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Molecular Detection and Antibiotic Susceptibility of *Bacillus cereus* Isolated from Periodontitis Patients in Misan City

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ABSTRACT

This research aims to detect and isolate *Bacillus cereus*, a facultative anaerobic Gram-positive bacillus, from the oral cavities of patients aged 18 to 23 years with dental diseases such as gingivitis or periodontitis. Furthermore, this research addresses the gap in understanding the role of Bis-(2-ethylhexyl) phthalate in periodontal therapy and explores the pathogenic pathways of *B. cereus* in dental biofilms. Additionally, the study investigates the minimum inhibitory concentration (MIC) of *B. cereus* strains isolated from dental patients in Al-Amarah City. Bis-(2-ethylhexyl) phthalate was identified as an antibacterial agent derived from *Penicillium digitatum* and was evaluated for its effectiveness against *B. cereus*. The isolates were identified using phenotypic and biochemical tests, as well as polymerase chain reaction (PCR). All isolates showed resistance to Optochin, Doxycycline, Flucloxacillin, Tetracycline, Nalidixic Acid, and Ciprofloxacin. Besides, the six *B. cereus* strains that were tested showed a fair level of sensitivity to Ciprofloxacin (CIP.5 µg) with an inhibition zone of 21 mm per disk. Based on FT-IR analysis followed by GC-MS, the active compound was identified to be Bis-(2-ethylhexyl) phthalate. As well, the current findings show that the antibiotic Bis-(2-ethylhexyl) phthalate as a secondary metabolite is effective against *B. cereus.* Finally, the study showed the possibility of treating infections caused by Gram-positive pathogenic bacteria and Gram-negative pathogens like *Escherichia coli* using these bacteria that have been isolated from the mouths of patients with dental illness.

1. INTRODUCTION

Periodontal disease is a chronic multifactorial inflammatory illness associated with dysbiotic plaque biofilm, characterized by the degradation of the tooth-supporting tissues [1]. Periodontal diseases, both marginal and apical, arise from virulence factors produced by the dental plaque microbiota. These microorganisms release proteolytic enzymes and toxic byproducts that damage the periodontium's cellular and structural integrity. Notably, bacterial proteases degrade critical matrix components, including collagen, elastin, fibronectin, fibrin, and other elements of epithelial and connective tissue [1]. Dental biofilms develop as communities of interacting bacteria that are physically and functionally organized. These interactions, which can also be antagonistic or synergistic, result in the formation of a biofilm that shields the tooth surfaces [2, 3]. *B. cereus* is a microbe that causes opportunistic infections in humans and is primarily associated with periodontal diseases. The *B. cereus* group consists of six species: *B. weihenstephanensis*, *B. cereus*, *B. thuringiensis*, *B. mycoides*, and *B. pseudomycoides* [4]. *Bacillus cereus* is widespread in various habitats and can be easily isolated from foods such as meat, grains, and dairy products. They are large gram-positive bacilli forming spores, usually arranged in pairs or chains. They can move by peritrichous flagella; cells of *B. cereus* are 1-1.2 µm in width and 3-5 µm in length. It is a facultative aerobe with the optimum growth between 35-40°C [5].

The organism is frequently implicated in invasive *B. cereus* infections, including pneumonia and bacteremia [6]. Hospital environments have been linked to reports of eye infections [7] and soft tissue infections [8]. *B. cereus* is a significant contributor to morbidity and mortality. As soon as *B. cereus* is detected, appropriate empirical therapy should begin [9]. Furthermore, the evolution of antibiotic resistance in *B. cereus* raises concerns since resistant strains of the bacteria can aid in the enrichment and emergence of further strains resistant to antibiotics.

Penicillium is a diverse genus of ascomycetous fungi found in various terrestrial environments, including soil [10, 11]. Certain species play a significant role in producing bioactive compounds, including antifungal, antibacterial, and immunosuppressant agents [12]. These fungi have been widely studied for their potential in pharmaceutical applications, yet antifungal drug development remains limited compared to antibiotics [13]. Given the increasing need for novel antimicrobial solutions, investigating Penicillium-derived compounds may offer valuable insights relevant to this study's focus on bioactive agents [14-16].

Bis-(2-ethylhexyl) phthalate, previously isolated from *P.*

digitatum, has been identified as an antimicrobial compound; however, its specific role in regenerative therapy, especially in the aspect of periodontal therapy has not been investigated. Its mechanism of action and effectiveness against periodontal pathogens, especially *Bacillus cereus*, a potential causative agent of dental infections, are poorly studied. Therefore, this study was designed to address these knowledge gaps by investigating the contribution of *B. cereus* to periodontal disease pathogenesis, its interaction with dental biofilms, and its Minimum Inhibitory Concentration (MIC) in *B. cereus* strains isolated from dental patients in Al-Amarah City. Additionally, this study unveils novel insights on the antimicrobial effect of 2-(2-ethylhexoxy)-1-methoxy-2-propyl-2,4-hexadiene (another bioactive compound isolated from *P. digitatum*) against *B. cereus* and other periodontal pathogens. The present findings broaden our understanding of the potential use of compounds derived from *P. digitatum* as therapeutics for periodontal disease by elucidating these mechanisms.

2. METHODOLOGY

2.1 Clinical diagnoses of the patients

The patient was taking non-steroidal anti-inflammatory drugs; however, no antibiotics were administered during the previous dental procedure. On examination, facial asymmetry and palpable pterygomandibular swelling were observed.

