Elimination of Biofilm Produced by *Klebsiella Pneumonia* Using Strontium Radioactive Sources (Sr^{90})

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Abstract

The aim of this study was conducted to elimination biofilm production from *K. pneumonia* by Sr^{90} radiosources inorder to removal biofilm that contained with *K. pneumonia* infection by beta ray. Study design and study populations of 100 isolates of *K. pneumonia* that identified by Vitek2GN, results of identification for all isolates approve are *K. pneumonia*. Study production of biofilm achieved by Congo red agar test, positive results by formation black colony on Congo red agar and negative results with red colony on Congo red agar. Influence Strontium Sr^{90} on *K. pneumonia* with dose D*(1hr.) $0.73251*10^{-2}$ msV; D*(2hr.) $1.46502*10^{-2}$ msV; D*(3hr.) $2.19753*10^{-2}$ msV; D*(4hr.) $2.93004*10^{-2}$ msV in different hours percentage of killing for this bacteria reach to 90% with control 300 colony, elimination of biofilm production from this bacteria by all doses in different time with formation pink color of colony compared with control formation black colony on Congo red agar, this results of affect by Strontium cause lost production biofilm by turn off into pink color when exposed to different doses of beta rays emitted from Strontium compared with control with black color.

Key Words: Radiation, Bacteria and Biofilm.

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Introduction

K. pneumoniae is non-motile and gram-negative. In addition, it is encapsulation-fermenting with optional anaerobic bacteria. It takes the form of rod-shaped causing a hemorrhagic necrotizing lung consolidation. Sometimes, it could induce urinary tract infection with focal lesion bacteremia when patients compromised connected to hospital infection such as malignantness, bacteremia, biliary diseases, cirrhosis, infections of biliary tract, endothalmitis, meningitids, pyogenic cerebrum boil, urinary, pneumonia, diabetes mellitus osteomas , bacteremia and alcoholism can impair to protect people and makes the risk of *K. pneumoniae* infection bigger (Jasim & Farhan, 2020; Tsai et al., 2010) resulting pneumonia, pyogenic liver abscesses and bloodstream infection in mammals (Newire, Ahmed, House, Valiente, & Pimentel, 2013; Siu et al., 2011). In spite of antibiotic therapy is a common tool to treat infections resulted from *K. pneumonia* (Guo et al., 2015; White, Zhao, Simjee, Wagner, & McDermott, 2002).

Biofilm-forming bacteria appear on the outer layer of the tissues and biomaterials at regions of persistent infections (Costerton, Montanaro, & Arciola, 2005). Medical implants are vulnerable to biofilm formation. The same is true with catheters. This is due to immune responses expressively decreased close to foreign bodies (Edmiston, McBain, Roberts, & Leaper, 2015).

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Biofilm formation is the primary reason for implant failure and usually restricts the lifetime of several indwelling medical devices (Ahmed & Darouiche, 2015). Biofilms works as protective measure for microorganisms against opsonization by "antibodies, phagocytosis and removal via the ciliary action of epithelial cells, bacterial populations in biofilms are considerably more resistant than free-living planktonic cells are to antibacterial agents" (Anderl, Franklin, & Stewart, 2000; Ghafourian et al., 2012). A biofilm could be a microorganism community with cells are could be embedded in "self-produced matrix of an extra-cellular polymer (EPS)" material with acylated homoserine lactone (AHL) in quorum sensing due to their limited mobility (Li, Lau, Lee, Ellen, & Cvitkovitch, 2001). Biofilm extracellular polymers, known as slime, could be polymeric accumulation normally consisting of extracellular DNA, polysaccharides and proteins. Biofilms is applicable to the (non) living widely utilized in nature, industry, and hospitals (Lear & Lewis, 2012).

There is a wide use of Radiation sterilization, as a physical cold process and in the developed and developing world for sterilizing in health industry. In a historical review, there is a clear ionizing radiation use to treat several infections before the appearance of antibiotics (Calabrese & Baldwin, 2000).

