAA TAC CTC-3') (BIONEER) with amplicon size 230 bp were used in this study. PCR was performed using *Taq* PCR Master Mix Kit (QIAGEN®) and the method described by Hussain *et al*. [20]. DNA was amplified with T Professional basic Theromcycler (Biometra). A total of 30 PCR cycles were run as follows: DNA denaturation at 95°C for 1 min (5 min for the first cycle), primer annealing at 50°C for 1 min and DNA extension at 72°C for 2 min. After the final cycle the reaction was terminated by holding at 72°C for 10 min. The amplified product sizes were estimated by comparison with GelPilot 1 kb Plus Ladder (100) cat. No. 239095 (QIAGEN, US) using a 1% ethidium bromide-stained agarose gel.

Antimicrobial Susceptibility Testing of *S. Aureus* Isolates Before and after *in vitro* X- Irradiation: The *in vitro* antimicrobial susceptibility of all *S. aureus* isolates were determined by the disk diffusion method on Mueller-Hinton agar (Oxoid) according to Bauer *et al*. [23]

and Acar and Goldstein [24]. For each organism, the test was set up from cell suspension equivalent to a 0.5 McFarland turbidity standard [prepared by mixing 0.05 mL of 1 % $BaCl_2$, with 9.95 mL of 1% H_2SO_4 and produces approximately 1.5 x 10^8 CFU/ml]. The following antimicrobial discs (µg) (Oxoid) representing different groups of antibiotics were included in this study; amoxicillin-clavulanic acid (30µg) [β-lactam/β-lactamase inhibitor combination], chloramphenicol (30µg) [phenicols], levofloxacin (5µg) [fluoroquinlones], linezolid (30µg) [oxazolidinones], meropenem (10µg) [carbapenems], minocycline (30µg) [tetracyclines], mupirocin (5µg) [Pseudomonic Acid A], oxacillin (1µg) [penicillins], penicillin (10 units) [penicillins], quinupristin/dafloprístin (15µg) [streptogramins], teicoplanin (30µg) [glycopeptides] and vancomycin (30µg) [glycopeptides]. Methicillin susceptibility was determined with oxacillin discs. The zone diameters of each antibiotic (except for mupirocin) were interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards or NCCLS) [25]. For mupirocin (5µg) the interpretative zone diameter criteria published by Fuchs *et al*. [26] and Malaviolle *et al*. [27]: susceptibility corresponding to ≥ 14 mm was used in this study.

Statistical Analysis: IBM SPSS statistics (V. 20.0, IBM Corp., USA, 2011) was used for data analysis. Data were expressed as both number and percentage for categorized data. The following tests were done: 1) Chi-square test to study the association between each 2 variables or comparison between 2 independent groups as regards the categorized data and 2) Comparison between 2 proportions as regards univariant categorized data. The probability of error at 0.05 was considered significant, while at 0.01 and 0.001 are highly significant.

RESULTS

Detection of the *eap* Gene in *S. aureus* Isolates Before and After 2441 cGy /1 Fraction *In vitro* X- Irradiation: By using the previously designed *eap*- specific primers, referred as EAP-CON1 and EAP-CON2 with sensitivity and specificity values were 100% [20], all the 63 (100%) *S. aureus* isolates tested before X-irradiation were positive for the *eap* gene. A single amplification product of the *eap* gene with the expected size 230 bp was detected for all of them before irradiation. However, 35 (55.56%) only express the *eap* 230 bp band after *in vitro* X-irradiation; i.e., the *eap* was not detectable in 28 X-irradiated clinical isolates (Figs. 1a and b). The difference in the rate of *eap* positivity among *S. aureus* isolates before and after X-irradiation was clear and highly significant ($P< 0.001$).