2.2 Collection of gingivalis samples

Twenty-two swabs of the bacterial isolate were collected from 12 male and 10 female patients attending a clinic at the Special Dental Center in Misan City, Iraq; the swabs were taken from pocked lesions of gingivalis following diagnosis by certified dentists (gingivitis, periodontitis). The collected samples were immediately sent to the Microbiology laboratory in the Department of Biology, College of Science, University of Misan. The specimens are collected from acute gingivitis and patients aged from 2-70 years, had samples taken. From January 1, 2023, until April 30, 2023. Twenty samples were collected from a total of twenty-two oral cavities (gingivitis, periodontitis).

2.3 Isolation and identification of bacterial isolated strains *Bacillus cereus*

One strain selected (B2) from seven strains (B1, B2, and B7) belonging to *Bacillus cereus* bacterial isolated strains, were identified by 16S r DNA sequenced, extracted and DNA amplified using PCR, sequenced, and aligned with other identified strains in Gene Bank database by using BLAST tool an online to determine the similarity score.

2.4 Preparation of media

The culture media was prepared according to the instructions of the manufacturer.

2.4.1 Blood agar

About 40 g of blood agar was dissolved in 1 liter of distilled water (DW) and heated to the boiling point to ensure complete dissolution of the powder; the medium was sterilized for 15 min at 121°C, then cooled to 50°C before 5% sterile blood was aseptically added. The mixture was mixed thoroughly and poured into sterile agar plates.

2.4.2 Chocolate agar

About 40 g of blood agar was dissolved in 1 liter of distilled water (DW) and brought to boiling point to ensure complete dissolving of the powder; the media was sterilized for 15 min at 121°C and cooled to 50°C before aseptically adding 5% sterile blood to the media. The medium was placed in a water bath at 75-80°C with gentle swirling until it turned dark brown. The medium was aseptically poured into sterile Petri dishes after being cooled to approximately 50°C.

2.4.3 MacConkey agar

About 51.5 g of the powder (i.e., MacConkey agar) was added to 1 liter of DW and boiled to ensure it completely dissolved; the mixture was sterilized for 15 min at 121°C and cooled to 50°C before being aseptically poured into Petri dishes.

2.4.4 Nutrient agar

About 28 g of the powder (i.e., Nutrient agar) was added to 1 liter of DW and boiled to ensure it completely dissolved; the medium was sterilized for 15 min at 121°C, cooled to 50°C, and then poured into aliquots in sterile Petri dishes.

2.4.5 Potato Dextrose Agar (PDA)

About 200 g of peeled potatoes were boiled in 1 liter of distilled water, and the infusion was filtered to remove solid particles. To the potato infusion, 20 g of dextrose was added, and the mixture was autoclaved for 15-20 minutes at 121°C. The medium was then cooled to 50°C before being aseptically poured into sterile Petri dishes.

2.5 Identification of *Penicillium digitatum*

Pure mold colonies were grown on temporary slides and analyzed under a microscope. Mycelia and spore structures of *Penicillium digitatum* were examined for their unique morphological characteristics. The morphological characteristics of the fungi captured in the photographs were identified using a combination of in-depth literature research and expert opinion.

Distinct morphological features Colonies grown at 32°C on PDA are initially white but rapidly become black during conidial development.

2.6 Antimicrobial susceptibility test

The antibacterial activity of the fungal compound was determined using the disc diffusion assay technique against the Gram-positive (MDR *Bacillus cereus*, and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) isolates. Each bacterial strain was suspended to a turbidity of 0.5 McFarland and plated on Muller-Hinton Agar. Discs containing concentrations of 100 µg, 200 µg and 400 µg of the fungal compound were placed on the agar plates, and incubated at 37°C for 24h. The diameter of the inhibitory zones was measured.

2.7 Cultivation and extraction

A fungal strain and culture medium were obtained from citrus fruits infected with characteristic green mold symptoms.

The *P. digitatum* isolates were then cultivated on potato dextrose agar (PDA) at 27°C for seven days before being stored at 4°C. Mature spores were collected by rinsing the PDA surface with autoclaved distilled water. The hyphae were then removed by filtering the spore suspensions through a sterilized cotton ball placed in a funnel. The spore suspension of *P. digitatum* was quantified using a Countess-II FL automatic cell counter (Thermo Fisher Scientific, Waltham, MA) and adjusted to 1 × 10^6 CFU/mL using autoclaved distilled H_2O.

2.7.1 UV-visible

UV-visible spectroscopy was employed in this study (λ_max = 277 nm, corresponding to the C=O carbonyl group). Additionally, the IR spectrum exhibited a strong absorption at 3446.7–3377.3 cm^{-1} and 3130.4 cm^{-1}, corresponding to the strong N-H stretching vibration of amines. Absorptions at 3094.38–2950.48 cm^{-1} correspond to aliphatic C-H stretching vibrations. A strong peak at 1718.58 cm^{-1} corresponds to the C=O stretching vibration of the carbonyl group. A peak at 1575.84 cm^{-1} corresponds to the aromatic C=C stretching vibration. Absorptions at 1124.50–1136.07 cm^{-1} indicate the presence of C-O stretching vibrations. Figure 1 shows the FTIR spectrum of the bioactive compound isolated from *P. digitatum* cultured on spoiled citrus fruits, and Table 1 presents the identified chemical compounds in the extract.

