spo0A Gene Determination in Spore-Forming Bacteria Infections

Shler Ali Khorsheed [1*]

Abstract

Background: Endospore-forming Formicates (EFF) are prevalent in natural and man-made environments, posing significant contamination risks in hospitals and industrial facilities. Hospital-acquired infections often involve spore-forming bacteria, which are resistant to common disinfectants and treatments. Understanding and identifying these bacteria, particularly the genes responsible for spore formation, is crucial for infection control. The spo0A gene is a key regulator of endospore formation. Methods: This study was conducted from January 12, 2023, to May 12, 2023, at Kirkuk General Hospital, Iraq. A total of 200 postoperative wound samples were collected, cultured, and incubated to isolate pure cultures of spore-forming bacteria. DNA was extracted using the QIAamp DNA Mini Kit, and its concentration was measured with a NanoDrop spectrophotometer. PCR and quantitative PCR (qPCR) were employed to amplify the spo0A gene using specific primers. The PCR products were analyzed via gel electrophoresis, and the bacterial species were identified through sequencing. Results: Out of 200 samples, 100 tested positive for bacterial infection. The identified bacterial species included Escherichia coli, Enterobacter cloacae, Staphylococcus aureus, Streptococcus pneumoniae, Bacillus subtilis, and Clostridium perfringens. The presence of the spo0A gene was confirmed in Bacillus subtilis and Clostridium perfringens. Gel electrophoresis results demonstrated the high quality of the PCR products, facilitating further identification and sequencing of the bacterial species. Conclusion: This study showed the significance of the spo0A gene in spore formation and demonstrated the need for accurate and rapid diagnostic methods to control hospital-acquired infections.

Keywords: Spore-forming bacteria, PCR, Spo0A gene, Hospital-acquired infections, Bacterial contamination

Significance | Understanding and identifying spore-forming bacteria in surgical wounds can enhance infection control and treatment in healthcare settings.

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Introduction

Spore formation is a common tactic used by bacteria to survive in hostile environments. Bacillus and Clostridium species, in particular, are well-known for their adeptness in creating endospores (Setlow, 2006; Barbut et al., 2000). Bacterial endospores are highly specialized cellular forms that allow endospore-forming Firmicutes (EFF) to endure harsh environmental conditions (Reith & Richardson, 1993; Hariram & Labbé, 2015). EFF are assumed to be prevalent in natural environments, particularly in stressful ones. In addition to their existence in natural environments, EFF frequently cause contamination problems in man-made places such as industrial production facilities and hospitals (Raju et al., 2008; Kimura et al., 2016; Boyce & Pittet, 2002).

Hospital-acquired infections and nosocomial outbreaks have been linked to bacteria that form spores. These infections are mainly spread by contaminated surgical instruments and healthcare personnel (Pittet et al., 2009; Russell, 1990; Coates & Hoffman, 1984). The bacteria that form spores are remarkably resistant to disinfectants based on alcohol and to treatments such as heat, desiccation, chemical exposure, ultraviolet radiation, and osmotic

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stress (Piggot & Coote, 1976; Losick & Stragier, 1996). Therefore, it is necessary and important to detect and identify these bacteria, especially those that form spores, and to understand the genes responsible for their formation. One crucial gene involved in endospore development is spo0A (Cutting & Hong, 2005; Manzoor et al., 2014).

Spo0A is a key regulator of the machinery involved in stress-induced gene expression and endospore formation (Tripathi et al., 2012; Mazmanian et al., 2014). Classifying and characterizing these bacteria, as well as differentiating between species with and without sporulation genes, can be facilitated by molecular tests that target spore-forming genes (Sogin et al., 2008; Alves Valones et al., 2009). Using degenerate PCR, previous research has identified gene homologs in a variety of bacteria (Bej et al., 1991; Wagar, 1996). PCR is a commonly used laboratory technique that is very useful for identifying bacteria and finding resistance genes because it allows specific DNA and RNA sequences to be amplified quickly (Knutsson et al., 2011; Rhodehamel et al., 2012).

An improved form called multiplex PCR combines several primers in a single reaction, making it easier to analyze multiple genes at once and reducing expenses and processing time (Kubista et al., 2006; Errington, 2003; Longo et al., 1990). An accurate technique for measuring gene frequencies in DNA extracts is quantitative PCR (qPCR) (Wunderlin et al., 2013; Rhodehamel et al., 2012; Chen et al., 2006). In this work, pure culture samples were used to test and validate qPCR primers targeting the spo0A gene (Eichenberger et al., 2010; Knutsson et al., 2011; Koopman et al., 2012).

