ISSN No:-2456-2165

Isolation and Determination of the Spore -Forming Gene in Pathogenic Bacteria by PCR Technique at the Republican Hospital in Kirkuk

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Abstract:- This study focuses on the isolation and molecular quantification of bacteriostatic genes in pathogenic bacteria prevalent in the Republican Hospital in Kirkuk. Spore formation plays a pivotal role in the virulence and persistence of pathogenic bacteria, making it essential to understand the presence and diversity of spore-forming genes in the hospital environment. Using polymerase chain reaction (PCR) technology, we aimed to develop a targeted and effective method to detect bacteriostatic genes within bacterial isolates obtained from clinical samples from 200 patients of different ages and genders at the Republican Hospital. Bacterial isolates were collected from various clinical sources using standard microbiological protocols, and their identification was confirmed through traditional microbiological methods. *Bacillus subtilis, Clostridium perfringens, Pseudomonas aeruginosa Escherichia coli, Streptomyces aureus. Enterobacter cloacae* were isolated from 50% of patients. Genomic DNA extraction was performed, and PCR primers were designed to specifically amplify the spore-forming gene region. The resulting PCR products were visualized using gel electrophoresis to confirm the presence of target genes. The result was the isolation of the .spo0A gene. The study not only aimed to determine the prevalence of spore-forming genes in pathogenic bacteria, but also explored potential associations with clinical outcomes and antibiotic resistance profiles. In addition, bioinformatics tools were used to analyze genetic evolution, highlighting the genetic diversity and evolutionary aspects of bacteriostatic genes among isolates. This research, conducted at the Republican Hospital in Kirkuk, provides valuable insights into the molecular characteristics of pathogenic bacteria in the healthcare setting. The findings contribute to our understanding of microbial dynamics within hospitals and may inform infection control strategies. Furthermore, the PCR-based approach provides a rapid and sensitive diagnostic tool to detect spore-forming genes, facilitating targeted interventions to mitigate the impact of spore-forming bacteria on patient health.

Keywords:- *Polymerase Chain Reaction Technique (PCR)- Spore-Forming Bacteria – Genome- Spo0a Gene.*

I. INTRODUCTION

Bacterial survival mechanisms in harsh environmental conditions often involve the formation of spores, with Gram-positive bacteria, particularly Bacillus and Clostridium species, being known for their proficiency in producing endospores .The extremely specialized cellular forms known as bacterial endospores enable endospore-forming Formicates (EFF) to withstand extreme environmental conditions. [1,2] .EFF are thought to be common in natural settings, especially those that experience stress. Apart from their presence in natural settings, EFF frequently leads to contamination issues in man-made locations like hospitals or industrial production units. [3,4]. Spore-forming bacteria such as *Clostridium perfringens Bacillus cereus*, and *Bacillus subtilis* have been implicated in hospital-acquired infections and nosocomial outbreaks, primarily through transmission via healthcare workers and contaminated surgical instruments [5,6,7]. These spore-forming bacteria exhibit remarkable resistance to alcohol-based disinfectants and various treatments that typically eliminate vegetative cells, including desiccation, heat, UV and γ-radiation, mechanical stress, chemical exposure, hydrostatic pressure, and osmotic stress [8,9]. Hence, it is imperative to identify these spore-forming bacteria and elucidate the genes responsible for spore formation, with spo0A being a pivotal gene involved in endospore development [10,11]. The ubiquitous presence of the regulatory protein Spo0A is a hallmark of stress-induced gene expression machinery and endospore formation [12,13]. Molecular tests targeting spore-forming genes can aid in classifying and characterizing these bacteria, distinguishing between species with and without sporulation genes [14,15]. Previous studies have determined gene homologs in various bacteria using degenerate PCR [16,17]. PCR, a widely used laboratory technique, enables the rapid amplification of specific DNA and RNA sequences, making it invaluable for bacterial identification and detecting resistance genes [18]. Multiplex PCR, an enhanced version, incorporates multiple primers in a single reaction, facilitating the simultaneous analysis of multiple genes and reducing costs and time [19,20,21]. Quantitative PCR (qPCR) is a precise method for quantifying gene frequencies in DNA extracts [22,23,24]. In this study, qPCR primers targeting the spo0A gene were employed, tested, and validated in pure culture samples [25,26,27]. In summary, understanding the mechanisms and genes involved in bacterial spore formation is crucial for

