Validation Efficiency of Radiation on *Serratia* Marcescens

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Abstract

Background: *Serratia marcescens* is a pathogen bacterium (gram negative) that has a rod shape; it is considered as high risk bacteria as antibiotic resistance, and a lot of studies were carried out to limit *S. marcescens* activity using radiation. **Objective:** This research aimed to determine the activity of *S. marcescens* against certain antibiotic, and the radiation affect on this bacterium. **Materials and Methods:** 25 of diagnosed *S. marcescens* cross-sectional study design, were collected from Baghdad hospitals from different patients, the patients were vestige to radiation by cultivated on media at 37°C to reach the culture of the stationary-phase, then centrifugation (for 10 minutes at 5000 rpm), and suspended in saline. Then exposed to diverse types of radiations (using only one millimeter), the collected data then compared with group that was not exposed to radiation (control group), rediluted and inoculated in media, cultivated within test tube containing distilled water 5ml, then exposing to an different radiosources including CS^{137} and CO^{60} with activity 1-10 µci at different radiation doses for one hour. **Results:** The results exhibition of gamma, beta rays emitted by CO^{60} with activity 1 µci on *S. marscences* inclusive D (1hrs.)=$1.55599*10^{-5}$, D (2hrs.)=$3.111989*10^{-5}$, D (3hrs.)=$4.86798*10^{-5}$ were viable cell in 1hr and compared with control=196. The results of exhibition of Gamm, Beta rays emitted by CO^{60} with activity 10 µci on *S. marcescens* inclusive D(1hrs.)= $2.3323*10^{-5}$, D (2hrs.)= $4.6647*10^{-5}$, D (3hrs.)= $6.99705*10^{-5}$ were viable cell in 1hr, and compared with control=196. Same measurement was carried out for gamma, Beta rays emitted by Cs^{137} with activity 1 µci and 10 µci and compared with control=196. Statistical Analysis System-SAS program all results are significant. **Conclusions:** *S. marcescens* shows high resistance as a multidrug resistance, radiation emitted from semiconductor laser have high efficiency for killing *S. marcescens*.

Key Words: Impress, Gamma Rays, *Serratia* spp. Beta Rays, Iraqi Patients.

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Introduction

Serratia marcescens (*S. marcescens*) is a nosocomial pathogen bacterium (gram negative) which referred to enterobacteriaceae (Yersiniaceae) family; it is nonpathogenic, innocuous, water organism (saprophytic). *S. marcescens* is used as a biomarker due to its simply identifiable via the formation of red colour colonies (Merlino and Bizios 1990).

S. marcescens is epidemics microorganism that colonized in Intensive care units (ICU), it is important in the urinary tracts, digestive tract, perineum of neonates and the respiratory tract, etc., and as known, the sources of epidemics could be, sinks, blood products, medications, antiseptics, lotions and medical equipment's (Khanna et al., 2013).

The rate of mortality caused by infection with endocarditis and meningitis in nosocomial blood stream is quite high; a number of *S. marcescens* strains of are able of produce pigment known as "prodigiosin", its colour ranged from pink to dark red upon the colony age.

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Few strains have capability in producing beta lactamase which present resistance and struggling toward beta lactum antibiotics (Yoon et al., 2005; Mahlen 2011). The major risk factor is in the hospitalization throughout intravenous placement, intraperitoneal, and catheters of catheters as well as the earlier respiratory tract instrumentation (Gonzalez-Juarbe et al., 2015). *S. marcescens* pathogen is involved with around 5% of urinary infections and blood stream infections (CLABSI) Central Line-Associated Blood Stream Infections (Apostolopoulou et al., 2014).

The survival benefit of *S. marcescens* colonies is the formation of biofilms (Satpathy et al., 2016), which are communities that considered as complex surface-adhered to dead and viable bacteria; it is enclosed with polysaccharide extracellular matrix, extracellular DNA and protein (Flemming et al., 2016). Additionally, *S. marcescens* adhered to the epithelial cells of the host is throughout the biofilm formation (Nandre et al., 2012), the biofilms have capability to increase its pathogenicity, this can increase antimicrobial resistance due to many reasons such as slow growth rates, and antibiotic penetration (Srinivasan et al., 2016).

Materials and Methods

Clinical examination include study design, study population, identification by VITECK2-GN, antimicrobial susceptibility tests for antibiotics, validation efficiency of radiation on *S. marcescens*, validation efficiency of radiation on biofilm *S. marscences* and statistical analysis.