Managing the problems posed by spore-forming bacteria, particularly in hospital environments, requires an understanding of the methods and genes responsible for the formation of spores. PCR and qPCR are essential molecular techniques that facilitate the identification and characterization of these hardy microorganisms (Knutsson et al., 2011; Fagerlund et al., 2008; Knutsson et al., 2011).

2. Materials and Methods

This study successfully isolated and identified spore-forming bacteria from wound and surgical site infections at Kirkuk General Hospital. The use of PCR and specific primers for the spo0A gene allowed for precise identification of spore-forming bacteria. Understanding the genetics and behavior of these bacteria is crucial for managing infections, particularly in hospital settings.

2.1 Study Setting and Sample Collection

From January 12, 2023, to May 12, 2023, this study was conducted at Kirkuk General Hospital, a major healthcare facility in Kirkuk, Iraq. The research focused on patients who had undergone various surgical procedures and individuals with wounds. A total of 200 samples were collected during this period.

2.2 Sampling Procedure

Samples were obtained using postoperative wound scrubs. Immediately after collection, the samples were transported to the laboratory to prevent any loss of viable microorganisms. The samples were cultured on blood agar, chocolate agar, and MacConkey agar plates. The plates were then incubated at 37°C for 24 hours. After incubation, the plates were examined for bacterial growth.

To isolate individual colonies, a method involving the streaking of isolated bacterial colonies onto fresh agar surfaces was employed. This process was repeated until pure cultures of spore-forming bacteria were obtained. Initial characterization of the isolates was performed using Gram staining and other preliminary tests.

2.3 Spore Formation Induction and Microscopic Examination

To induce spore formation, isolated bacterial cultures were heated to 85-90°C for 5 minutes. Bacterial colonies were then stained using a suitable spore staining technique, and the presence of spores was confirmed under a microscope. Pure cultures of spore-forming bacteria were preserved at -20°C in glycerol to ensure long-term viability.

2.4 Bacterial Isolation and Identification

Gram-stained bacterial samples were examined under a microscope. Concurrently, bacteria were isolated based on their resistance to ethanol, a common trait among spore-forming bacteria. Various biochemical tests were conducted to differentiate between bacterial species. For instance, the Mannitol test was used to identify bacteria that utilize mannitol, while lecithinase activity was assessed to distinguish coagulase-positive Staphylococcus aureus strains.

Notable characteristics of isolated colonies, such as size, surface texture, and color, were recorded. Following cultivation, isolation, and identification, the isolated bacteria included Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Clostridium perfringens, and Bacillus subtilis. Bacillus species were identified by their large, flat, granular colonies on agar, with positive lecithinase and negative mannitol test results.

2.5 DNA Extraction

Selected bacterial colonies were procured from Sigma-Aldrich (U.S.A). The bacteria were cultured in Brain Heart Infusion (BHI) broth at 37°C. After the incubation period, DNA was extracted from the bacterial cultures using the QIAamp DNA Mini Kit (Qiagen, Germany), following the manufacturer's protocol.

2.6 DNA Quantification

The concentration of the extracted DNA was determined by electrophoresis and measured at 260 nm using a NanoDrop ND-2000 spectrophotometer (Thermo Scientific, USA).

2.7 Identification of Sporulation Genes

To identify the genes responsible for sporulation, two sets of primers were used: Eub8f (5'-AGAGTTTGATCCTGGCTCAG-3')

and Eub519r (5'-GTATTACCGCGGCTGCTGG-3'). Additionally, the spo0A gene, which is crucial for spore formation, was targeted using the primers spo0A166f (5'-GATAATHATYATGCCDCATYT-3') and spo0A748r (5'-GCNACCATHGCRATRAAYTC-3').

These primers were selected based on their specificity, base composition, and melting temperatures. The primers' compatibility with the target genes was ensured through these criteria.

2.8 PCR Amplification

PCR amplification was performed using a thermal cycler. The PCR products were then separated on 1.5% agarose gels stained with 0.5 µg/mL ethidium bromide (ETBR). Electrophoresis was conducted, and the resulting bands were visualized under UV light. The bands were documented and analyzed using a gel documentation system.