Antimicrobial Susceptibility Testing of *S. aureus* Isolates Before and After 2441 cGy/1 Fraction *In vitro* X-Irradiation: In this study, the 63 S. *aureus* isolates were tested before and after *in vitro* X- irradiation against 12 different antimicrobial agents; amoxicillin-clavulanic acid, chloramphenicol, levofloxacin, linezolid, meropenem, minocycline, mupirocin, oxacillin, penicillin, quinupristin/ dafloprístin, teicoplanin and vancomycin by the disk diffusion method. Out of the 63 isolates tested, a total of (1.59%) was fully susceptible to all the 12 antimicrobial agents before and after irradiation while, (69.84%) and (60.32%) were multiple-resistant (resistant to two or more antibiotics) before and after *in vitro* X-irradiation, respectively. All isolates were susceptible to linezolid and vancomycin before irradiation. Methicillin resistant *S. aureus* (MRSA) was distributed among the tested isolates before and after irradiation being (46.03%) and (52.38%), respectively. The rate of mupirocin resistance was (12.70%) and (22.22%) before and after irradiation, respectively. All the mupirocin resistant isolates before irradiation were also resistant to six or more other antibiotics.

Association Between Antimicrobial Susceptibility Pattern and *eap* Existence Investigated at the DNA Level Among *S. aureus* Isolates Before and After *In vitro* X-Irradiation: Tables 1 & 2 represent the antimicrobial susceptibility patterns of the 35 *eap*-positive and the 28 *eap*-negative X-irradiated *S. aureus* isolates with relation to *in vitro* X-irradiation, respectively. As can be seen in these tables, higher rates of resistance were demonstrated for *S. aureus* isolates harbored the *eap* gene after X- irradiation (35/63) as compared with those before irradiation ($P<0.001$) versus amoxicillin/ clavulanic acid (74.29% vs. 31.43%), meropenem (74.29% vs. 31.43%), minocycline (71.43% vs. 25.71 %) and oxacillin (74.29% vs. 31.43%), while ($P < 0.05$) versus mupirocin (34.29% vs. 11.43%) and penicillin (74.29% vs. 48.57%). Interestingly, the overall percentage susceptibilities to the tested antibiotics after *in vitro* X-irradiation showed high significant increase ($P<0.01$) for *S. aureus eap*-negative

Table 1: Antimicrobial susceptibility patterns of the 35 *eap*-positive X-irradiated *S. aureus* isolates with relation to *in vitro* X – irradiation

	Susceptible n (%)	Intermediate n (%)	Resistant n (%)	Susceptible n (%)	Intermediate n (%)	Resistant n (%)	
Antimicrobial Agent	Before X- irradiation			After X- irradiation			*P*-value
Amoxicillin/ clavulanic acid 2:1	24 (68.57%)	--	11 (31.43%)	9 (25.71%)	--	26 (74.29%)	< 0.001*
Chloramphenicol	16 (45.71%)	4 (11.43%)	15 (42.86%)	12 (34.29%)	1 (2.86%)	22 (62.86%)	> 0.05
Levofloxacin	13 (37.14%)	5 (14.29%)	17 (48.57%)	10 (28.57%)	--	25 (71.43%)	> 0.05
Linezolid	35(100.00%)	--	--	34 (97.14%)	--	1 (2.86%)	> 0.05
Meropenem	24 (68.57%)	--	11 (31.43%)	9 (25.71%)	--	26 (74.29%)	< 0.001*
Minocycline	26 (74.29%)	3 (8.57%)	6 (17.14%)	10 (28.57%)	1 (2.86%)	24 (68.57%)	< 0.001*
Mupirocin	31 (88.57%)	--	4 (11.43%)	23 (65.71%)	--	12 (34.29%)	< 0.05?
Oxacillin	24 (68.57%)	--	11 (31.43%)	9 (25.71%)	--	26 (74.29%)	< 0.001*
Penicillin	18 (51.43%)	--	17 (48.57%)	9 (25.71%)	--	26 (74.29%)	< 0.05?
Quinupristin/dalfopristin	26 (74.29%)	--	9 (25.71%)	24 (68.57%)	1 (2.86%)	10 (28.57%)	> 0.05
Teicoplanin	30 (85.71%)	1 (2.86%)	4 (11.43%)	27 (77.14%)	--	8 (22.86%)	> 0.05
Vancomycin	35 (100%)	--	--	33 (94.29%)	--	2 (5.71%)	> 0.05

*$P < 0.001$ highly significant, ? $P < 0.05$ significant , $P > 0.05$ non- significant

Table 2: Antimicrobial susceptibility patterns of the 28 *eap*-negative X-irradiated *S. aureus* isolates with relation to *in vitro* X – irradiation