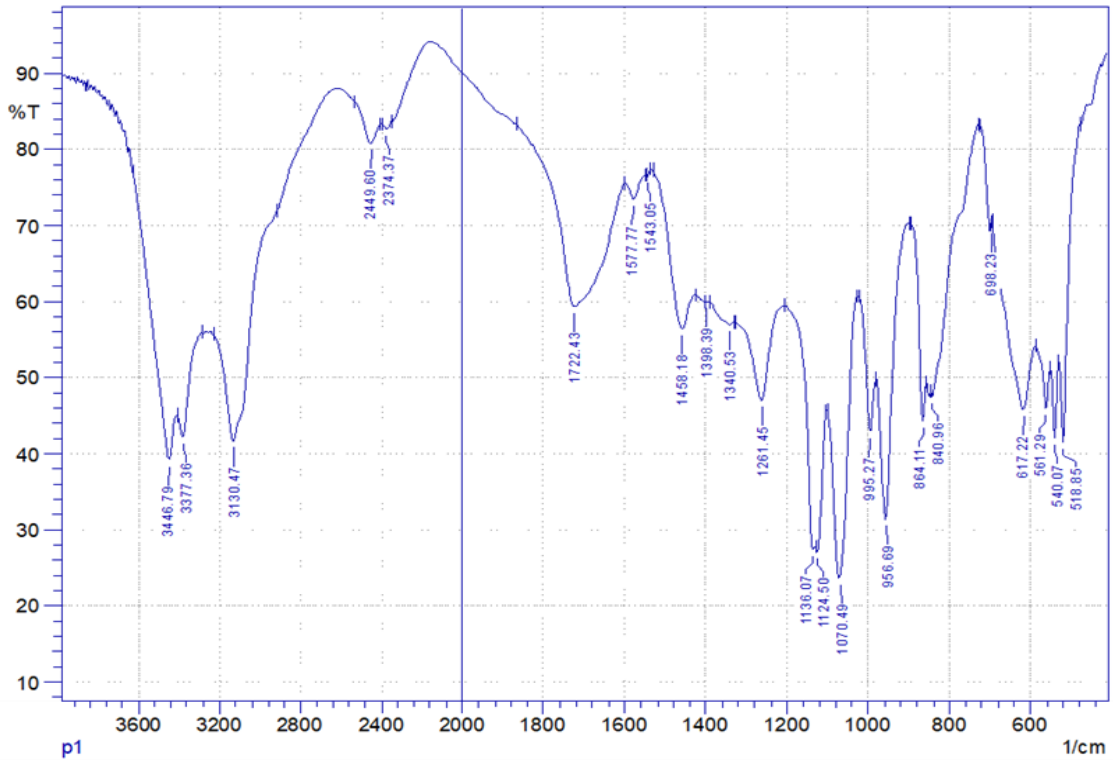


Figure 1. FTIR spectrum of the bioactive compound isolated from *P. digitatum* cultured on citrus spoilage fruits

Table 1. Some of the identified compounds in the *P. digitatum* cultured on spoiled citrus [17]

No.	Compounds	Molecular Weight	Chemical Formula	RT (min)	Area (%)
1.	Bis-(2-ethylhexyl)-phthalate	390.50	C_6H_{42}	34.460	5.750
2.	Bis-(2-ethylhexyl)-Sebacate	426.60	$C_{26}H_{50}O_4$	37.740	1.300
3.	5-Eicosene	282.50	$C_{20}H_{42}$	20.470	4.100
4.	1-Octadecene	252.50	$C_{18}H_{36}$	24.130	5.670
5.	Squalene	422.80	$C_{30}H_{50}$	38.050	53.540
6.	2-(phenyl)thio)-octanal	216.30	$C_{14}H_{16}O_5$	44.460	2.570
7.	Cyclotetradecanetrimethyl	280.50	$C_{20}H_{40}$	48.540	4.310

2.7.2 GC-MS analysis

The aqueous extract of *P. digitatum* was analyzed using GC-MS. A 1-µL aliquot of the extract was injected into the chromatographic column (30 m × 0.32 mm × 0.25 µm) via the Autosampler AS 1300. For the first analysis, the initial temperature was set to 60°C and then gradually increased to 240°C. It was further increased to 290°C and maintained for 2 minutes. Helium was used as the carrier gas at a flow rate of 1 mL/min. Mass spectra were obtained using electron ionization (EI) at 70 eV in full scan mode over an m/z range of 40–1000. The compounds were identified by comparing their mass spectra and retention times (RT) with those retrieved from the National Institute of Standards and Technology (NIST) library [15]. Figure 2 presents the GC-MS chromatogram of the isolated bis(2-ethylhexyl) phthalate from *P. digitatum*. Figure 3 displays the determined MIC of the crude penicillin extract isolated from *P. digitatum* cultured on spoiled citrus against MDR *B. cereus*.

2.7.3 Antimicrobial susceptibility test

The antibacterial activity of Bis-(2-ethylhexyl) phthalate isolated compound from *Penicillium digitatum* was decisive

by disc diffusion methods against the Gram-positive MDR *B. cereus* and *Staphylococcus aureus* and Gram-negative (*Escherichia coli*) [16]. Each bacterial isolate was suspended to a turbidity of 0.5 McFarland and plated on Muller-Hinton Agar. Discs containing concentrations of 100, 200, and 400 µg of the antibacterial activity of Bis-(2-ethylhexyl) phthalate compound were placed on the agar plates and incubated at 37°C for 24 h. The zone of inhibition was detected.