addressing the challenges spore-forming bacteria poses, especially in healthcare settings. Molecular techniques such as PCR and qPCR play pivotal roles in this endeavour, enabling the identification and characterisation of these resilient microorganisms [28].

II. METHODOLOGY

A. Samples

This research was conducted in one of Kirkuk's hospitals, which is Kirkuk General Hospital in Iraq, from January 12, 2023 to 12 of May, 2023. A total of 200 samples were collected from the wounds of patients who underwent various surgical operations.

B. Sample Collection:

Samples were collected by swabbing post-operative wounds. These collected samples were immediately transported to the laboratory to ensure their microbial integrity. Samples were inoculated onto blood agar, chocolate agar, and MacConkey agar plates. After that, the plates were incubated for 24 hours at 37°C. After incubation, observe the agar plates for bacterial growth. Isolate individual bacterial colonies by streaking them onto fresh agar plates using the quadrant streak method or a similar technique To obtain pure cultures, sub-culturing streak isolated colonies onto new agar plates. Repeat this process until a pure culture of the spore-forming bacteria is obtained. Perform Gram staining and other preliminary tests to identify the general characteristics of the isolated bacteria. Subject the isolated bacteria to heat treatment (e.g., 80-85°C for 10 minutes) to induce sporulation. stain the bacterial smears with malachite green or another suitable spore stain and observe under a microscope for the presence of spores. Preserve pure cultures of spore-forming bacteria by storing them at -80°C or in appropriate culture storage conditions, using a suitable cry protect ant like glycerol. This protocol provides a basic guideline for isolating spore-forming bacteria. [29].. In order to identify spore-forming bacteria among diverse bacterial species, suspected colonies were subjected to a series of biochemical tests, including catalase production, motility assessment, root growth assessment, citrate utilization reactions, and hemolysis assays. These species identification test procedures are well documented in the scientific literature [30, 31]

C. Isolation of Spore-Forming Bacteria :

The bacterial samples were examined under a microscope due to their Gram stain composition. Simultaneously, the isolation procedure concentrated on detecting bacteria exhibiting ethanol resistance, a feature frequently linked to spore-forming bacteria. After isolation, the size of the colony was measured, and a series of tests was used to distinguish between different kinds of bacteria. The Mannitol test produced positive results, demonstrating that the bacteria were using Mannitol. On the other hand. the lecithinase activity of S. aureus is used in detection of coagulase-positive strains, because of high link between lecithinase activity and coagulase activity. Can be used to differentiate between certain species within the genus .Bacillus the lecithinase test produced negative results, meaning that lecithinase activity was absent. The isolated colonies were notable for their notable features, which included a wrinkled surface on the agar medium, a notable size, and yellow coloring. As a result of these distinguishing features, the isolated bacteria were identified as *Bacillus subtilis , Clostridium perfringens, Pseudomonas aeruginosa Escherichia coli, Streptomyces aureus. Enterobacter cloacae*. Conversely, Mannitol-negative, lecithinase-positive, large, flat, and grainy colonies on the agar surface were identified as belonging to the Bacillus species [32,33].

D. DNA Extraction:

Characteristic bacterial colonies were selected and inoculated into a 5 mL Brain Heart Infusion (BHI) solution obtained from (Sigma-Aldrich, USA). The inoculated solution was incubated overnight at 35°C. Subsequently, DNA extraction was performed from the cultured solution using the (QIA amp DNA mini kit) following the manufacturer's instructions for the kit.