Methodology

Clinical examination includes study populations from different clinical sources, sample collections, isolation and identification by Vitek2-GN, study production of biofilm, elimination of biofilm production from *K. pneumonia* using Sr^{90} radiosources (Collins & Lawson, 2000).

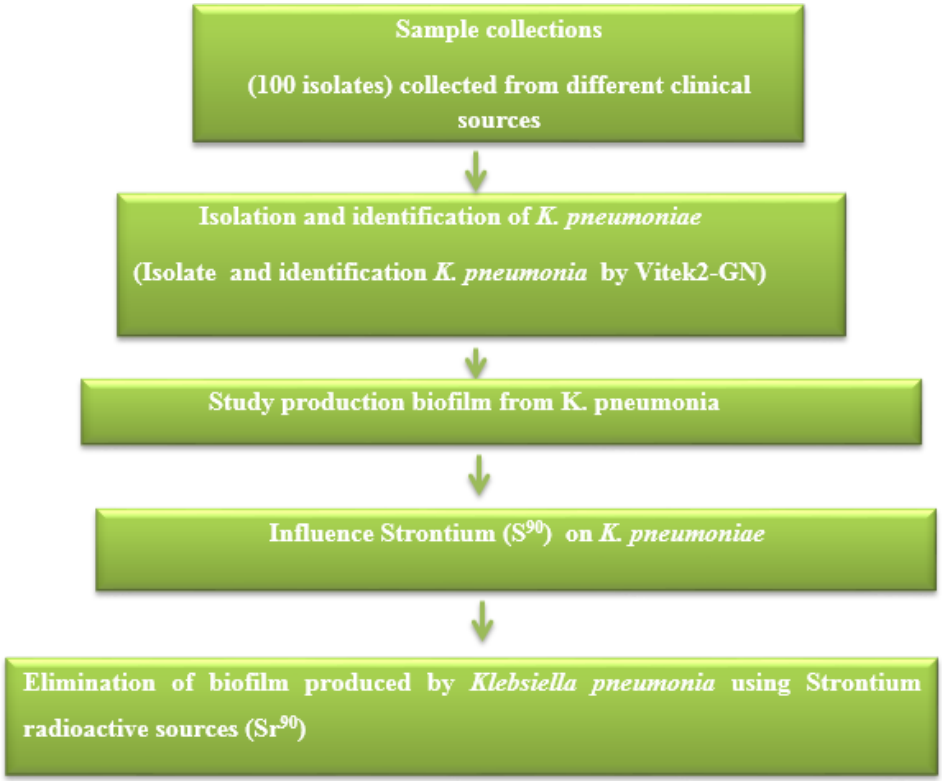

Figure 1. Scheme for Study design of this research

Study Populations

A total of 100 *K. pneumonia* isolates were gathered from sputum from Baghdad hospitals in 2021. There is a clear identification of these isolates by conventional biochemical reactions based on the the criteria of the isolates inoculated a Nutrient agar. The outcomes remained for 24 h of incubation at 37°C (Forbes, Sahm, & Weissfeld, 2007).

A. Isolation and Identification of K. pneumoniae

In this work, there were 100 *K.* isolated *pneumoniae* from sputum. The isolate dentification was conducted by Vitek2 GN. There are freshly subcultured isolates on "brain-heart infusion (BHI) agar" before each test (Tille, 2013).