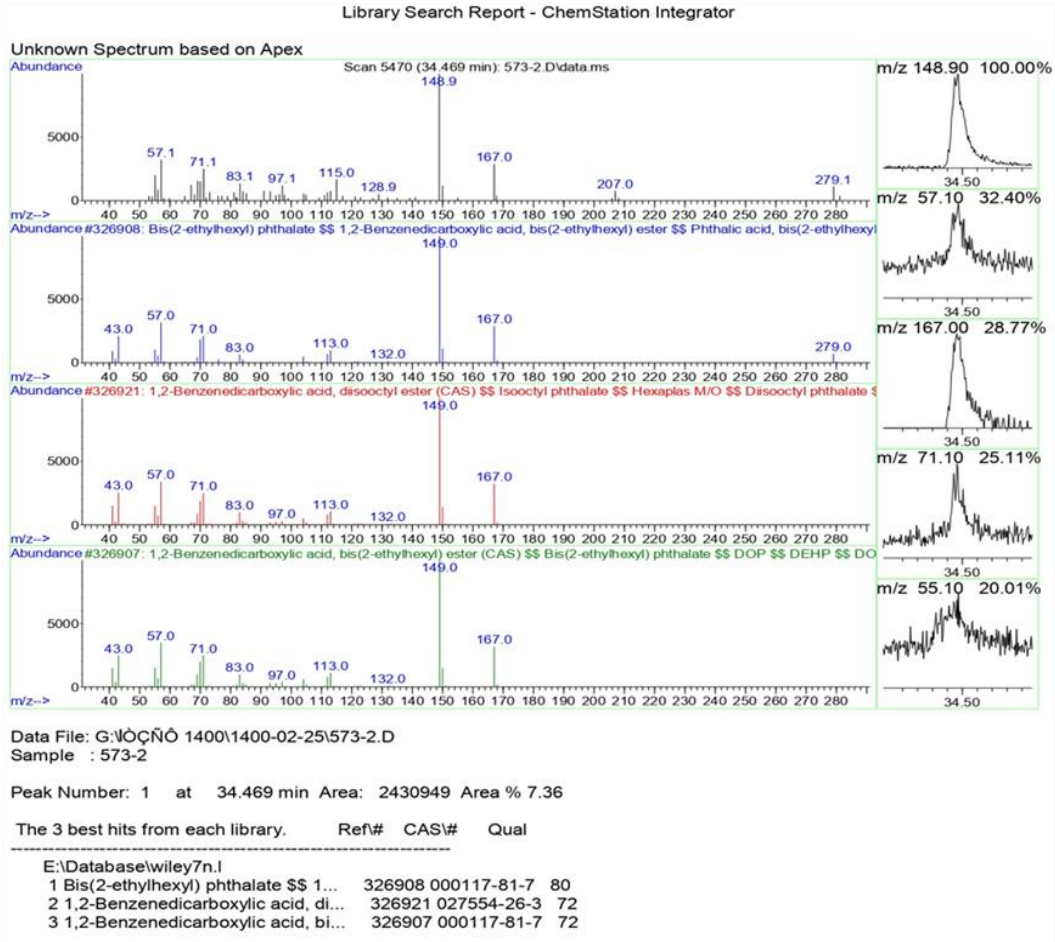


Figure 2. GC-MS chromatogram of the isolated and identified Bis-(2-ethylhexyl) phthalate from *P. digitatum* cultured on spoiled citrus [17]

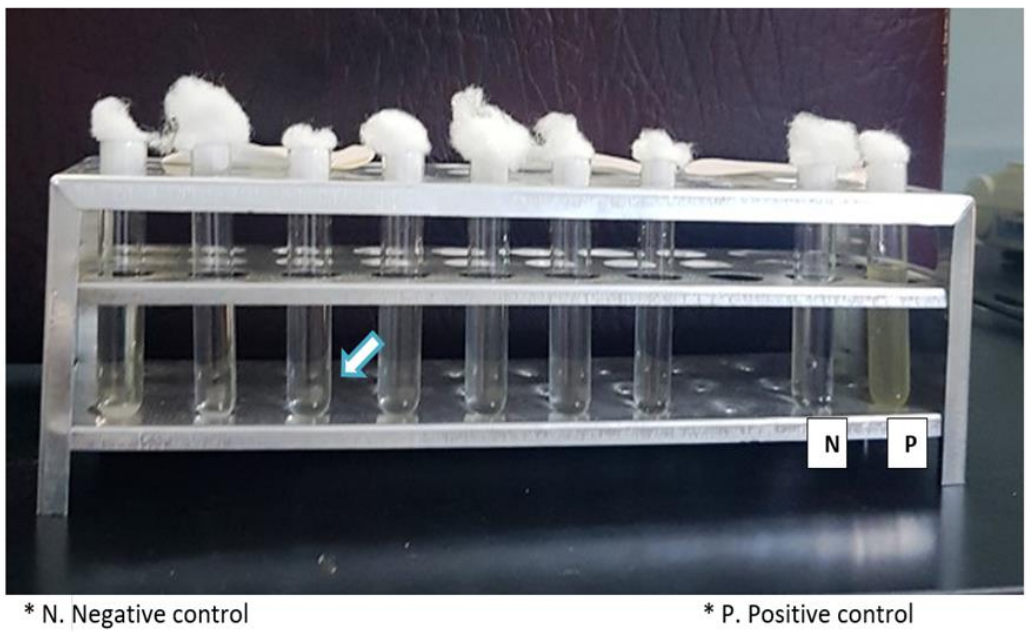


Figure 3. MIC of crude *Penicillin* compound extracted from *P. digitatum* cultured on spoiled citrus against MDR *B. cereus* (Arrow pointed to MIC ≥ 16 mg/mL)

2.7.4 MIC

The MIC of Bis-(2-ethylhexyl) phthalate was detected using the dilution assay [18].

2.8 Identification of bacterial isolates

The culture features of the bacteria were used to identify them, and a Gram stain was used to confirm the identification. Gram-positive and Gram-negative ID kits from Biomerieux, France, were used in conjunction with an automated microbiological system called Vitek2 for the identification process.