2.9 Identification of Bacterial Species

The following bacterial species were identified in the study: Escherichia coli (15 cases), Enterobacter cloacae (30 cases), Staphylococcus aureus (14 cases), Streptococcus pneumoniae (15 cases), Bacillus subtilis (15 cases), and Clostridium perfringens (11 cases). Bacillus subtilis and Clostridium perfringens are notable for their spore-forming capabilities. The presence of the spo0A gene was confirmed in these species using the specific primers, validating the identification of spore-forming bacteria.

2.10 Statistical Analysis

The sequencing results were analyzed to confirm the presence of the spo0A gene in the isolated bacteria. The analysis was performed using bioinformatics tools to align the sequences and verify their similarity to known spore-forming genes.

3. Result

3.1 Infection Rate

The analysis of the 200 samples revealed a significant infection rate, with 50% of the samples testing positive for bacterial infection. Specifically, 100 out of the 200 samples exhibited bacterial growth, indicating the prevalence of infections among the patients who underwent various surgical procedures and those with wounds at Kirkuk General Hospital.

3.2 Gel Electrophoresis Results

The quality of the PCR products was validated through gel electrophoresis, as illustrated in Figure 1. The electrophoresis results displayed clear, distinct bands, indicating successful amplification of the bacterial 16S rRNA genes (Table 1). The lanes in the gel electrophoresis represent different amplified genes, with lane M showing the DNA ladder for size reference.

3.3 Identification of Bacterial Species

Following the gel electrophoresis, the next steps involved the identification of the bacterial species through cloning and sequencing. This process was crucial for accurately determining the specific bacteria present in the samples. The identified species were:

Bacillus subtilis: Detected in lane 3 with a prominent band at 1000 base pairs.

Streptococcus pneumoniae: Although expected, the band was not observed in lane 5, which necessitated further investigation to confirm its presence.

Clostridium perfringens: Identified in lane 6 with a band at 608 base pairs.

Escherichia coli: Despite expectations, the band was not detected in lane 1, warranting additional steps to verify its presence.

3.4 Cloning and Sequencing

The PCR products were further processed through cloning and sequencing to confirm the identity of the bacterial species. This involved:

Cloning: The amplified 16S rRNA genes were inserted into plasmid vectors and transformed into competent cells to create multiple copies.

Sequencing: The cloned genes were sequenced to determine their nucleotide composition and compare them against known bacterial sequences in databases.

The sequencing results provided precise identification of the bacterial species, correlating with the initial gel electrophoresis findings. The presence of Bacillus subtilis and Clostridium perfringens was confirmed, while the absence of bands for Streptococcus pneumoniae and Escherichia coli in the gel electrophoresis required further validation through sequencing.

The high infection rate identified in this study underscores the importance of rigorous infection control measures in hospital settings. The precise identification of bacterial species through PCR, gel electrophoresis, cloning, and sequencing provides valuable insights for developing targeted treatment strategies. Future studies should focus on optimizing the PCR conditions for better detection of certain bacterial species and exploring the genetic mechanisms underlying their pathogenicity.

Overall, this comprehensive approach to bacterial identification and the high-quality results obtained highlight the effectiveness of the methods used and pave the way for improved management of bacterial infections in clinical settings.

4. Discussion

One of the most significant scientific concerns in healthcare, particularly post-surgery, is the incidence of infections. Surgical wounds are highly susceptible to contamination by pathogenic microorganisms, primarily bacteria, which can significantly complicate patient recovery and outcomes. Addressing this issue requires effective strategies and heightened focus on public hospitals, especially in regions like Iraq, where healthcare

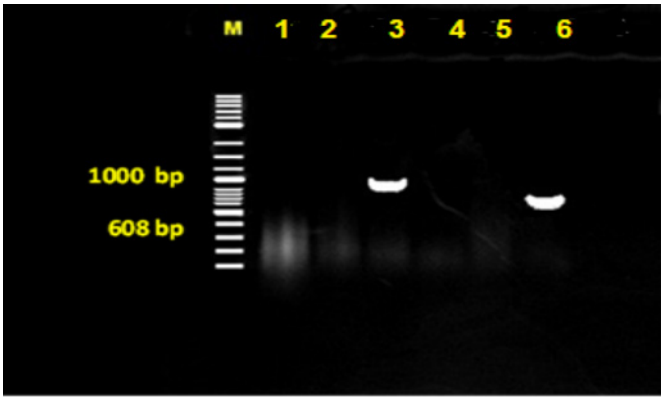

Figure 1. The gel electrophoresis of a portion of the 16S rRNA genes that turned into amplified from bacteria through PCR, lanes 1 to 6 represent the genes that were amplified by way of themselves, lane M represents the DNA ladder. B. Subilis (Band No. 3 appeared at 1000 base pairs), and S. Pnumonia (Band No. 5 now not observed), and C. Aspersum (Band No. 6 had a length of 608