E. DNA Quantification:

To evaluate the quality of the extracted DNA, agarose gel electrophoresis was employed, and the concentration of the isolated DNA was assessed at 260 nm using a Nano drop ND 2000 spectrophotometer from (Thermo Scientific, USA). [34,35,36].

F. DNA Sequences for Spore Forming Genes:

To investigate the bacterial genome, particularly focusing on spore-forming genes, we employed primers targeting the 16S rRNA gene, generating fragments of approximately 500 bp. These primers, Eub8f (5'-AGAGTTTGATCCTGGCTCAG-3') and Eub519r (5'-GTATTACCGCGGCTGCTGG-3'), were used for this purpose [37,38 ,39].

While the 16S rRNA gene provides phylogenetic information about the presence of various bacterial species, we also needed to examine a functional indicator related to sporulation, specifically the spo0A gene. To achieve this, we utilized primers designed for the spo0A gene. These primers, spo0A166f (5′-GATATHATYATGCCDCATYT-3′) and spo0A748r (5′-GCNACCATHGCRATRAAYTC-3′), were chosen based on their specificity, amplification efficiency, fragment length, and sequence compatibility with the spo0A gene, which plays a central role in the spore-forming process [40,41].

G. PCR Technique:

The reaction mixture consisted of 20 µL of Master Mix (2X) from Thermo Fisher, USA, along with 8.3 µL of distilled water, 1 µL (10 µM) of each primer, and 2 µL (approximately 50 ng) of template DNA. This reaction mixture was then placed in a thermal cycler programmed for one cycle at 92 °C for 18 minutes, followed by 30 cycles of denaturation at 92 °C for 30 seconds, annealing at 52°C for 30 seconds, and extension at 70°C for 1.5 minutes. Finally, a single extension step at 70°C for 1.5 minutes was performed. [42].

The PCR products were subsequently loaded onto a 1.5% agarose gel and stained with ethidium bromide (ETBR) at a concentration of 0.5 µg/mL for the electrophoresis step. The PCR samples were visualized using an ultraviolet trans illuminator, and the resulting bands were analyzed using a gel documentation system for documentation and analysis. [43,45].

- *The Diagnosis Species were as Follows*

The identified bacterial species in the study included Escherichia coli (found in 15 cases), Enterobacter cloacae (found in 30 cases), Bacillus subtilis (15 cases), staphylococcus aureus . (14 cases), Pseudomonas aeruginosa (15 cases), and Clostridium perfringens (11 cases).

Among these identified bacterial species, Bacillus subtilis and Clostridium perfringens are well-known for their spore-forming capabilities. The primers used in this study targeted the spo0A gene, recognized as the key gene responsible for initiating spore formation. The sequencing results confirmed the presence of the spo0A gene in these spore- forming bacteria isolated from Kirkuk General Hospital [46]., as summarized in Table 1.

III. RESULT

The results indicate that out of the total 200 samples analyzed, 100 of them tested positive for bacterial infection, confirming an infection rate of 50% based on the sample set. The gel electrophoresis results, depicted in (Figure 1), demonstrate the quality of the PCR products. Following the subsequent steps of cloning and sequencing, the bacterial species were successfully identified.

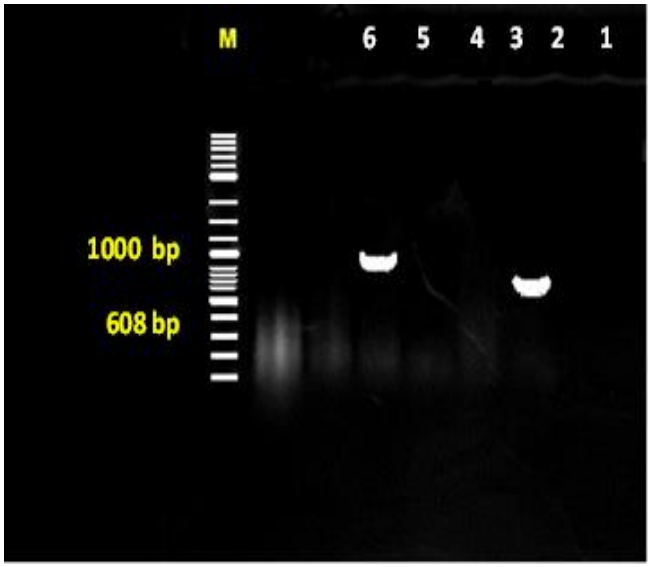

Fig. 1: Agarose Gel Electrophoresis of PCR-Amplified Partial 16S rRNA Genes from Bacteria