B. Procedure for identification of K. pneumonia by Vitek2-GN

Identification of *K. pneumonia* isolates was conducted using Vitek2 GN system according to the procedure suggested by the manufacturing company. 64 biochemical tests were used to measure enzymatic activities, carbon source utilization, and antibiotics resistance. This system is designed for the performance from one purified isolate colony. The bacterial suspension was made by Vitek2 GN suspension solution. In addition, the turbidity was modified by 0.5 McFarland (1.5×10^{8}CFU/ml). The Gram (GN) cnegative card was for the automated identifying of most important Gram negative bacteria. The GN identification card was utilized to establish biochemical methods, results from the concept of the card to provide anaerobic conditions and other microbes with little need for air, according to the suitability of each test and as instructed by the company Biomerieux. French and newly developed substrates with a sterile Pasteur pipette, were inoculated based on the manufactures instructions. Following the incubation at 37°C for 24hrs., we identified the isolate by the automatic analytical, rapid identification at species level. The tubes of suspension and card of GN were placed in the cassette with one negative control well**.** The device works through a period of cuddling on the analysis and storage of biochemical patterns are subjective and cuddling analyzed the device software these patterns printed diagnostic report for each card inside Reader/ Incubator according to instructions Biomerieux company (Barros, Carvalho, Peralta, Facklam, & Teixeira, 2001; Carlton, Noordman, Biswas, De Meester, & Loessner, 2005; Chang et al., 2002; corporation, 2010).

Study Production Biofilm from *K. pneumoniae*

A. Prepare Congo–red-agar Medium

This is made through dissolving 37g of Brain heart Infusion broth 50 g Sucrose and 10 g Agar agar in (900 ml) distilled water. It is then sterilized through the autoclaving process. We made the Congo-red stain by dissolving 0.8 g in 100 ml of distilled water being sterilized through autoclaving mixed with media into 55°C. put into sterile petri plates. This approach helps in the detection of producing biofilm(Freeman, Falkiner, & Keane, 1989).

B. Production Biofilm on Congo Red Test

A study by (Mathur et al., 2005) showed a method to screen the biofilm formation. The inoculation of plates was conducted by single colony incubating aerobically for 24 - 48 hr at 37C°. The positive outcomes appeared by black colonies with a dry crystalline steadiness. Weak slime producers often remained pink, yet with some darkening at the centers of colonies. A darkening black colony with no dry crystalline colonial morphology showed an indeterminate outcome.

Effectiveness Strontium Sr^{90} Radiosources on *Klebsiella pneumoniae*

A. Exposure Strontium Sr^{90} to K. pneumoniae

The grown "isolates were in LB broth for 24 h. on shaker (150 rpm) at 30ºC". The centrifuging process was conducted on the well grown bacterial culture at 8000 rpm for quarter of an hour. There was a suspension of "the supernatant decanted and the pellets in sterile saline". The suspended cells remained in a clean sterile flask for the sake of forming pools. The suspension of pool bacteria (5ml) was put in a clean screw of sterile cap test tubes at various doses D*(1hr.) $0.73251*10^{-2}$ msV; D*(2hr.) $1.46502*10^{-2}$ msV; D*(3hr.) $2.19753*10^{-2}$ msV; D*(4hr.) $2.93004*10^{-2}$ msV of Beta radiation . The irradiated cultures and the non-irradiated control were serially diluted. They were also plated on the outer layer of TSA agar plates. In addition, the colonies were measured and inhibition effects were examined with the measured percent reduction of bacterial growth by equation (Trampuz, Piper, Steckelberg, & Patel, 2006).
The percentage of Killing, calculated from equation

$$\text{Percentage of Killing \%} = \frac{\text{Control} - \text{treated}}{\text{Control}} * 100$$

B. Elimination Biofilm Production by Strontium Sr^{90} from K. pneumonia

The irradiation facility used was Beta (β) that emitted from (Sr^{90}) isotope, after exposure *K. pneumoniae* to Strontium Sr^{90} in different doses measure biofilm production on Congo red agar medium, results detected positive with black colony and negative results with pink colony (Mathur et al., 2005).

Statistical Analysis

We utilized "SPSS version 22.0" to conduct the statistical analysis. The "Chi-square analysis" compared the biofilm production capacity with the clinical specimen type of (Collins & Lawson, 2000).

Results and Discussions

A. Study Design and Study Populations

The study design complies with a plan or a protocol for direct study for the translation of the conceptual hypothesis into an operational one. Also formulating trials and experiments, observations in medical, clinical etc. (e.g: epidemiological) such as human beings (Porta, 2014) in the study cross-sectional design.