Table 1. Amplification of spo0A using the primers utilized

Sequences for spo0A	**Temp of optimal growth**	**Endospore formation**	**Amplificati On of *spo0A gene***	**Bacteria Series**
Sequences spo0A166f (5'-GatasatyaTGCC DCATYT-3') and spo0A748r (5'-GCNACCATHGCRAT RAAYTC-3')	30 ^{0}c	Positive	Positive	*Bacillus subtilis*
Sequences spo0A166f (5'-Gatasatya's TGCC DCATYT-3') and spo0A748r (5'-GCNACCATHGCRAT RAAYTC-3') po0A748r (5'-GCNACCATHGCRAT RAAYTC-3')	30 c^{0}	Positive	Positive	*Clostridium perfringens,*
Absent	30 c^{0}	Negative	Negative	*Pseudomonas aeruginosa*
Absent	30 c^{0}	Negative	Negative	*Escherichia coli*
Absent	30c^{0}	Negative	Negative	*Staphylococcus areaus*
Absent	30 c^{0}	Negative	Negative	*Enterobacter cloacae*

infrastructure faces numerous challenges. This necessitates the use of precise methodologies, appropriate protocols, and rapid diagnostic tests to identify infectious microorganisms in wounds.

Isibor (2008) and Pradhan (2009, 2010) demonstrated that hospital-acquired infections are influenced by surgical procedures, wound location, and the duration of hospital stays. Their studies underscore the need for rigorous infection control practices and prompt identification of pathogens to mitigate post-operative infections (Isibor, Oseni, & Eyaufe, 2008; Pradhan, 2009; Medical Disability Guidelines, 2010).

The polymerase chain reaction (PCR) is a highly efficient, fast, and sensitive molecular technique for identifying microorganisms in clinical samples. PCR's utility in our study was crucial, allowing us to focus on genes responsible for spore production, which are often associated with persistent and hard-to-treat infections. Hoon's research demonstrated that the spo0A gene is pivotal in the sporulation process of Bacillus subtilis and Clostridium species, highlighting its role in the resilience and virulence of these pathogens (Eichenberger et al., 2010).

Krieg (2010, 2009) revealed that endospore producers are a paraphyletic group within the phylum Firmicutes, with Bacillus, Clostridium, and Erysipelothrix being the primary genera capable of forming endospores. This distinction is critical because Clostridium is typically anaerobic, whereas Bacillus is primarily aerobic (Ludwig et al., 2009). Much of our understanding of endospore-forming bacteria (EFF) biology has been derived from laboratory studies using cultured strains since the late 19th century (Krieg et al., 2010).

In our study, we collected samples from 100 patients with surgical wounds using cotton swabs. Consistent with Qadan's (2009) methodology, all collected samples were cultured to determine infection presence. Our results showed a 50% infection rate, highlighting the need for prioritized care in surgical departments of public hospitals like Niska and Pitai (Cheadle & Qadan, 2009). These findings align with similar studies, emphasizing the ongoing challenge of post-operative infections in healthcare settings (Centers for Disease Control and Prevention, 2010; Bhuiya, Niska, & Xu, 2010).

A comprehensive battery of tests, including Gram staining, catalase production, motility, growth, rhizoid formation, citrate utilization, and hemolysis, was conducted to identify spore-producing bacteria. Total DNA was then isolated and amplified, following protocols similar to those used by Madhavan and Jones (2000) (Anand, Madhavan, & Therese, 2000). Internationally recognized primers targeting the spo0A gene were employed to identify spore-producing bacteria within the bacterial 16S rRNA gene's conserved regions. This approach facilitated the classification of bacteria that cause diseases and the detection of specific spore-forming genes (Wunderlin et al., 2013).