As Lane M represents the DNA ladder , where lanes from 1 to 6 represent the PCR amplified genes for the isolated bacteria , *Escherichia coli* (No 1 no band found) , Entrobacter cloacae (No 2 no band found) , *Bacillus subtilis* (No 3 band appeared 1000 bp) *Staphylococcus aureus* (No 4 no band). , *Pseudomonas aeruginosa* (No 5 no band found), *Clostridium perfringens* (No 6 band appeared 608 bp) respectively.

IV. DISSCUTION

The occurrence of post-operative infections caused by microbial agents, particularly spore-forming bacteria, poses a significant medical challenge. Addressing this issue requires effective interventions and increased attention to public hospitals in Iraq. Consequently, the adoption of accurate techniques, proper procedures, and swift detection methods for identifying infectious microbes from wounds is imperative According to studies conducted in 2008 by Isibor and in 2009 and 2010 by Pradhan, wound infections happen in hospitals following surgical procedures, and the degree of contamination in the wound, where it is located, and how long the patient stays there can all affect how well the patient does. [47,48, 46].

Polymerase chain reaction (PCR) stands out as a sensitive, specific, and rapid molecular method extensively employed for detecting microbial species in medical specimens. In our current study, PCR is indispensable, as it allows us to target a specific gene, namely the spore-forming gene.spo0A in *bacillus sabtilis* and clostridium *perfringes* as in the study of Hoon and Krieg in 2010 and 2009 that within the Firmicutes, endospore formers constitute a paraphyletic group [50]. Only the first two classes of this phylum—Bacilli, Clostridia, and Erysipelotrichi—contain species that generate endospores. Bacilli are primarily aerobic bacteria, while Clostridia are primarily anaerobic kinds of bacteria [51].

The majority of our understanding of the biology of endospore-forming Firmicutes (EFF) has come from investigations conducted in laboratories using cultivable strains since the late 19th century [51]. PCR's capabilities encompass gene extraction, gene sequence comparison, and determination of a specific gene's presence within bacterial genomes.

Our examination involved collecting samples from 100 patients with post- operative wounds using cotton swabs. All collected samples underwent cultivation to assess the presence of infection as in the study of Qadan in 2009 [52]. The results revealed that 50% of the wounded individuals were infected, underscoring the urgency of enhancing the surgical department's attention in public hospitals each of Niska and Pittet have They have results close to those of our study [53,54,55].. Various reactions, such as gram staining (which identifies spore-forming bacteria as gram-positive) and tests for catalase production, motility, rhizoid growth, citrate reactions, and hemolysis, were conducted to achieve this goal. Subsequently, total DNA was extracted and employed as a template for amplifying the 16S rRNA sequence as in studying both Madhavan and Jones have results an approach to the results of this study [56,57, 58]. Additionally, the spo0A gene was targeted to identify spore-forming bacteria within mixed bacterial samples for the protection of regions within the

16S rRNA gene of bacteria, we utilized globally recognized primers this approach considered a group of bacteria responsible for infections. Furthermore, we employed forward and reverse primers for the master gene responsible for regulating spore formation, the spo0A gene. which were *Bacillus subtilis* , *Clostridium perfringens,* as shown in the study of Anand and Errington in (2001,2000). [59,60].