B. Bacterial Isolation and Identification

The study design and study populations of 100 isolates of *K. pneumonia* that identified by Vitek2GN, results of identification for all isolates approve are *K. pneumonia*.

C. Study Production of Biofilm from K. pneumoniae

Study production of biofilm from *K. pneumonia* achieved by Congo red agar Strontium as control, positive results by formation black colony on Congo red agar.

Figure 2. Study production of biofilm from *K. pneumonia* before exposure to Strontium Sr^{90} (Control).

Results in figure (2) explain production of biofilm from *K. pneumonia* before exposure to Strontium Sr^{90} (Control), give black color on Congo red agar medium.

There is a past study (Zheng et al., 2018). This work showed BF greater in isolates of the young adults than in the seniors. This contradicts past works of sputum samples were there was a greater BF among isolates of patients older than 70 years than in those younger than 70 (Yang & Zhang, 2008). This difference is linked to patients younger than 40 years old having good immune systems putting a pressure on the bacteria for forming biofilms for evading the host immunity. This work found that BF is linked to insistent bacteremia infection after 72 h treatment conforming the difficulties in treating the bacteria (Gunn, Bakaletz, & Wozniak, 2016).

D. Effectiveness Strontium Radiosouces (Sr^{90}) on K. pneumonia

The irradiation effect on cell membrane, DNA, cytoplasmic membrane and cause damage to this bacterium. Gamma rays damage at *K. pneumonia* a cellular level being penetrated, VG. Gamma rays are lower in ionising than Beta of Alpha particles as less penetrating (Rothkamm & Löbrich, 2003).

Table 1. The inhibition effect of irradiation on *S.aureus* growth after exposure to Gamma irradiation

Isotope	Dose (D*)msV				
	Type of decaye	1 hr	2 hr	3 hr	4 hr
^{90} Sr	β	$0.73251*10^{-2}$	$1.46502*10^{-2}$	$2.19753*10^{-2}$	$2.93004*10^{-2}$

Results in table (1) represents different doses with different hours for Influence Strontium Sr^{90} on *K. pneumonia* with dose D*(1hr.) $0.73251*10^{-2}$ msV; D*(2hr.) $1.46502*10^{-2}$ msV; D*(3hr.) $2.19753*10^{-2}$ msV; D*(4hr.) $2.93004*10^{-2}$ msV in different hours percentage of killing for this bacteria reach to 90% with control 300 colony.

E. Elimination Biofilm Produced from K. pneumonia after Exposure to Strontium Radiosouces (Sr^{90})

Biofilm production of *K. pneumonia* after exposure to Strontium Sr^{90} radiosources examine on Congo red agar medium in different doses.

Figure 3. Study production of biofilm from *K. pneumonia* after exposure to Strontium Sr^{90} (Control) leading to elimination biofilm production

Results in figure (3) explain production of biofilm from *K. pneumonia* after exposure to Strontium on Congo red to pink agar medium this results explain negative results for production biofilm from *K. pneumonia* when exposed to radiation, Sr^{90} this results indicates turn off *K. pneumonia* into less virulent by produce biofilm with black colony turn off into less virulent that lost production biofilm with red to pink color on Congo red agar medium, this results efficiency to elimination biofilm production.

Influence Strontium Sr^{90} on *K. pneumonia* with dose D*(1hr.) $0.73251*10^{-2}$ msV; D*(2hr.) $1.46502*10^{-2}$ msV; D*(3hr.) $2.19753*10^{-2}$ msV; D*(4hr.) $2.93004*10^{-2}$ msV in different hours percentage of killing for this bacteria reach to 90% with control 300 colony, elimination of biofilm production from this bacteria by all doses in different time with formation pink color of colony compared with control formation black colony on Congo red agar, this results of affect by Strontium cause lost production biofilm by turn off into pink color when exposed to different doses of beta rays emitted from Strontium compared with control with black color.

