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Changes in antibiotic susceptibility of *Staphylococcus aureus* due to different stress factors

Staphylococcus *aureus* is one of the important pathogenic bacteria from the viewpoint of food safety. It is a Gram-positive bacterium that can be easily degraded by heat treatments (like cooking or pasteurization). However, it can secrete heat-stable toxins if food is cross-contaminated by S. *aureus*. In 2019, the world health organization (WHO) considered anti-microbial resistance (AMR) as one of the top 10 global public health threats facing humanity. Nevertheless, methicillin-resistant S. *aureus* (MRSA) is one of the two main causative agents for bloodstream infections. In 2020, the total number of reported outbreaks of S. *aureus* toxins in the EU were 43, where 402 human cases were reported. S. *aureus*'s ability to evolve is quite extensive. It can effectively adapt to stress factors such as high salinity, and it is resistant to many antibiotics by the acquisition of resistance genes. Gamma irradiation is one of the most effective tools to achieve safe food with minimal effects on food. It is necessary, especially in the era of antibiotic resistance, to study its effect on the resistance of S. *aureus* to different antibiotics.

My research aimed at examining the response of methicillin resistant S. *aureus* to antibiotics after being exposed to different environmental stress. High osmolarity (salinity) and sublethal dose of gamma irradiation were proposed as stressing factors. The susceptibility/resistance to diverse antibiotics before and after stressing the bacterium cells was determined, and a genotypic examination was also performed to detect the presence of the *mecA* gene responsible for antibiotic resistance in S. *aureus* after environmental stressors.

Two S. *aureus* strains were selected for examination: their susceptibility was compared using agar disk diffusion test in the presence of ten various antibiotics (Piperacillin 100µg, Nalidixic Acid 30µg, Erythromycin 15µg, Meropenem 10µg, Colistin 10µg, Piperacillin 30µg, Gentamycin 10µg, Ampicillin 10µg, Chloramphenicol 30µg, and Ciprofloxacin 5µg). The most resistant strain was further challenged with a concentration of 6 % NaCl. Gamma irradiation was applied with a sublethal dose of 0.6 kGy; the dose was introduced as an individual treatment or combined with

the saline. Treatments were compared to a control sample containing no additional salt and not irradiated. Changes in surviving bacterial counts were determined, and antibiotic resistance due to treatments was detected by measuring the clearing zones around antibiotic-containing disks. A decision was taken considering the test results to further increase the NaCl concentration to 10 %, and 12%, with or without irradiation, with the antibiotic meropenem only. A traditional PCR test was performed for all treated samples to amplify the *mecA* gene, which is responsible for antibiotic resistance in MRSA. The detection targeted the gene fragments present in the intra and extracellular DNA.

The results identified one of the two strains as Methicillin susceptible S. *aureus* (MSSA), which was susceptible to all antibiotics except for Colistin 10 µg and nalidixic acid 30 µg. The other strain was confirmed as Methicillin resistant S. *aureus* (MRSA) because of its resistance to all challenged antibiotics except for Chloramphenicol 30 µg and Ciprofloxacin 5 µg. The susceptibility of the bacterium to antibiotics was changed by applying the stress factors; the bacteria showed increased susceptibility against some antibiotics, and the opposite was true for others. There were also antibiotics where the resistance did not change. Meropenem 10 µg showed a promising change for the treatments. The 6%, 10 %, and 12% increase in NaCl changed the resistance to intermediate. The microbe became susceptible after the 0.6 kGy of gamma irradiation alone or combined with high osmolarity (6%, 10%, and 12 % NaCl). Furthermore, the PCR test revealed the presence of the *mecA* gene in all the sample treatments, confirming no genetic change. The bacterial total count decreased by one log/ml for all salt concentrations and four log/ml for the irradiated samples, even when used alone or combined with high osmolarity.

In conclusion, although high salinity and low doses of gamma irradiation decreased antibiotic resistance to meropenem, *mecA* gene from iDNA fraction of B.02174 strain was amplifiable. Thus, the loss of resistance can be explained by either the inhibition of gene expression or the altered permeability of the membrane. A previous study found that NaCl at a relatively high concentration (>0.3 M) could enhance the porin permeability and/or damage bacterial membranes, which enhances bacterial uptake of diverse molecules from the outer environment. It can be hypothesized that irradiation had an indirect effect on S. *aureus* DNA (by forming free radicals), and accumulated effect of the two applied stressors contributed to the change of resistance into sensitivity in the case of meropenem, a carbapenem antibiotic.