Table 1: Test for Spo0A Amplification through the used Primers

Bacteria Series	**Temp of Optimal Growth**	**Endospore Formation**	**Amplificatio n of *Spo0a Gene***	**Sequences for Spo0a**
Bacillus subtilis	30 ^{0}c	Positive	Positive	sequences spo0A166f (5′-GATATHATYATGCC DCATYT-3′) and spo0A748r (5′-GCNACCATHGCRAT RAAYTC-3′)
Clostridium perfringens,	30 c^0	Positive	Positive	sequences spo0A166f (5′-GATATHATYATGCC DCATYT-3′) and spo0A748r (5′-GCNACCATHGCRAT RAAYTC-3′)
Pseudomonas aeruginosa	30 c^0	Negative	Negative	Absent
Escherichia coli	30 c^0	Negative	Negative	Absent
Staphylococcus areaus	30c^0	Negative	Negative	Absent
Enterobacter cloacae	30 c^0	Negative	Negative	Absent

V. CONCLUSION

The isolation and determination of the spore-forming gene in pathogenic bacteria using the Polymerase Chain Reaction (PCR) technique at the Republican Hospital in Kirkuk hold significant implications for understanding and managing bacterial infections. This sophisticated molecular biology approach allows for the identification and characterization of spore-forming genes, providing crucial insights into the virulence and persistence mechanisms of pathogenic bacteria. By employing PCR, the researchers can specifically amplify and detect the spore-forming gene, enhancing diagnostic capabilities for identifying bacterial strains with increased resistance and survival mechanisms. This information is vital for tailoring effective treatment strategies, as spore formation often contributes to bacterial resilience against conventional therapies. This study confirmed the success of isolating and diagnosing spore-forming bacteria in certain species and genera of bacteria using various traditional diagnostic methods. *Pseudomonas aeruginosa, Escherichia coli, and Streptomyces aureus were diagnosed. Enterobactercloacae, Bacillus subtilis and Clostridium perfringens* which does not form sepals. Moreover, the gene responsible for the formation of spores was identified in both *Bacillus subtilis and Clostridium perfringens* was positive as for everyone of *Pseudomonas aeruginosa, Escherichia coli, and Streptomyces aureus were diagnosed. Entero bactercloacae* were negative using PCR technology. The efficiency of PCR in identifying this gene of this bacterium has been demonstrated through the use of spo0A genetic primers Moreover, this research at the Republican Hospital in Kirkuk contributes to the broader field of microbiology and public health. Understanding the genetic basis of spore formation in pathogenic bacteria can aid in the development of targeted therapies, vaccines, and preventive measures to combat the spread of infectious diseases. Additionally, the findings may have implications for infection control practices within healthcare settings. In conclusion, the isolation and determination of the spore-forming gene using PCR at the Republican Hospital in Kirkuk represent a significant step forward in advancing our understanding of bacterial pathogenesis. This research has the potential to inform clinical practices, guide treatment strategies, and contribute to the overall improvement of public health in the region.

REFERENCES

[1]. Barbut F, et al.(2000). Epidemiology of recurrences or reinfections of Clostridium difficile-associated diarrhea. J. Clin. Microbiol. 38:2386 – 2388.
[2]. Setlow P. (2006). Spores of Bacillus subtilis: their resistance to and killing by radiation, heat and chemicals. J. Appl. Microbial. 101 514–525. 10.1111/j.1365- 2672.2005.02736.x
[3]. Richardson JF, Reith S. Characterization of a strain of methicillin-resistant Staphylococcus aureus (EMRSA-15) by conventional and molecular methods. J Hosp Infect 1993; 25: 45–52.
[4]. Labbé R and Hariram U,.(2015) Spore prevalence and toxigenicity of Bacillus cereus and Bacillus thuringiensis isolates from U.S. retail spices. J Food Prot. Mar;78(3):590-6.
[5]. Sarker MR Paredes-Sabja D, Raju D, Torres JA,. (2008) Role of small, acid-soluble spore proteins in the resistance of Clostridium perfringens spores to chemicals. Int J Food Microbiol. Mar 20;122(3):333-5.