Biofilm production of *K. pneumoniae* gathered from clinical specimens has a capability showing a 93% of isolates from biofilm. Biofilm that form bacteria could result in infections in catheter- and implant-linked infections and life-threatening disorders in people suffering from w cystic fibrosis, chronic otitis media and chronic wounds. Millions of people suffer from these bacteria worldwide annually at a big mortality rate (Bjarnsholt, 2013). The nosocomial opportunistic microorganisms *K. pneumoniae* production of biofilms on outer layer tissue of the host is important in developing the infections. Developing biofilm influences the efficiency of antimicrobial treatements and the next infection results (Borges, J Saavedra, & Simoes, 2015; Murphy & Clegg, 2012).

Conclusions

1. Strontium Sr^{90} very efficiency for elimination biofilm production from *K. pneumonia* in different doses.
2. Irradiated *K. pneumoniae* turn off from produce biofilm with black color into non produce biofilm with pink color that leading to turn off from virulent into non virulent bacteria.

References

Ahmed S, Darouiche RO. Anti-biofilm agents in control of device-related infections. *Biofilm-based healthcare-associated infections* 2015: 137-146.

Anderl JN, Franklin MJ, Stewart PS. Role of antibiotic penetration limitation in Klebsiella pneumoniae biofilm resistance to ampicillin and ciprofloxacin. *Antimicrobial agents and chemotherapy* 2000; 44(7): 1818-1824.

Barros RR, Carvalho MDGRS, Peralta JM, Facklam RR, Teixeira LM. Phenotypic and genotypic characterization of Pediococcus strains isolated from human clinical sources. *Journal of clinical microbiology* 2001; 39(4): 1241-1246.

Bjarnsholt T. The role of bacterial biofilms in chronic infections. *Apmis* 2013; 121: 1-58.

Borges A, Saavedra MJ, Simoes M. Insights on antimicrobial resistance, biofilms and the use of phytochemicals as new antimicrobial agents. *Current medicinal chemistry* 2015; 22(21): 2590-2614.

Calabrese E, Baldwin L. Radiation hormesis: the demise of a legitimate hypothesis. *Human & experimental toxicology,* 2000; 19(1): 76-84.

Carlton R, Noordman W, Biswas B, De Meester E, Loessner MJ. Bacteriophage P100 for control of Listeria monocytogenes in foods: genome sequence, bioinformatic analyses, oral toxicity study, and application. *Regulatory Toxicology and Pharmacology* 2005; 43(3): 301-312.

Chang YH, Han JI, Chun J, Lee KC, Rhee MS, Kim YB, Bae KS. Comamonas koreensis sp. nov, a non-motile species from wetland in Woopo, Korea. *International Journal of Systematic and Evolutionary Microbiology* 2002; 52(2): 377-381.

Collins MD, Lawson PA. The genus Abiotrophia (Kawamura et al.) is not monophyletic: proposal of Granulicatella gen. nov, Granulicatella adiacens comb. nov, Granulicatella elegans comb. nov. and Granulicatella balaenopterae comb. nov. *International Journal of Systematic and Evolutionary Microbiology* 2000; 50(1): 365-369.

Costerton J, Montanaro L, Arciola CR. Biofilm in implant infections: its production and regulation. *The International journal of artificial organs* 2005; 28(11): 1062-1068.

Edmiston CE, McBain AJ, Roberts C, Leaper D. Clinical and microbiological aspects of biofilm-associated surgical site infections. *Biofilm-based healthcare-associated infections*, 2015: 47-67.

Forbes BA, Sahm DF, Weissfeld AS. *Diagnostic microbiology*: Mosby St Louis 2007.

Freeman D, Falkiner F, Keane C. New method for detecting slime production by coagulase negative staphylococci. *Journal of clinical pathology* 1989; 42(8): 872-874.

Ghafourian S, Mohebi R, Rezaei M, Raftari M, Sekawi Z, Kazemian H, Sadeghifard N. Comparative analysis of biofilm development among MRSA and MSSA strains. *Roumanian archives of microbiology and immunology* 2012; 71(4): 175-182.