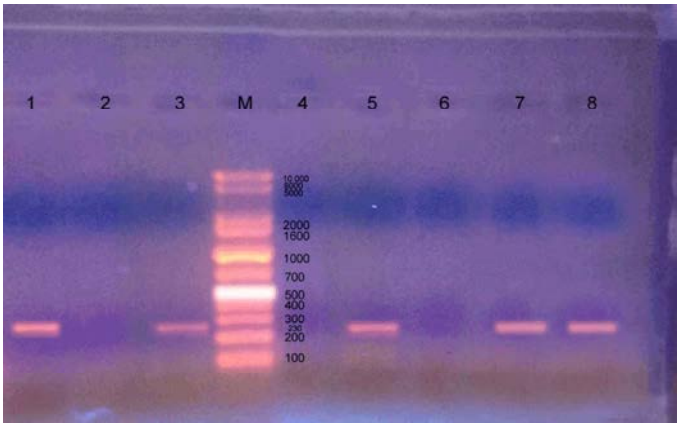
	Susceptible n (%)	Intermediate n (%)	Resistant n (%)	Susceptible n (%)	Intermediate n (%)	Resistant n (%)	
Antimicrobial Agent	Before X- irradiation			After X- irradiation			*P*-value
Amoxicillin/ clavulanic acid 2:1	10 (35.71%)	--	18 (64.29%)	21 (75.00%)	--	7 (25.00%)	< 0.01 *
Chloramphenicol	12 (42.86%)	4 (14.29%)	12 (42.86%)	22 (78.57%)	2 (7.14%)	4 (14.29%)	< 0.01 *
Levofloxacin	11 (39.29%)	4 (14.29%)	13 (46.43%)	20 (71.43%)	1 (3.57%)	7 (25.00%)	< 0.01 *
Linezolid	28(100.00%)	--	--	28 (100.0%)	--	--	--
Meropenem	10 (35.71%)	--	18 (64.29%)	21 (75.00%)	--	7 (25.00%)	< 0.01 *
Minocycline	16 (57.14%)	1 (3.57%)	11 (39.29%)	22 (78.57%)	2 (7.14%)	4 (14.29%)	< 0.05 ?
Mupirocin	24 (85.71%)	--	4 (14.29%)	26 (92.86%)	--	2 (7.14%)	> 0.05
Oxacillin	10 (35.71%)	--	18 (64.29%)	21 (75.00%)	--	7 (25.00%)	< 0.01 *
Penicillin	5 (17.86%)	--	23 (82.14%)	19 (67.86%)	--	9 (32.14%)	< 0.001 *
Quinupristin/dalfopristin	13 (46.43%)	2 (7.14%)	13 (46.43%)	24 (85.71%)	--	4(14.29%)	< 0.01 *
Teicoplanin	19 (67.86%)	2 (7.14%)	7 (25.00%)	23 (82.14%)	1 (3.57%)	4 (14.29%)	> 0.05
Vancomycin	28 (100.00%)	--	--	28 (100.00%)	--	--	--

* $P < 0.001$ highly significant, ? $P < 0.05$ significant, $P > 0.05$ non- significant, * $P < 0.01$ highly significant

cases (28/63) versus amoxicillin/clavulanic acid (75.00% vs. 35.71%), chloamphenicol (78.57% vs. 42.86%), levofloxacin (71.43% vs. 39.29%), meropenem (75.00% vs. 35.71%), oxacillin (75.00% vs. 35.71%), quinupristin/dalfopristin (85.71% vs. 46.43%) and penicillin (67.86% vs. 17.86%, $P<0.001$). The rates of linezolid and vancomycin susceptibilities were similar (100.00%) among both groups before X-irradiation and for the *eap* gene-negative cases after *in vitro* X-irradiation while, they were (97.14%) and (94.29%) for the *eap* gene-positive cases after irradiation, respectively.