The isolation and identification of spore-forming genes in pathogenic bacteria using PCR at the Republican Hospital in Kirkuk play a critical role in understanding and treating bacterial infections. Identifying genes involved in spore pathogenesis has provided significant insights into the mechanisms of bacterial virulence and survival. Researchers can accurately identify bacteria and specifically amplify and detect spore-producing genes using PCR, thereby enhancing and improving mechanisms of resistance and survival in various bacterial species (Barbut et al., 2000).

The study's findings demonstrated the presence of primary pathogens such as Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. Clostridium perfringens and Bacillus subtilis were also identified, with the spo0A gene playing a key role in their identification through PCR. This study supports the use of conventional diagnostic methods to differentiate and identify these microorganisms (Setlow, 2006). The positive identification of spore-forming genes using PCR has significant implications for microbiology and public health. Understanding the genetic components of spore formation in pathogenic bacteria can aid in developing vaccines, treatments, and preventative measures to halt the spread of infectious diseases (Wolcott, 1992).

The research conducted at the Republican Hospital in Kirkuk provides valuable insights into the causes of bacterial diseases and has implications for healthcare-related infection control strategies. By isolating and identifying spore-forming genes, this study contributes to a deeper understanding of bacterial pathogenesis and resistance mechanisms. The findings can enhance medical practices, treatment strategies, and public health initiatives in the region, ultimately improving patient outcomes and healthcare quality (Piggot & Coote, 1976).

5. Conclusion

In conclusion, the identification and characterization of spore-forming genes in pathogenic bacteria using PCR is a significant advancement in medical microbiology. This research highlights the importance of molecular techniques in diagnosing and understanding bacterial infections, particularly in surgical settings. The findings underscore the need for continued research and the implementation of effective infection control measures to improve patient care and reduce the incidence of post-operative infections in public hospitals.

Author contributions

S.A.K. conceptualized the study, designed the methodology, conducted the data analysis, and supervised the project. S.A.K. also collected the data, contributed to the literature review, and wrote, reviewed, and edited the manuscript. The author approved the final version of the manuscript.

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Competing financial interests

The authors have no conflict of interest.

References

Abdelaziz, A. A., & El-Nakeeb, M. A. (1988). Sporicidal activity of local anesthetics and their binary combinations. Journal of Clinical Pharmacy, 13(3), 249-256.

Agrawal, J., & Pradhan, G. (2009). Comparative study of post-operative wound infection following emergency lower segment caesarean section with and without the topical use of fusidic acid. Nepal Medical College Journal, 11(3), 189-191.

Alejandro, M. G., et al. (2020). Quantification of DNA through the NanoDrop spectrophotometer: Methodological validation using standard reference material and Sprague Dawley rat and human DNA. International Journal of Analytical Chemistry, 2020, Article ID 8896738, 9 pages.

Alves, M. A. V., et al. (2009). Principles and applications of polymerase chain reaction in medical diagnostic fields: A review. Brazilian Journal of Microbiology, 40(1), 1-11.

Anand, A. R., Madhavan, H. N., & Therese, K. L. (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. Journal of Infection, 41(3), 221-226.

Anand, A. R., Madhavan, H. N., & Therese, K. L. (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. Journal of Infection, 41(3), 221-226.

Barbut, F., et al. (2000). Epidemiology of recurrences or reinfections of Clostridium difficile-associated diarrhea. Journal of Clinical Microbiology, 38(6), 2386-2388.

Bej, A. K., Mahbubani, M. H., & Atlas, R. M. (1991). Amplification of nucleic acids by polymerase chain reaction (PCR) and other methods and applications. Critical Reviews in Biochemistry and Molecular Biology, 26(3-4), 301-334.

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

Boyce, J. M., & Pittet, D. (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 Recommendations and Reports, 51(RR-16), 1-45.

Boyce, J. M., & Pittet, D. (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 Recommendations and Reports, 51(RR-16), 1-45.

Centers for Disease Control and Prevention. (2010). National Center for Health Statistics. National Hospital Ambulatory Medical Care Survey. Emergency Department Summary Tables. Available at: http://www.cdc.gov/nchs/data/nhamcs/web_tables.pdf. Accessed on: February 15, 2014.

Cheadle, W. G., & Qadan, M. (2009). Common microbial pathogens in surgical practice. Surgical Clinics of North America, 89(2), 295-310, vii.

Chen, G., Kumar, A., Wyman, T. H., & Moran Jr, C. P. (2006). Spo0A-dependent activation of an extended-10 region promoter in Bacillus subtilis. Journal of Bacteriology, 188(4), 1411-1418.