2.8.1 Bacterial morphological characterization

The gram-stained bacteria were examined under a microscope and categorized into different shapes (cocci, bacilli, irregular single, pairs, chains, or clusters) using the gram staining concept.

2.8.2 Detection of *B. cereus* by PCR

All PCR reactions were carried out in 50 µL reaction volume. The reaction mixture was prepared in a PCR tube. The primer used were:

Forward: 5-AGAGTTTGATCCTGGCTCAG-3

Backward: 5-GGTTACCTTGTTACGACTT-3

The PCR reaction included a step at 95℃ as an initial denaturation for 5 min, followed by 35 cycles of denaturation at 95℃ for 30 sec; primer annealing was done at 55℃ for 30 sec and extension at 75℃ for 30 sec; then, the final extension was done at 75℃ for 5 min.

2.8.3 Phylogenetic tree analysis

The 16S rDNA gene sequences were used to carry out BLAST with the database of the NCBI gene bank and were provisionally identified.

3. RESULTS AND DISCUSSION

Six *Bacillus* spp. (27% of the total) were identified in this study. This result is in line with previous research showing that *Bacillus* spp. comprise 20% of clinical bacterial isolates from dental diseases [19]. Additionally, the current results agree with previous research, showing that various bacterial species are prevalent in dental plaque [20]. Based on morphological and biochemical properties, six isolates were identified as Gram-positive, motile, rod- or short rod-shaped bacteria, and all tested positive for IMViC. The results showed that none of the six isolates produced indole, but the spore-forming isolates were positive for nitrate reduction. The ability to hydrolyze starch was positive, while the ability to hydrolyze gelatin was negative. Table 2 and Figures 4 and 5 showed that all six isolates tested negative for oxidase and positive for catalase. *Bacillus* spp. are Gram-positive, rod-shaped, endospore-forming bacteria that were initially identified in the nineteenth century by Cohn and Koch. Gram-positive isolates C1–C6 were used in the present investigation. *B. subtilis* and short-rod-shaped *B. cereus* were also isolated and identified from oral samples [21].

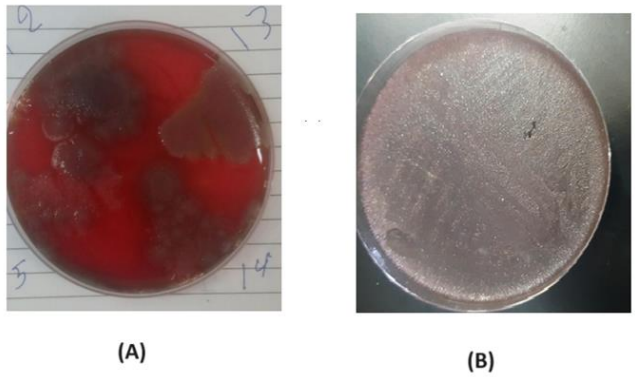


Figure 4. Colony of *B. cereus* on blood agar (a) after 3 days of incubation at 37℃, (b) after 7 days of incubation at 37℃

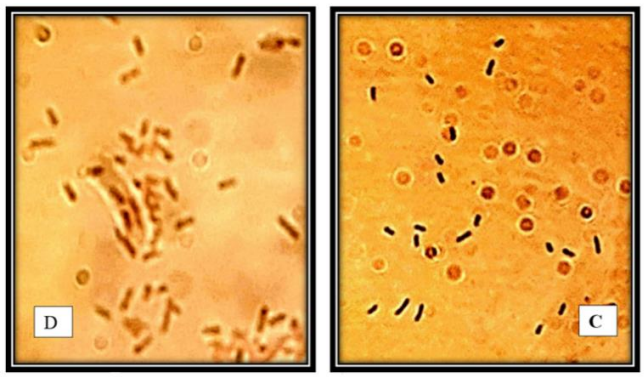


Figure 5. *B. cereus* under microscope (100x)

Table 2. Biochemical characterization of *B. cereus*

Number of Isolates	Gram Stain	Catalase	Oxidase	Indol	Motility	Spore
1	+ rods	+	-	-	+	+

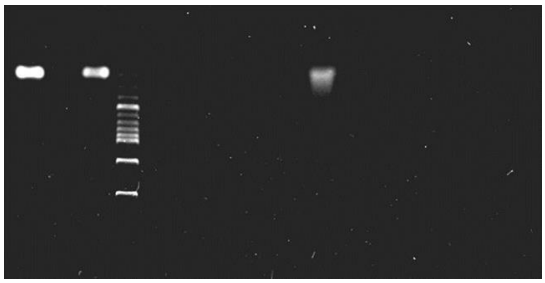
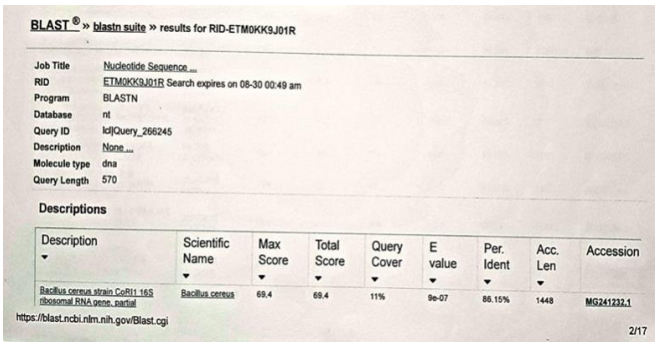


Figure 6. Agarose gel electrophoresis showing *16S rDNA* amplicon of *B. cereus* at lanes 1, 2, and 3, where M refers to a 2 kb ladder

Six strains were amplified by polymerase chain reaction (PCR), and the identification was obtained by a comparative study of 16S rDNA gene sequences. When resolved on agarose, a single distinct PCR amplicon band of 1500 pb was seen in Figure 6. The results of identifying the *B. cereus* using NCBI Blast nucleotide sequences were shown in Figure 7 for detection and Table 3 for identification. In a study by Anitha et al. [19], the results for the identification of the *B. cereus* using NCBI Blast nucleotide sequence and comparison with reference strains in the same study are displayed in Table 3.