25 of *S. marscences* isolates were gathered hospitals of Baghdad in 2019. The isolates were characterized via biochemical reactions as explained by (Apostolopoulou et al., 2014), and inoculated on agar at 37 °C for 24 hours.

Identification of S. marscences by VITECK2-GN

The gram Negative (GN) card was applied for identification of most GN bacteria, according to the biochemical procedures. 64 biochemical tests were carried out to identify the enzymatic activities, carbon source utilization and the resistance to the antibiotics as depicted by (Collins and Lawson, 2000; Barros *et al.*, 2001). Final results of identification were available after 8 hours as (BioMerieux, 2010).

Procedure

After isolation of the pure culture, and after checking the purity of the cultures, the following steps were followed:

1. 3 ml of sterile aqueous saline (0.475% NaCl, pH from 4.5 to 7.0).
2. A adequate number of colonies were transferred to the step 1 saline to prepare the suspension that must be homogenous organisms and the density must be equivalent to a McFarland No. 0.70 to 0.110.

Antimicrobial Susceptibility Test by Discs Diffusion Methods

The susceptibility of the antimicrobial test was done using discs diffusion method of antibiotic discs for 8 antibiotics to 25 isolates as shown in table 1 and compared with the recommendation CLSI, 2020 (The Clinical & Laboratory Standards Institute).

Table 1. Antibiotics Code and Concentrations of discs

No.	Discs concentrations (µg/disc)	Symbol	Antimicrobial agents	Company
1	10	NA	Nalidixic acid	Bioanalyse (Turkey)
2	10	CN	Gentamycin	
3	10	TOB	Tobramycin	
4	30	ATM	Aztreonam	
5	25	AX	Amoxicillin	
6	10	LOM	Lomefloxacin	
7	5	LEV	Levofloxacin	
8	10	CIP	Ciprofloxacin	
9	10	NOR	Norfloxacin	

Validation Efficiency of Radiation on S. marscences

S. marscences cultivation was done according to (Satpathy et al., 2016) with some modifications, cultivated in nutrient broth for 24 hours at 37°C to reach the stability (stationary-phase), after that centrifugation for 10 minutes at 5000 rpm, then suspended in saline (150 ml of saline was used), only 1 ml of the suspended solution was exposed to radiation in diverse time (1, 2, 3) hrs, comparison with control (without exposure). The following equation was used for calculating the killing rate of *S. marscences*:

$$Killing\ of\ \ S.marscences\ \% = \frac{Control - Patronlize}{Control}\ x\ 100$$

Validation Efficiency of Radiation on Biofilm of S. marscences

This was synthesized by dissolving only 37 grams of brain heart infusion broth, then only 10 grams of

agar was added to distilled water (900 ml), sterilizing in autoclave, then congo-red stain was added as comes in (Freeman *et al*, 1989).

Results and Discussions

Antimicrobial Susceptibility Test by Discs Diffusion Methods

The antibiotic resistance for Ciprofloxacin 25(100%), Norfloxacin 24 (95%), Gentamycin 21 (85%), Aztreonam 4 (15%), Amoxicillin 4 (15%), Nalidixic acid 3 (10%), Lomefloxacin 1 (5%) and Levofloxacin 1 (5%) showed in table1, while figure 1 shows the inhibition zone around antibiotics using the discs diffusion methods for *S. marscences.*

Table 2. Number and percent of resistance *S. marscences* against antibiotics

Resistance	Antibiotic							
	Ciprofloxacin	Norfloxacin	Gentamycin	Aztreonam	Amoxicillin	Nalidixic acid	Lomefloxacin	Levofloxacin
Resistance number	25	24	21	4	4	3	1	1
Resistance %	100%	95%	85%	15%	15%	10%	5%	5%

Figure 1. Antibiotic susceptibility test for *S. marscences*

The resistance to antimicrobial is significantly limit to the options of therapeutic patients that were infected, particularly its resistance to the carbapenem class, the discovery of new therapies and its developments, the combinations and the existing antimicrobial regimens. It is important in emphasis on the prevention of bacterial infection transmission.