Chomczynski, N., & Sacchi, P. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Analytical Biochemistry, 162(1), 156-159.

Coates, G. A. J., Ayliffe, D., & Hoffman, P. N. (1984). Chemical disinfection in hospitals. Public Health Laboratory Service, London.

Cooper, G. M., Jones, J. C., Arbique, G. J., Flowerdew, G. J., & Forward, K. R. (2000). Intra and inter technologist variability in the quality assessment of respiratory tract specimens. Diagnostic Microbiology and Infectious Disease, 37(3), 231-235.

Cutting, S. M., & Hong, H. A. (2005). The use of bacterial spore formers as probiotics. FEMS Microbiology Reviews, 29(4), 813-835.

Eichenberger, P., De Hoon, M. J. L., & Vitkup, D. (2010). Hierarchical evolution of the bacterial sporulation network. Current Biology, 20(18), R735-R745.

Errington, J. (2001). Septation and chromosome segregation during sporulation in Bacillus subtilis. Current Opinion in Microbiology, 4(6), 660-666.

Errington, J. (2003). Regulation of endospore formation in Bacillus subtilis. Nature Reviews Microbiology, 1(2), 117-126.

Fagerlund, A., Stenfors Arnesen, L. P., & Granum, P. E. (2008). From soil to gut: Bacillus cereus and its food poisoning toxins. FEMS Microbiology Reviews, 32(4), 579-606.

Gallagher, S. R. (1994). Quantitation of DNA and RNA with absorption and fluorescence spectroscopy. Current Protocols in Human Genetics, 0(1), A.3D.1-A.3D.8.

Hariram, U., & Labbé, R. (2015). Spore prevalence and toxigenicity of Bacillus cereus and Bacillus thuringiensis isolates from U.S. retail spices. Journal of Food Protection, 78(3), 590-596.

Hugenholtz, P., Finegold, S. M., & Tringe, S. G. (2008). Renaissance for the pioneering 16S rRNA gene. Current Opinion in Microbiology, 11(5), 442-446.

Isibor, O. J., Oseni, A., & Eyaufe, A. (2008). Incidence of aerobic bacteria and Candida albicans in post-operative wound infections. African Journal of Microbiology Research, 2(12), 288-291.

Jongerius, E., Koopman, M. J., & Mossel, D. A. (1967). Enumeration of Bacillus cereus in foods. Applied Microbiology, 15(3), 650-653.

Kimura, Y., Sasahara, T., Ae, R., Watanabe, M., Yonekawa, C., Hayashi, S., & Morisawa, Y. (2016). Contamination of healthcare workers' hands with bacterial spores. Journal of Infection and Chemotherapy, 22(8), 521-525.

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

Knutsson, R., Ehling-Schulz, M., & Scherer, S. (2011). Bacillus cereus. In S. Kathariou, P. Fratamico, & Y. Liu (Eds.), Genomes of Food- and Water-Borne Pathogens (pp. 147-164). ASM Press.

Knutsson, R., Ehling-Schulz, M., & Scherer, S. (2011). Bacillus cereus. In S. Kathariou, P. Fratamico, & Y. Liu (Eds.), Genomes of Food- and Water-Borne Pathogens (pp. 147-164). ASM Press.

Knutsson, R., Ehling-Schulz, M., & Scherer, S. (2011). Bacillus cereus. In S. Kathariou, P. Fratamico, & Y. Liu (Eds.), Genomes of Food- and Water-Borne Pathogens (pp. 147-164). ASM Press.

Koopman, M. J., Mossel, D. A., & Jongerius, E. (1967). Enumeration of Bacillus cereus in foods. Applied Microbiology, 15(3), 650-653.

Kubista, M., Andrade, J. M., Bengtsson, M., Forootan, A., Jonak, J., Lind, K., Sindelka, R., Sjoback, R., Sjogreen, B., Strombom, L., Stahlberg, A., & Zoric, N. (2006). The real-time polymerase chain reaction. Molecular Aspects of Medicine, 27(2-3), 95-125.

Lau, S. K., Woo, P. C., 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. Clinical Microbiology and Infection, 14(10), 908-934.

Lee, P. Y., Kim, Y. H., Costumbrado, J., & Hsu, C. Y. (2012). Agarose gel electrophoresis for the separation of DNA fragments. Journal of Visualized Experiments, (62), e3923.