The results obtained before irradiation showed that, among the target gene-negative cases, for amoxicillin/clavulanic acid, meropenem and oxacillin (64.29%) were resistant and (35.71%) were susceptible vs. (31.43%) and (68.57%) among positive cases, for penicillin (82.14% and 17.86% vs. 48.57% & 51.43%), for quinupristin/ dalfopristin (53.57% & 46.43% vs. 25.71% and 74.29%). While, after irradiation for amoxicillin/ clavulanic acid (25.00% & 75.00% vs. 74.29 % &25.71%), for chloramphenicol (21.43% & 78.57 vs. 65.72% & 34.29%), for levofloxacin (28.57% & 71.43% vs. 71.43% 28.57%), for meropenem (25.00% &75.00% vs.74.29% & 25.71%) and minocycline (21.43% & 78.57% vs. 71.43% & 28.57%), for mupirocin (7.14% & 92.86% vs. 34.29% & 65.71%), for oxacillin (25.00% & 75.00% vs. 74.29 % &25.71%) and for penicillin (32.14% & 67.86% vs. 74.29 % &25.71%). Figs. 2a-h demonstrate the relations between the presence of Eap-encoding gene (*eap*) and its antibiotic susceptibility testing before and after *in vitro* X-irradiation, which were highly significant for amoxicillin/clavulanic acid ($P = 0.000$), chloamphenicol ($P = 0.006$), levofloxacin ($P = 0.003$), meropenem ($P = 0.000$), minocycline ($P = 0.000$), oxacillin ($P = 0.000$), penicillin ($P = 0.002$) and significant ($P = 0.038$) for quinupristin/dalfopristin.

(a)



(b)

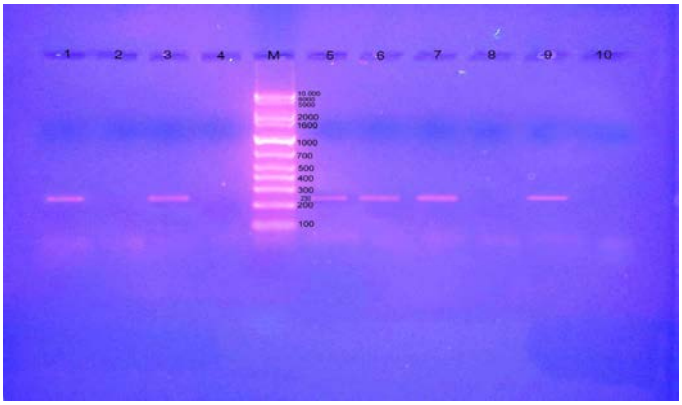


Fig. 1: Agarose gel electrophoresis patterns of PCR products of the *eap* gene using genomic DNA of different *Staphylococcus aureus* isolates before and after *in vitro* X-irradiation. Lane M, mol. wt marker (GelPilot 1 kb Plus Ladder (100), QIAGEN); (a) lanes 1&2, 3&4, 5&6, isolates nos.1, 4 &10 before and after irradiation respectively (*eap* gene-negative *S. aureus* isolates after X-irradiation); lanes 7&8, isolate no. 33 before and after irradiation respectively (*eap* gene-positive isolate after X-irradiation). (b) lanes 1&2, 3&4, 7&8, 9&10, isolates nos.17, 20, 23 & 28 before and after irradiation respectively (*eap* gene-negative isolates after X-irradiation); lanes 5&6 isolate, no. 51 before and after irradiation respectively (*eap* gene-positive isolate after X-irradiation)

DISCUSSION

Cancer patients are at high risk for severe infections due to the immunosuppression that typically exists in these patients and the use of more intensive therapeutic regimens [28]. For many years, *S. aureus* has been recognized as a cause of serious infections, especially among patients with cancer and it is associated with substantial mortality [29-31]. *S. aureus* has an extraordinary repertoire of virulence factors that allows it to survive extreme conditions within the human host [32]. The extracellular adherence protein (Eap) is a multifunctional *S. aureus* protein and broad-spectrum adhesin for several host matrix and plasma proteins [33]. In agreement with the findings of this study, high positivity (100%) of the Eap-encoding gene (*eap*) was detected among all *S. aureus* (n=63) isolated from cancer patients tested before 2441cGy single dose *in vitro* X- irradiation [20], thus allowing the use of this gene for molecular diagnostics of *S. aureus* in such patients before receiving radiotherapy. However, of particular interest, positive results were detected for only (55.56%) of the tested isolates after irradiation ($P<0.001$). Detection of *eap* gene-negative cases (44.44%) after X-irradiation could be attributed to the effect of X-radiation, as well as other forms of ionizing radiations which interact with matter to generate free electrons and unstable ions. Furthermore, continued collisions of these free electrons with other atoms produce more ions, which could break the DNA backbone, causing physical breaks and mutations. In addition to the generation of mutations through direct breakage of the DNA backbone, however,