[6]. Sasahara, T., Ae, R., Watanabe, M., Kimura, Y., Yonekawa, C., Hayashi, S., & Morisawa, Y. (2016). Contamination of healthcare workers' hands with bacterial spores. Journal of Infection and Chemotherapy, 22(8), 521-525.
[7]. Pittet D, Boyce JM,(2002). Healthcare Infection Control Practices Advisory Committee, Hand Hygiene Task Force. Guideline for hand hygiene in health-care settings. Recommendations of the healthcare infection control practices advisory committee and the hand hygiene task force. MMWR Recomm Rep;51:1e45.
[8]. Allegranzi B,Pittet D, Boyce J, (2009) .World Health Organization World Alliance for Patient Safety First Global Patient Safety Challenge Core Group of Experts. The world health organization guidelines on hand hygiene in health care and their consensus recommendations. Infect Control Hosp Epidemiol;30:611e22.
[9]. Russell AD. (1990) .Bacterial spores and chemical sporicidal agents. Clin Microbiol Rev; 3:99e119.
[10]. Coates Ayliffe, D., G. A. J., and P. N. Hoffman. (1984). Chemical disinfection in hospitals. Public Health Laboratory Service, London.
[11]. Piggot PJ, Coote JG.(1976). Genetic aspects of bacterial endospore formation. Bacteriol. Rev. 40:908 –962. 2.
[12]. Stragier P, Losick R. (1996). Molecular genetics of sporulation in Bacillus subtilis. Annu. Rev. Genet. 30:297–341.
[13]. Hong HA, , Cutting HSM. 2005. The use of bacterial spore formers as probiotics. FEMS Microbiol. Rev. 29:813– 835. Abdelaziz, A. A., and M. A. El-Nakeeb. 1988. Sporicidal
[14]. Abdelaziz, A. A., and M. A. El-Nakeeb.(1988). Sporicidal preservatives. activity of local anesthetics and their binary combinations with J. Clin. Pharm.. 13:249-256.
[15]. Niazi, A.; Manzoor, S.; Asari, S.; Bejai, S.; Meijer, J.; (2014) Bongcam-Rudloff, E. Genome analysis of Bacillus amyloliquefaciens subsp. plantarum UCMB5113: A rhizobacterium that improves plant growth and stress management. PLoS ONE, 9, e104651.
[16]. Chauhan, D.K Tripathi, D.K.; Singh, V.P.; Kumar, D.;. (2012) Impact of exogenous silicon addition on chromium uptake, growth, mineral elements, oxidative stress, antioxidant capacity, and leaf and root structures in rice seedlings exposed to hexavalent chromium. Acta Physiol. Plant. , 34, 279–289.
[17]. Debelius J Sharon G, Garg N, , Knight R, Dorrestein PC, Mazmanian SK. (2014). Specialized metabolites from the microbiome in health and disease. Cell Metab 20:719–730.
[18]. Relman DA Dethlefsen L, Huse S, Sogin ML,: (2008) .The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLoS Biol, 6:e280. 210.137.
[19]. Marcela Agne Alves Valones, et al (2009).. Principles and applications of polymerase chain reaction in medical diagnostic fields: a review .Braz J Microbiol.; 40(1): 1–11.
[20]. Mahbubani M. H., Bej, A. K., and R. M. Atlas.(1991). Amplification of nucleic acids by polymerase chain reaction (PCR) and other methods and applications. Crit. Rev. Biochem. Mol. Biol. 26:301–334.
[21]. Wagar, E. A. (1996). Direct hybridization and amplification applications for the diagnosis of infectious diseases. J. Clin. Lab. Anal. 10:312–325
[22]. Wolcott, M. J. (1992). Advances in nucleic acid-based detection methods. Clin. Microbiol. Rev. 5:370–386
[23]. Ehling-Schulz, M.; Knutsson, R. and Scherer, S. (2011): “Bacillus cereus” In: Kathariou S., P. Fratamico, and Y. Liu Editors.Genomes of Food- and Water-Borne Pathogens. ASM Press. Washington D.C., USA. 147-164.
[24]. Ehling-Schulz, M.; Knutsson, R. and Scherer, S. (2011): “Bacillus cereus” In: Kathariou S., P. Fratamico, and Y. Liu Editors.Genomes of Food- and Water-Borne Pathogens. ASM Press. Washington D.C., USA. 147-164
[25]. Tallent, S.M., Rhodehamel, E.J., Harmon, S.M., and Bennett, R.W. (2012): Bacteriological analytical manual (BAM); methods for specific pathogens. U.S. Food and Drug Administration. Chapter 14 Bacillus cereus.
[26]. Andrade JM, Kubista M, Bengtsson M, Forootan A, Jonak J, Lind K, Sindelka R, Sjoback R, Sjogreen B, Strombom L, Stahlberg A, and Zoric N.(2006). The real-time polymerase chain reaction. Mol. Aspects Med. 27:95–125
[27]. Koopman M.J.Mossel D.A., Jongerius E. (2012). Enumeration of Bacillus cereus in foods. Appl Microbiol. 1967; 15: 650-653
[28]. Errington J. 2003. Regulation of endospore formation in Bacillus subtilis. Nat. Rev. Microbiol. 1:117–126
[29]. Stenfors Arnesen, L.p.; Fagerlund, A. and Granum, P.E. (2008): From soil to gut: Bacillus cereus and its food poisoning toxins. FEMS Microbiol. Rev. 32 (4):579– 606.
[30]. Ehling-Schulz, M.; Knutsson, R. and Scherer, S. (2011): “Bacillus cereus” In: Kathariou S., P. Fratamico, and Y. Liu Editors.Genomes of Food- and Water-Borne Pathogens. ASM Press. Washington D.C., USA. 147-164.
[31]. Tallent, S.M., Rhodehamel, E.J., Harmon, S.M., and Bennett, R.W. (2012): Bacteriological analytical manual (BAM); methods for specific pathogens. U.S. Food and Drug Administration. Chapter 14 Bacillus cereus.
[32]. ,Kotewicz K.M. Strain E.A. Tallent S.M. Bennett R.W. (2012) Efficient isolation and identification of Bacillus cereus group. J AOAC Int. ; 95: 446-451
[33]. Mossel D.A.,Koopman M.J. Jongerius E. (2012). Enumeration of Bacillus cereus in foods. Appl Microbiol. 1967; 15: 650-653.