Validation Efficiency of Radiation on S. marscences

The results exhibition of Gamm, Beta rays emitted by CO ^{60} with activity 1 µci on *S. marscences* inclusive D (1hrs.) = $1.55599*10^{-5}$, D (2hrs.) = $3.111989*10^{-5}$, D (3hrs.) = $4.86798*10^{-5}$ were viable cell in 1hr with percentage of homicide compared with control= 196. The results of exhibition of gamma, beta rays emitted by CO ^{60} with activity 10 µci on *S. marscences* inclusive D (1hrs.) = $2.3323*10^{-5}$, D (2hrs.) = $4.6647*10^{-5}$, D (3hrs.) = $6.99705*10^{-5}$ were viable cell in 1 hrs with percentage of homicide compared with control=196.

The results of exhibition of gamma, beta rays emitted by Cs ^{137} with activity 1 µci on *S. marscences* inclusive D(1hr.) = $6.28903*10^{-5}$, D(2hrs.) = $12.57806*10^{-5}$, D(3hrs.) = $18.867099*10^{-5}$ were viable cell in 1hr with percentage of homicide compared with control=196. The results of exhibition of gamma, beta rays emitted by Cs ^{137} with activity 10 µci on *S. marscences* inclusive D (1hr.) = $11.728*10^{-5}$, D (2hrs.) = $23.456*10^{-5}$, D (3hrs.) = $35.1843*10^{-5}$ were viable cell in 1hrs. with percentage of homicide compared with control=196.

The results of exhibition of beta rays emitted by CO ^{60} with activity 1 µci on *S. marscences* inclusive D (1hrs.) = $1.24532*10^{-5}$, D (2hrs.) = $2.490655*10^{-5}$, D (3hrs.) = $3.735982*10^{-5}$ were viable cell in 1hr with percentage of homicide and compared with control=196. The results of exhibition of beta rays emitted by CO ^{60} with activity 10 µci on *S. marscences* inclusive D (1hr.) = $1.866686*10^{-5}$, D (2hrs.) = $3.733172*10^{-5}$, D (3hrs.) = $5.6000586*10^{-5}$ were viable cell in 1hr with percentage of homicide compared with control=196.

The results of exhibition of beta rays emitted by Cs ^{137} with activity 1 µci on *S. marscences* inclusive D (1hrs.) = $3.4478394*10^{-5}$, D (2hrs.) = $6.835678*10^{-5}$, D (3hrs.) = $10.2535183*10^{-5}$ were viable cell in 1hr with percentage of homicide compared with control=196. The results of exhibition of beta rays emitted by Cs ^{137} with activity 10 µci on *S. marscences* inclusive D (1hr.) = $6.367058*10^{-5}$, D (2hrs.) = $12.7341148*10^{-5}$, D (3hrs.) = $19.1011747*10^{-5}$ were viable cell in 1hr, with percentage of homicide compared with control=196.

The Vestige of irradiation on the viability of *S. marscences* appear by count viability with calculate

of killing percent, the viable cells number of *S. marscences* is few compared before exhibition to irradiation, but percentage of killing is higher compared with before exposure to irradiation, irradiation is efficient for killing *S. marscences*.

The results illustrate that 100% of isolates (25) were producing a well built slime layer pointed to the black colonies formation before radiation exhibition and little biofilm production after exposure to radiation, Statistical Analysis System-SAS program all results are significant.

Results in table 2 and figure 2 exhibit the number of *S. marscences* in Iraqi laboratory with percentage of killing on nutrient agar medium for exhibition for different types of radiations from different radio sources.

Table 2. Vestige different rays with proportion viability with percentage of homicide of *S. marscences* exposed to radioactive radiations

Radiosources radiation	Activity (µci)	Types of radiation	Dose for 1 hours/ msv	Dose for 2 hours/ msv	Dose for 3 hours/ msv
Cs^{137}	10 µci	β,ɣ	$11.728*10^{-5}$	$23.456*10^{-5}$	$35.1843*10^{-5}$
Cs^{137}	10 µci	B	$6.367058*10^{-5}$	$12.73411648*10^{-5}$	$19.1011747*10^{-5}$
Cs^{137}	1 µci	β,ɣ	$6.28903*10^{-5}$	$12.56806*10^{-5}$	$18.867099*10^{-5}$
Cs^{137}	1 µci	B	$3.4478394*10^{-5}$	$6.835678*10^{-5}$	$10.2535183*10^{-5}$
Co^{60}	10 µci	β,ɣ	$2.3323*10^{-5}$	$4.6647*10^{-5}$	$6.99705*10^{-5}$
Co^{60}	10 µci	B	$1.866686*10^{-5}$	$3.733172*10^{-5}$	$5.6000586*10^{-5}$
Co^{60}	1 µci	β,ɣ	$1.55599*10^{-5}$	$3.111989*10^{-5}$	$4.86798*10^{-5}$
Co^{60}	1 µci	B	$1.24532*10^{-5}$	$2.490655*10^{-5}$	$3.735982*10^{-5}$