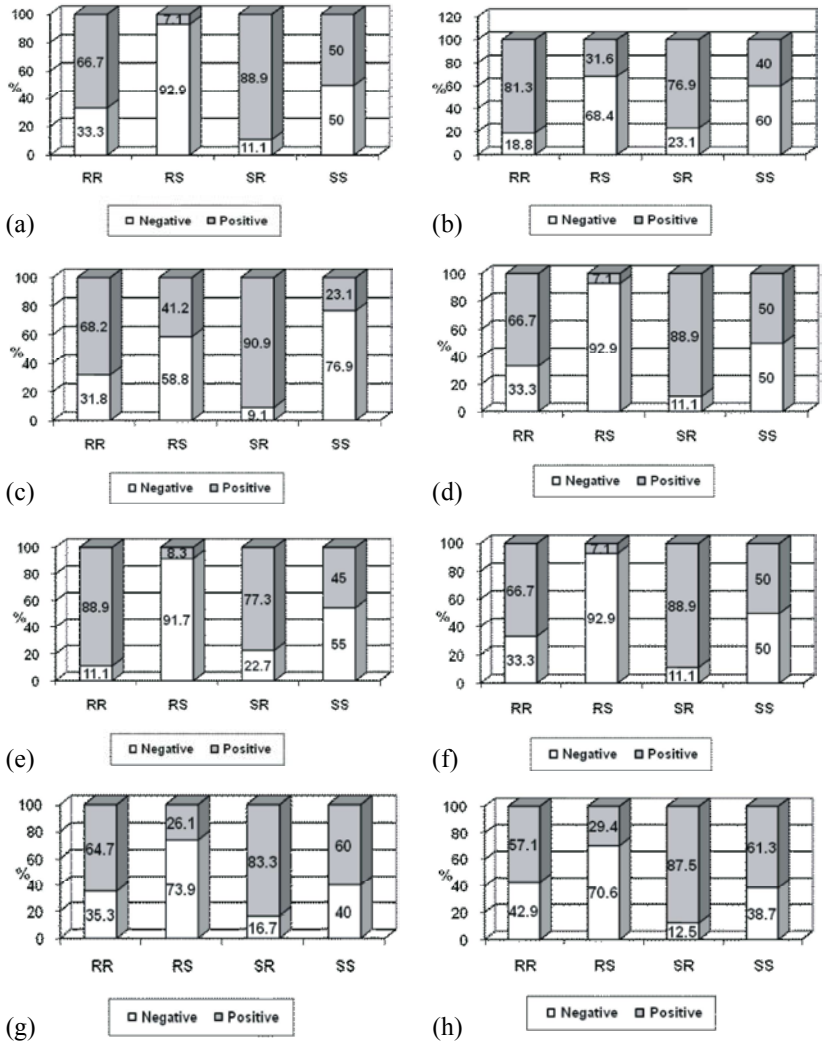


Fig. 2: Relations between the presence of Eap-encoding gene (*eap*) and its antibiotic susceptibility testing before and after *in vitro* X-irradiation for amoxicillin/ clavulanic acid (a); chloramphenicol (b); levofloxacin (c); meropenem (d); minocycline (e); oxacillin (f); penicillin (g) & quinupristin/dalfopristin (h). RR: Resistant before and after irradiation; RS: Resistant before and sensitive after irradiation; SR: Sensitive before and resistant after irradiation & SS: Sensitive before and after irradiation. Negative: *eap* -negative X-irradiated isolates & Positive: *eap* -positive X-irradiated isolates

what effect low-to-medium energy X-ray radiation would have on microorganisms with respect to their viability and damage to the genome was unknown [34].