The activity testing on Mueller Hinton agar was conducted using six different antibiotic discs (Oxoid) that included the

following antibiotics: Optochin (Op.5 µg), Doxycycline (DO.10 µg), Flucloxacillin (FOX.5 µg), Ciprofloxacin (CIP.5 µg), Tetracycline (TE.30 µg), and Nalidixic acid (NA. 5 µg). Based on the results of the zone of inhibition measurements, the susceptibility of the *B. cereus* bacterial isolates to the antibiotics was classified as sensitive, intermediate, or resistant according to the criteria set out by EUCAST. Ciprofloxacin (CIP.5 µg) was the sole antibiotic that proved effective against all six strains of *B. cereus* strains tested in this investigation while Optochin (OP.5 µg), Doxycycline (DO.10 µg), Flucloxacillin (FOX.5 µg), Tetracycline (TE.30 µg), and Nalidixic acid (NA.5 µg) all proved ineffective. Ciprofloxacin (CIP.5 µg) was found to have a 21 mm inhibition zone against all six strains of *B. cereus* (Figure 9 and Tables 4 and 5). All six bacterial strains of *B. cereus* exhibited high sensitivity to the antibiotic. One class of antibiotics known as fluoroquinolones includes ciprofloxacin (CIP.5 µg). There are other documented cases of invitation as well by Horii et al. [22]. It has been demonstrated by Citron and Appleman [23]. *B. cereus* usually shows resistance to penicillin and other β-lactam antibiotics.


BLAST® » blastn suite » results for RID-ETM0KK9J01R

Job Title Nucleotide Sequence ...
RID ETM0KK9J01R Search expires on 08-30 00:49 am
Program BLASTN
Database nt
Query ID lcl|Query_266245
Description None ...
Molecule type dna
Query Length 570

Descriptions

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Bacillus cereus strain CoRI1 16S ribosomal RNA gene, partial	Bacillus cereus	69.4	69.4	11%	9e-07	86.15%	1448	MG241232.1

https://blast.ncbi.nlm.nih.gov/Blast.cgi 2/17

Figure 7. Data of the identification of the *B. cereus* using NCBI Blast nucleotide sequence

Table 3. Data of the identification of the *B. cereus* using NCBI Blast nucleotide sequence and comparison with the reference strains

Description	Score	E Value	Accession
Buxilinx screen	N/A	N/A	N/A
Bacillinx screen, strain CoRIT, 155 ribosomal RNA gene, partial sequence	4.4	0.407	MR341231
Uncoloured bacterium	N/A	N/A	N/A
Uncoloured bacterium clone 200_312, 155 ribosomal RNA gene, partial sequence	4.4	0.409	K203284
Uncoloured bacterium clone DolO2, 45654, 153 ribosomal RNA gene, partial sequence	4.4	0.007	J2031931
Uncoloured bacterium clone RNA/MP3N-155 ribosomal RNA gene, partial sequence	4.4	0.009	K0033931
Uncoloured bacterium and full 15x DNA gene, clone D14815627	4.4	0.007	DR99329
Uncoloured bacterium clone OTU_161, 155 ribosomal RNA gene, partial sequence	4.6	0.007	K004384
Uncoloured bacterium clone TTP-821, 155 ribosomal RNA gene, partial sequence	4.5	0.007	K203286
Uncoloured bacterium clone 2_14, 155 ribosomal RNA gene, partial sequence	4.5	0.007	R013137
Uncoloured bacterium clone 271424, 177 145 ribosomal RNA gene, partial sequence	4.5	0.007	J000022
Uncoloured bacterium clone S19, 415 ribosomal RNA gene, partial sequence	N/A	N/A	N/A

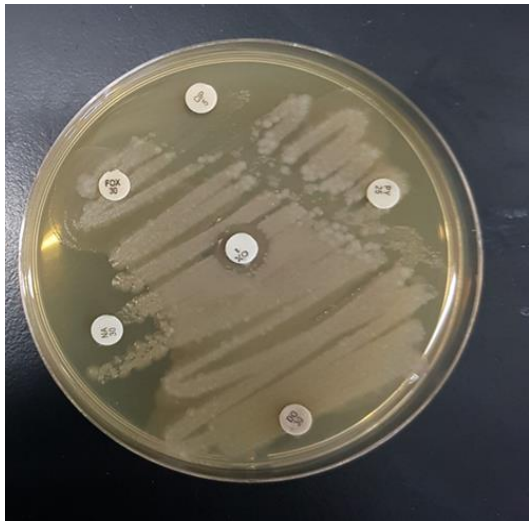


Figure 9. Effect of some antibiotics against B. cereus
Susceptibility testing: - OP = Optochin, DO= Doxycycline, NA= Nalidixic Acid, CIP= Ciprofloxacin, E= Erythromycin, FOX= Flucloxacillin