[34]. Alejandro M. G. *et al*,.(2020). Quantification of DNA through the Nano Drop Spectrophotometer: Methodological Validation Using Standard Reference Material and Sprague Dawley Rat and Human DNA. Hindawi International Journal of Analytical Chemistry Volume 2020, Article ID 8896738, 9 pages.

[35]. N. Sacchi, P. Chomczynski and "Single-step method of RNA isolation by acid guanidinium thiocyanate – phenol chloroform extraction," Analytical Biochemistry, vol. 162, no. 1, pp. 156–159, 1987.

[36]. S. R. Gallagher, "Quantitation of ADN and ARN with absorption and fluorescence spectroscopy," Current Protocols in Human Genetics, vol. 0, no. 1, pp. A.3D.1–A.3D.8, 1994.

[37]. Finegold S.M Hugenholtz, P. A .Tringe, S.G. (2008); renaissance for the pioneering 16S rRNA gene. Curr. Opin. Microbiol., 11, 442–446.

[38]. George W.L., Sutter V.L.Citron D.,. (1979) .Selective and differential medium for isolation of *Clostridium difficile*.*J Clin Microbiol*. ; 9: 214-219.

[39]. Woo, P.C.; Lau, S.K.; Teng, J.L.; Tse, H.; Yuen, K.Y. (2008). Then and now: Use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. Clin. Microbiol. Infect., 14, 908–934.

[40]. T. E. Meyer. 2002. Evolutionary analysis by wholegenome comparisons. J. Bacteriol. 184:2260–2272

[41]. Roussel-Delif, Wunderlin, T., Junier, T., L., Jeanneret, N., & Junier, P. (2013). Stage 0 sporulation gene A as a molecular marker to study diversity of endospore-forming F irmicutes. *Environmental Microbiology Reports*, *5*(6), 911-924.