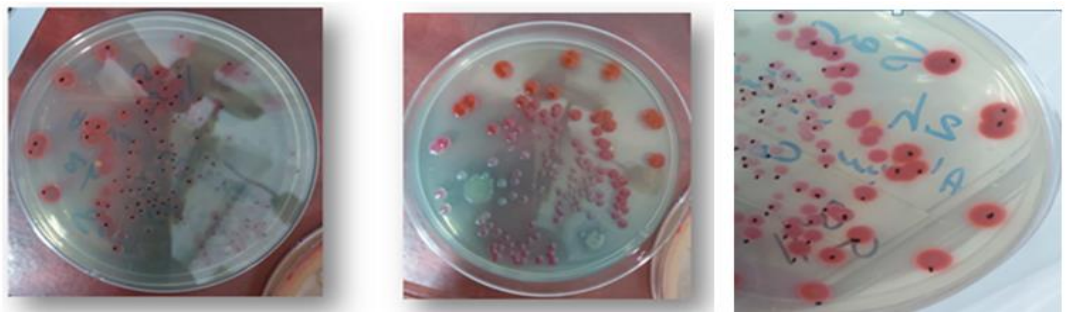

Figure 3. Number of *S. marscences* in Iraqi laboratory with percentage of killing on nutrient agar medium for exhibition for different radio sources

Many theories were anticipated and examined to understand the mechanism of how radiation causes damages to the cells. Some researchers suggest the radio toxins which could be the toxic materials that produced in the cells after irradiation causing cell mortality, while others suggest the direct damage to membrane of the cells caused by the radiation (direct radiation effect). Additionally, radiation has a sever effect on enzymes as well as on metabolism energy, also radiation has effect on the cytoplasm of cells which may play an extra role in mortality of the cells (Flemming et al., 2016).

Validation Efficiency of Radiation on Biofilm of S. marscences

The capability of *S. marscences* in adhering and in the formation of multilayered biofilms on tissues of the host surfaces is considered as a significant mechanism that causes diseases. The environment of the biofilms can increased the exchanges in genes and therefore it may provide extra resistance to the phenotypes (Srinivasan et al., 2016).

Figure 3 shows the colonies before and after exposed to the radiation.

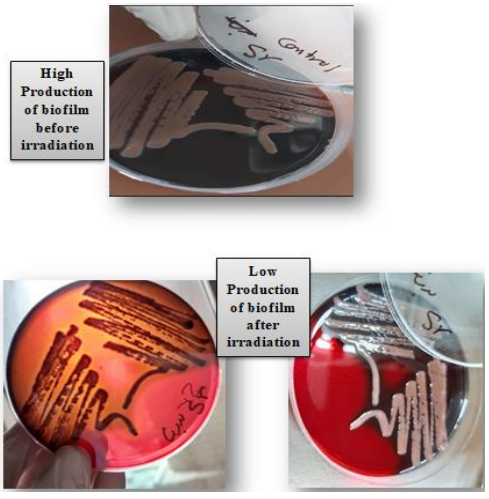

Figure 4. Production biofilm of *S. marscences* before and after exhibition to radiation

This outcome of the current study disagreed with (Rewatkar and Wadher 2013) that shows only 90% of isolates have colonies with black colour on congo-red agar, and the rest (10%) of the isolates were having colonies with a pink colour showing that no production to the biofilm.

However, this study agreed with the finding of (Nagaveni et al., 2010) that shows 72.7% of isolates had black colour while the rest (27.2%) present pink colour, which indicate there was no production of biofilm. Also (Go¨tz 2002) study the environment factors that affect the production of the slime layer such as, temperature, O_2, pH and further stress conditions which can give dissimilar outcomes (Cunha et al., 2002).

The formation of the biofilm was also screened by (Mathur et al., 2006) the study described different procedures by which inoculation time to the pure single isolated colony was aerobically at 37°C and for 48 hours, the black colonies were formed in a dry crystals, while the slime remained pink, and the dark colonies with no dry crystalline shows an indeterminate outcome.

Conclusions

1. There are high numbers of *S. marscences* resistance for antibiotics.
2. Validation of radiation emitted from semiconductor laser has high efficiency for killing Multidrug resistance *S. marscences*.

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