Table 4. Types of antibiotics under consideration in this study

No.	Antibiotics	Concentration (mcg)	Antibiotics Inhibition Zone
1	Optochin	OP.5	R
2	Doxycycline	DO.10	R
3	Flucloxacillin	FOX. 5	R
4	Ciprofloxacin	Cip.5	S
5	Tetracycline	TE.30	R
6	Nalidixic acid	NA.5	R

*R = Resistant, S = Sensitive

Table 5. The inhibition zone of some antibiotics and Bis-(2-ethylhexyl)-phthalate against MDR *B. cereus* isolated from some patients of dental diseases

No.	Bacterial Isolate	Inhibition Zone (mm)
1	*Bacillus cereus*	14.00
2	*Staphylococcus aureus*	14.33
3	*Escherichia coli*	12.82

Table 6. MIC for antimicrobial compound Bis-(2-ethylhexyl)-phthalate against MDR *B. cereus* isolated from some patients of dental diseases

Sample	Dilution of Bis-(2-ethylhexyl) Phthalate Compound (mg/ml)						
Bacillus cereus	≥ 64	≥ 32	≥ 16	≥ 8	≥ 4	≥ 2	≥ 1
	-	-	-	+	+	+	+

According to a study by Khalifa et al. [24], a secondary metabolite of the antibacterial compound Bis-(2-ethylhexyl)

phthalate showed antimicrobial activity against Gram-positive pathogenic bacteria *B. cereus* and Gram-negative pathogens isolates *E. coli,* suggesting its potential use in treating infections caused by these bacterial strains (Tables 4 and 5). Table 6 recorded ≥ 16 mg/ml as the MIC for the antimicrobial activity compound Bis-(2ethylhexy) phthalate against MDR *B. cereus*. The inhibition zones recorded for MDR *B. cereus, S. aureus*, and *E. coli* were 14 mm, 14.33 mm, and 12.82 mm, respectively. According to Tsoupras and Davi [25], the importance of fungi as a source of new molecules for drug discovery is clear; however, it is crucial to further investigate the compounds that have already been described in other to obtain more effective derivatives or analogs.

4. CONCLUSION

This study demonstrated that *B. cereus* accounts for 27% of the total bacterial isolates from patients with dental diseases, underscoring its role in dental infections. Early clinical resolution was closely associated with the use of appropriate empirical antibiotic therapy. Among the tested antibiotics, Ciprofloxacin (CIP.5 µg) emerged as the most effective agent for empirical treatment of *B. cereus* infections, although further clinical studies are required to substantiate these findings. Moreover, Bis-(2-ethylhexyl) phthalate, a microbial secondary metabolite from *P. digitatum,* exhibited significant activity against *B. cereus.* This compound holds promise as a potential therapeutic agent against infections caused by both Gram-positive pathogens like *B. cereus* and Gram-negative bacteria, such as *E. coli*. However, additional clinical and pharmacological studies are necessary to fully elucidate its therapeutic potential and to explore its application in treating dental and other systemic infections.

ACKNOWLEDGMENTS

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ETHICAL STANDARD

This study was achieved by the research ethics committee at the University of Misan.

REFERENCES

[1] Helmi, M.F., Huang, H., Goodson, J.M., Hasturk, H., Tavares, M., Natto, Z.S. (2019). Prevalence of periodontitis and alveolar bone loss in a patient population at Harvard School of Dental Medicine. BMC Oral Health, 19: 254. https://doi.org/10.1186/s12903-019-0925-z

[2] Sanz, M., Beighton, D., Curtis, M.A., Cury, J.A., Dige, I., et al. (2017). Role of microbial biofilms in the maintenance of oral health and in the development of dental caries and periodontal diseases. Consensus report of group 1 of the Joint EFP/ORCA workshop on the boundaries between caries and periodontal disease. Journal of Clinical Periodontology, 44(S8): S5-S11. https://doi.org/10.1111/jcpe.12682

[3] Wijnands, L.M., Dufrenne, J.B., Zwietering, M.H., Van Leusden, F.M. (2006). Spores from mesophilic *Bacillus cereus* strains germinate better and grow faster in simulated gastro-intestinal conditions than spores from psychrotrophic strains. International Journal of Food Microbiology, 112(2): 120-128. https://doi.org/10.1016/j.ijfoodmicro.2006.06.015

[4] Pilo, P., Frey, J. (2011). *Bacillus anthracis*: Molecular taxonomy, population genetics, phylogeny and patho-evolution. Infection, Genetics and Evolution, 11(6): 1218-1224. https://doi.org/10.1016/j.meegid.2011.05.013

[5] Horst, J.A., Tanzer, J.M., Milgrom, P.M. (2018). Fluorides and other preventive strategies for tooth decay. Dental Clinics of North America, 62(2): 207-234. https://doi.org/10.1016/j.cden.2017.11.003

[6] Avashia, S.B., Riggins, W.S., Lindley, C., Hoffmaster, A., Drumgoole, R., Nekomoto, T., et al. (2007). Fatal pneumonia among metalworkers due to inhalation exposure to *Bacillus cereus* containing Bacillus anthracis toxin genes. Clinical Infectious Diseases, 44(3): 414-416. https://doi.org/10.1086/510429

[7] Rishi, E., Rishi, P., Sengupta, S., Jambulingam, M., Madhavan, H.N., Gopal, L., Therese, K.L. (2013). Acute postoperative Bacillus cereus endophthalmitis mimicking toxic anterior segment syndrome. Ophthalmology, 120(1): 181-185. https://doi.org/10.1016/j.ophtha.2012.07.009