[42]. Longo, M.C., *et al*., (1990). Use of uracil DNA glycosylase to control carryover contamination in polymerase chain reactions, Gene, 93, 125-128,.

[43]. Sambrook, J. & Russell, D.W. Molecular Cloning, 3 rd edition (2001).

[44]. Kirkpatrick, F.H. Overview of agarose gel properties .(1991). Electrophoresis of large DNA molecules: theory and applications 9-22

[45]. Kim, Y.H. Citation: Lee, P.Y., Costumbrado, J., Hsu, C.Y., (2012) .Agarose Gel Electrophoresis for the Separation of DNA Fragments. J. Vis. Exp. (62), e3923,

[46]. Galperin MY. and Yutin N, (2013). A genomic update on clostridial phylogeny: Gram-negative spore formers and other misplaced clostridia. Environ Microbiol 15:2631–2641.

[47]. Isibor OJ, Oseni A, Eyaufe A. (2008). Incidence of aerobic bacteria and Candida albicans in post-operative wound infections. Afr.J. microbiol. Res. 2: 288-291

[48]. Pradhan G, Agrawal J. (2009).Comparative study of post-operative wound infection following emergency lower segment caesarean section with and without the topical use of fusidic acid. Nepal Med Coll J.; 11: 189-191

[49]. Medical Disability Guidelines. Wound infection, postoperative. (2010). Available at: http://www.mdguidelines.com/woundinfection-postoperative. Accessed on: June 24,

[50]. De Hoon MJL, Eichenberger P, Vitkup D. (2010). Hierarchical evolution of the bacterial sporulation network. Curr. Biol. 20:R735–R745.

[51]. D, Krieg NR, Ludwig W, Rainey FA, Schleifer K-H, Whitman WB. (2009). Bergey's manual of systematic bacteriology, 2nd ed. Springer, Dordrecht, Netherlands.

[52]. Qadan M, Cheadle WG.(2009)Common microbial pathogens in surgical practice. Surg Clin North Am.;89(2):295–310. vii..

[53]. Centers for Disease Control and Prevention. (2010) National Center for Health Statistics. National Hospital Ambulatory Medical Care Survey. Emergency Department Summary Tables.. [2/15/2014]; Available from.

[54]. Niska, R.; Bhuiya, F.; Xu, J. (2010) .National Ambulatory Medical Care Survey: Emergency Department Summary. National Center for Health Statistics; Hyattsville, MD:

[55]. Pittet D. and Boyce JM, (2002). Guideline for hand hygiene in health-care settings: recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. MMWR Recomm Rep.; 51(RR-16):1–45.

[56]. Madhavan H. N., Anand, A. R., and K. L. Therese. (2000) . Use of polymerase chain reaction (PCR) and DNA probe hybridization to determine the Gram reaction of the infecting bacterium in the intraocular fluids of patients with endophthalmitis. J. Infect. 41:221–226.

[57]. Jones, J. C,. Cooper, G. M., J. J.. Arbique, G. J. Flowerdew, and K. R. Forward. 2000. Intra and inter technologist variability in the quality assessment of respiratory tract specimens. Diagn. Microbiol. Infect. Dis. 37:231– 235.

[58]. Kumar A, Chen G Wyman TH, Moran Jr CP (2006) Spo0A-dependent activation of an extended-10 region promoter in Bacillus subtilis. J Bacteriol 188: 1411–1418.-

[59]. Errington J (2001) Septation and chromosome segregation during sporulation in Bacillus subtilis. Curr Opin Microbiol 4: 660–666.

[60]. Madhavan H. N., Anand, A. R., and K. L. Therese. 2000. Use of polymerase chain reaction (PCR) and DNA probe hybridization to determine the Gram reaction of the infecting bacterium in the intraocular fluids of patients with endophthalmitis. J. Infect. 41:221–226.