Multi-drug resistant *S. aureus* strains are still a considerable problem in medicine [35, 36]. A significant challenge for the clinician is how to effectively treat *S. aureus* infections, given the increasing diversity of drug-resistant pathogens and the diminishing treatment options [37, 38]. The high rate of susceptibility to linezolid and vancomycin in the tested *S. aureus* isolates was consistent with many studies [39-42]. A border-line statistical significant difference was recorded in case of minocycline (P=0.070) and quinupristin/dalfopristin (P=0.083) for all isolates (n=63; *eap*-positive and -negative cases) concerning the results obtained after *in vitro* X-irradiation which was associated with increased % of resistance from (33.3%) to (49.2%) in comparison to those before; while in the same time, decreased in % of susceptibility from (66.7%) to (50.8%) in case of minocycline. While, it was associated with decreased % of resistance from (38.1%) to (23.8%) in comparison to those before and in the same time, increased % of

susceptibility from (61.9%) to (76.2%) for quinupristin/ dalfopristin. Thus, pointing to a change in the behavior of both antibiotics after irradiation treatment ($p>0.05$ but <0.1, i.e. tend to be significant).

In this study, almost all the *S. aureus* isolates exhibiting *eap* gene after *in vitro* X-irradiation were exhibiting more resistance to most of the antibiotics tested after irradiation compared to the results before. Thus, findings of the current study detecting the absence of *eap* gene and the low prevalence of single and multiple antibiotic resistance after X–irradiation confirm previous observations regarding antibiotic resistant profile and the contributory role of the extracellular adherence protein (Eap) as an important virulence factor of *S. aureus* enhancing bacterial internalization into eukaryotic cells. Internalization of the bacteria into nonprofessional phagocytic cells has been associated with persistent and relapsing infections, because of their intracellular location, which shields the bacteria from host defense and antibiotic treatment and thus decreasing antibiotic susceptibility [10, 43- 45]. The relation between percentage of resistant & susceptible isolates among *eap* gene-negative (n=28) and -positive (n=35) cases before *in vitro* X-irradiation was highly significant for amoxicillin/ clavulanic acid, meropenem and oxacillin ($P = 0.009$); for penicillin ($P = 0.006$) while, significant ($P = 0.024$) for quinupristin/dalfopristin. This means that, if the results of these antibiotics against the tested isolates before irradiation showed resistance, so it is more liable to be missing the encoding gene after irradiation while, if it is susceptible it is more liable to be *eap* gene- positive after irradiation. Thus, the results of these antibiotics before irradiation could be used for predicting the existence of this gene after irradiation; resistance for *eap* gene-negative and susceptible for –positive cases. On the other hand, after irradiation this relation was highly significant for amoxicillin/clavulanic acid, chloramphenicol, levofloxacin, meropenem, minocycline, oxacillin and penicillin ($P = 0.000, 0.000, 0.001, 0.000, 0.000, 0.000$ and 0.001), respectively predicting *eap* gene- negative cases from susceptible results and –positive cases from resistant results. For Amoxicillin/ clavulanic acid, chloramphenicol, levofloxacin, meropenem, minocycline, oxacillin, penicillin and quinupristin/dalfopristin, regardless the susceptibility pattern obtained before irradiation for all of the isolates tested (n=63), resistant isolates after irradiation were found to be more liable to be Eap adhesin-bearing isolates while, susceptible isolates were *eap* gene-negative cases (Figs. 2a-h). Thus, the results of these antibiotics could be used for the prediction of *eap* gene-positive cases (shifting the results from susceptible to resistant) and *eap* gene-negative cases (shifting the results from resistant to susceptible) after irradiation.

In conclusion, the results of the present study support the idea that the *eap* gene has an important role as a virulence marker for *S. aureus* infections and could be used successfully for sensitive detection and identification of such infections in cancer patients before receiving X- ray therapy. X-irradiation may have an effect on *S. aureus eap* gene which may hamper the use of this gene for molecular diagnostics of *S. aureus* infections in X- ray treated cancer patients. *S. aureus* infections in such patients could be effectively treated with both of linezolid and vancomycin. Additionally, the results indicate the role of *S. aureus eap* gene in the internalization process and antibiotic treatment. Thus, the existence of the target gene in relation to X-ray therapy could be predicted from the antimicrobial sensitivity pattern.

Competing Interests: No competing interests to be declared.

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