Gunn JS, Bakaletz LO, Wozniak DJ. What's on the outside matters: the role of the extracellular polymeric substance of Gram-negative biofilms in evading host immunity and as a target for therapeutic intervention. *Journal of Biological Chemistry* 2016; 291(24): 12538-12546.

Guo C, Yang X, Wu Y, Yang H, Han Y, Yang R, Zhou, D. MLST-based inference of genetic diversity and population

structure of clinical Klebsiella pneumoniae, China. *Scientific reports* 2015; 5(1): 1-6.

Jasim ST, Farhan AS. Article Review: Klebsiella Pneumonia: Epidemiology, Virulence Factors and Treatment. *Flora* 2020; 14(2): 5-10.

Lear G, Lewis GD. Microbial biofilms: current research and applications 2012.

Li YH, Lau PC, Lee JH, Ellen RP, Cvitkovitch DG. Natural genetic transformation of Streptococcus mutans growing in biofilms. *Journal of bacteriology* 2001; 183(3): 897-908.

Mathur T, Singhal S, Khan S, Upadhyay D, Fatma T, Rattan A. Adverse effect of staphylococci slime on in vitro activity of glycopeptides. *Japanese journal of infectious diseases* 2005; 58(6): 353-357.

Murphy CN, Clegg, S. Klebsiella pneumoniae and type 3 fimbriae: nosocomial infection, regulation and biofilm formation. *Future Microbiology* 2012; 7(10): 1234-1234.

Newire EA, Ahmed SF, House B, Valiente E, Pimentel G. Detection of new SHV-12, SHV-5 and SHV-2a variants of extended spectrum beta-lactamase in Klebsiella pneumoniae in Egypt. *Annals of clinical microbiology and antimicrobials* 2013; 12(1): 1-12.

Porta M. *A dictionary of epidemiology*: Oxford university press 2014.

Rothkamm K, Löbrich M. Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses. *Proceedings of the National Academy of Sciences* 2003; 100(9): 5057-5062.

Siu LK, Fung CP, Chang FY, Lee N, Yeh KM, Koh TH, Ip, M. Molecular typing and virulence analysis of serotype K1 Klebsiella pneumoniae strains isolated from liver abscess patients and stool samples from noninfectious subjects in Hong Kong, Singapore, and Taiwan. *Journal of clinical microbiology* 2011; 49(11): 3761-3765.

Tille PBASS. Diagnostic Microbiology. *Elsevier Health Sciences* 2013.

Trampuz A, Piper KE, Steckelberg JM, Patel R. Effect of gamma irradiation on viability and DNA of Staphylococcus epidermidis and Escherichia coli. *Journal of medical microbiology* 2006; 55(9), 1271-1275.

Tsai SS, Huang JC, Chen ST, Sun JH, Wang CC, Lin SF, Huang YY. Characteristics of Klebsiella pneumoniae bacteremia in community-acquired and nosocomial infections in diabetic patients. *Chang Gung Medical Journal* 2010; 33(5): 532-539.

White DG, Zhao S, Simjee S, Wagner DD, McDermott PF. Antimicrobial resistance of foodborne pathogens. *Microbes and infection* 2002; 4(4): 405-412.

Yang D, Zhang Z. Biofilm-forming Klebsiella pneumoniae strains have greater likelihood of producing extended-spectrum β-lactamases. *Journal of Hospital Infection* 2008; 68(4): 369-371.

Zheng JX, Lin ZW, Chen C, Chen Z, Lin, FJ, Wu Y, Li, DY. Biofilm formation in Klebsiella pneumoniae bacteremia strains was found to be associated with CC23 and the presence of wcaG. *Frontiers in cellular and infection microbiology* 2018: 8.

Hameed SA, Saadmahdi Z, Jasim AN, Taha AF, Habeeb AA. Effect of thickness on structural and optical properties of cdo thin films prepared by chemical spray pyrolysis method. *NeuroQuantology* 2020; 18(4): 20-26.