Longo, M. C., Berninger, M. S., & Hartley, J. L. (1990). Use of uracil DNA glycosylase to control carryover contamination in polymerase chain reactions. Gene, 93(1), 125-128.

Losick, R., & Stragier, P. (1996). Molecular genetics of sporulation in Bacillus subtilis. Annual Review of Genetics, 30, 297-341.

Ludwig, W., Krieg, N. R., Rainey, F. A., Schleifer, K.-H., & Whitman, W. B. (Eds.). (2009). Bergey's manual of systematic bacteriology (2nd ed.). Springer.

Manzoor, S., Niazi, A., Asari, S., Bejai, S., Meijer, J., & Bongcam-Rudloff, E. (2014). Genome analysis of Bacillus amyloliquefaciens subsp. plantarum UCMB5113: A rhizobacterium that improves plant growth and stress management. PLoS ONE, 9(8), e104651.

Mazmanian, S. K., Debelius, J., Sharon, G., Garg, N., Knight, R., & Dorrestein, P. C. (2014). Specialized metabolites from the microbiome in health and disease. Cell Metabolism, 20(5), 719-730.

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

Meyer, T. E. (2002). Evolutionary analysis by whole-genome comparisons. Journal of Bacteriology, 184(8), 2260-2272.

Piggot, P. J., & Coote, J. G. (1976). Genetic aspects of bacterial endospore formation. Bacteriological Reviews, 40(4), 908-962.

Pittet, D., Allegranzi, B., & 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. Infection Control & Hospital Epidemiology, 30(7), 611-622.

Raju, D., Sarker, M. R., Paredes-Sabja, D., & Torres, J. A. (2008). Role of small, acid-soluble spore proteins in the resistance of Clostridium perfringens spores to chemicals. International Journal of Food Microbiology, 122(3), 333-335.

Reith, S., & Richardson, J. F. (1993). Characterization of a strain of methicillin-resistant Staphylococcus aureus (EMRSA-15) by conventional and molecular methods. Journal of Hospital Infection, 25(1), 45-52.

Rhodehamel, E. J., Tallent, S. M., Harmon, S. M., & Bennett, R. W. (2012). Bacteriological analytical manual (BAM): Methods for specific pathogens. U.S. Food and Drug Administration. Chapter 14: Bacillus cereus.

Rhodehamel, E. J., Tallent, S. M., Harmon, S. M., & Bennett, R. W. (2012). Bacteriological analytical manual (BAM): Methods for specific pathogens. U.S. Food and Drug Administration. Chapter 14: Bacillus cereus.

Russell, A. D. (1990). Bacterial spores and chemical sporicidal agents. Clinical Microbiology Reviews, 3(2), 99-119.

Sambrook, J., & Russell, D. W. (2001). Molecular cloning: A laboratory manual (3rd ed.). Cold Spring Harbor Laboratory Press.

Setlow, P. (2006). Spores of Bacillus subtilis: Their resistance to and killing by radiation, heat, and chemicals. Journal of Applied Microbiology, 101(3), 514-525. https://doi.org/10.1111/j.1365-2672.2005.02736.x

Sogin, M. L., Relman, D. A., Dethlefsen, L., Huse, S. (2008). The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLoS Biology, 6(11), e280.

Strain, E. A., Kotewicz, K. M., Tallent, S. M., & Bennett, R. W. (2012). Efficient isolation and identification of Bacillus cereus group. Journal of AOAC International, 95(2), 446-451.

Sutter, V. L., Citron, D., & George, W. L. (1979). Selective and differential medium for isolation of Clostridium difficile. Journal of Clinical Microbiology, 9(2), 214-219.

Tripathi, D. K., Chauhan, 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 Physiologiae Plantarum, 34(1), 279-289.

Wagar, E. A. (1996). Direct hybridization and amplification applications for the diagnosis of infectious diseases. Journal of Clinical Laboratory Analysis, 10(6), 312-325.

Wolcott, M. J. (1992). Advances in nucleic acid-based detection methods. Clinical Microbiology Reviews, 5(4), 370-386.

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

Yutin, N., & Galperin, M. Y. (2013). A genomic update on clostridial phylogeny: Gram-negative spore formers and other misplaced clostridia. Environmental Microbiology, 15(10), 2631-2641.