[8] Lee, Y.L., Shih, S.D., Weng, Y.J., Chen, C., Liu, C.E. (2010). Fatal spontaneous bacterial peritonitis and necrotizing fasciitis with bacteraemia caused by *Bacillus cereus* in a patient with cirrhosis. Journal of Medical Microbiology, 59(2): 242-244. https://doi.org/10.1099/jmm.0.011056-0

[9] Dellinger, R.P., Levy, M.M., Rhodes, A., Annane, D., Gerlach, H., et al. (2013). Surviving sepsis campaign: International guidelines for management of severe sepsis and septic shock. Critical Care Medicine, 41(2): 580-637. https://doi.org/10.1097/CCM.0b013e31827e83af

[10] Lotfy, W.A., Mostafa, S.W., Adel, A.A., Ghanem, K.M. (2018). Production of di-(2-ethylhexyl) phthalate by *Bacillus subtilis* AD35: Isolation, purification, characterization and biological activities. Microbial Pathogenesis, 124: 89-100. https://doi.org/10.1016/j.micpath.2018.08.014

[11] Blunt, J.W., Carroll, A.R., Copp, B.R., Davis, R.A., Keyzers, R.A., Prinsep, M.R. (2018). Marine natural products. Natural Product Reports, 35(1): 8-53. https://doi.org/10.1039/C7NP00052A

[12] Carroll, A.R., Copp, B.R., Davis, R.A., Keyzers, R.A., Prinsep, M.R. (2019). Marine natural products. Natural Product Reports, 36(1): 122-173. https://doi.org/10.1039/C8NP00092A

[13] Bridge, P., Spooner, B. (2001). Soil fungi: Diversity and detection. Plant and Soil, 232: 147-154. https://doi.org/10.1023/A:1010346305799

[14] Moawad, M.N., Ghobrial, M., Shabaka, S. (2022). The red alga *Grateloupia gibbesii*, as a valuable source of lipids: Lipids quality indices, spectroscopic characterization, and potential industrial applications. Aquaculture International, 30(3): 1185-1209. https://doi.org/10.1007/s10499-021-00813-3

[15] Gowsia, I., Mir, F.A., Banday, J.A. (2022). Preparation

and characterization of polyvinyl alcohol–piperic acid composite film for potential food packaging applications. Progress in Biomaterials, 11(3): 281-295. https://doi.org/10.1007/s40204-022-00195-6

[16] Tadeg, H., Mohammed, E., Asres, K., Gebre-Mariam, T. (2005). Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. Journal of Ethnopharmacology, 100(1-2): 168-175. https://doi.org/10.1016/j.jep.2005.02.031

[17] Ali, M.A.A., Darweesh, A.O., Matar, M.M. (2023). Isolation and characterization of an antibacterial compound Bis-(2-ethylhexyl) phthalate from the fungi Penicillium digitatum. Journal of Biomedicine, 43(5): 1607-1612. https://doi.org/10.51248/.v43i5.3264

[18] Ginocchio, C.C. (2002). Role of NCCLS in antimicrobial susceptibility testing and monitoring. American Journal of Health-System Pharmacy, 59(suppl_3): S7-S11. https://doi.org/10.1093/ajhp/59.suppl_3.S7

[19] Anitha, M., Reichardt, F., Tabatabavakili, S., Nezami, B. G., Chassaing, B., Mwangi, S., Vijay-Kumar, M., Gewirtz, A., Srinivasan, S. (2016). Intestinal dysbiosis contributes to the delayed gastrointestinal transit in high-fat diet fed mice. Cellular and Molecular Gastroenterology and Hepatology, 2(3): 328-339. https://doi.org/10.1016/j.jcmgh.2015.12.008

[20] Rawashdeh, R., Malkawi, H.I., Al-Hiyasat, A.S., Hammad, M. (2008). A fast and sensitive molecular detection of Streptococcus mutans and Actinomyces viscosus from dental plaques. Jordan Journal of Biological Sciences, 1(3): 135-139.

[21] Rani, A.A., Jeeva, S., Punitha, S.M.J., Lexshmi, N.C. J.P., Brindha, J.R. (2016). Biochemical and molecular analysis of bacteria isolated from dental caries. Journal of Chemical and Pharmaceutical Research, 8(3): 512-519.

[22] Horii, T., Notake, S., Tamai, K., Yanagisawa, H. (2011). Bacillus cereus from blood cultures: Virulence genes, antimicrobial susceptibility and risk factors for blood stream infection. FEMS Immunology & Medical Microbiology, 63(2): 202-209. https://doi.org/10.1111/j.1574-695X.2011.00842.x

[23] Citron, D.M., Appleman, M.D. (2006). In vitro activities of daptomycin, ciprofloxacin, and other antimicrobial agents against the cells and spores of clinical isolates of Bacillus species. Journal of Clinical Microbiology, 44(10): 3814-3818. https://doi.org/10.1128/jcm.00881-06

[24] Khalifa, M., Fayed, R.H., Ahmed, Y.H., Sedik, A.A., El-Dydamony, N.M., Khalil, H.M. (2024). Mitigating effect of ferulic acid on di-(2-ethylhexyl) phthalate-induced neurocognitive dysfunction in male rats with a comprehensive in silico survey. Naunyn-schmiedeberg's Archives of Pharmacology, 397(5): 3493-3512. https://doi.org/10.1007/s00210-023-02831-9

[25] Tsoupras, A., Davi, K.G. (2024). Bioactive metabolites from fungi with anti-inflammatory and antithrombotic properties: Current status and future perspectives for drug development. In Fungi Bioactive Metabolites: Integration of Pharmaceutical Applications, pp. 427-494. https://doi.org/10.1007/978-981-99-5696-8_14