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SCREENING AND MOLECULAR CHARACTERIZATION OF POST-CRANIOTOMY ASSOCIATED BETA-HEMOLYTIC PATHOGENIC BACTERIA AND MODULATION OF BACTERICIDAL POTENCY OF DIFFERENT ANTIBACTERIAL AGENTS AGAINST THESE ISOLATES

TRIAGEM E CARACTERIZAÇÃO MOLECULAR DE BACTÉRIAS PATOGÊNICAS BETA-HEMOLÍTICAS ASSOCIADAS À PÓS-CRANIOTOMIA E MODULAÇÃO DA POTÊNCIA BACTERICIDA DE DIFERENTES AGENTES ANTIBACTERIANOS CONTRA ESSES ISOLADOS

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Abstract
Background: Post-craniotomy neurosurgical infections pose a major challenge in everyday neurosurgery, impacting patient recovery and adding financial strain on healthcare systems. Even with improvements in surgical methods and infection prevention measures, these infections continue to lead to complications and deaths related to surgery. Objective: Screening and isolation of pathogenic strains from Post-craniotomy infections and sensitivity assessment against different antibacterial agents. Method: Collected samples were streaked onto Nutrient Agar medium and isolated colonies were streaked for pure culture. Pure cultures were subjected to Blood Agar Test for isolation of pathogenic bacteria (beta-hemolytic strains) from infections. Molecular characterization was done to confirm and identify the pathogenic strain and accession number was obtained by nucleotide blast at NCBI site. Sensitivity of pathogenic strains against antibiotics and plant

Resumo
Contexto: As infecções neurocirúrgicas pós-craniotomia representam um grande desafio na neurocirurgia diária, afetando a recuperação dos pacientes e aumentando a pressão financeira sobre os sistemas de saúde. Mesmo com melhorias nos métodos cirúrgicos e nas medidas de prevenção de infecções, essas infecções continuam a causar complicações e mortes relacionadas à cirurgia. Objetivo: Triagem e isolamento de cepas patogênicas de infecções pós-craniotomia e avaliação da sensibilidade a diferentes agentes antibacterianos. Método: As amostras coletadas foram espalhadas em meio ágar nutriente e as colônias isoladas foram espalhadas para cultura pura. As culturas puras foram submetidas ao teste de ágar sangue para isolamento de bactérias patogênicas (cepas beta-hemolíticas) de infecções. A caracterização molecular foi feita para confirmar e identificar a cepa patogênica e o número de acesso foi obtido por blast de nucleotídeos no site NCBI. A

extracts was checked and zone of inhibition was measured. ONE WAY ANOVA was applied for statistical analysis and Tuckey's PostHoc test for comparison. P>0.05 was set as significant. Results: *P. aeruginosa* exhibited resistance against Erythromycin while *B. subtilis* showed sensitivity against Erythromycin with 21.56±0.06 zone of inhibition, Levofloxacin exhibited maximum antibacterial activity against *P. aeruginosa* with 19.97±0.12 and 16.36±0.04 zone of inhibition exhibited by *B. subtilis* against Levofloxacin. Gentamicin, showed moderate antibacterial activity against both *P. aeruginosa* and *B subtilis* with 15.78±0.02 and 13.51±0.07 zone of inhibition, respectively, but antibiotics exhibited non-significant difference with p>0.05. Both *P. aeruginosa* and *B subtilis*, showed sensitivity against plant extracts and plant extracts showed significant difference with p<0.05, *C. zeylanicum* exhibited maximum 18.77±0.09 and 15.26±0.31 zone of inhibition against *B. subtilis* and *P. aeruginosa*, respectively. *Z. jujube* showed minimum but antibacterial activity with zone of inhibition 7.98±0.09 and 7.66±0.44 against *P. aeruginosa* and *B. subtilis*, respectively. Both *P. aeruginosa* and *B subtilis* showed intermediate sensitivity against *E. cardamomum* with zone of inhibition 10.11±0.13 and 12.13±0.08, respectively. Conclusion: The current study concludes that antibacterial agents should be used instead of antibiotics against microbes, as excessive use of antibiotics leads to side effects and their continuous use also contributes to microbial resistance.

Keywords: Post-craniotomy Neurosurgical Infections. Pathogenic Strains. Antibacterial Agents. Nutrient Agar Medium. Blood Agar Medium. Beta-hemolytic Strains. Molecular Characterization.

sensibilidade das cepas patogênicas a antibióticos e extratos de plantas foi verificada e a zona de inibição foi medida. A ANOVA unidirecional foi aplicada para análise estatística e o teste PostHoc de Tuckey para comparação. P>0,05 foi definido como significativo. Resultados: P. aeruginosa exibiu resistência contra a eritromicina, enquanto B. subtilis mostrou sensibilidade contra a eritromicina com zona de inibição de 21,56±0,06. A levofloxacina exibiu atividade antibacteriana máxima contra P. aeruginosa com zona de inibição de 19,97±0,12 e 16,36±0,04 exibida por B. subtilis contra a levofloxacina. A gentamicina apresentou atividade antibacteriana moderada contra P. aeruginosa e B. subtilis, com zonas de inibição de 15,78 ± 0,02 e 13,51 ± 0,07, respectivamente, mas os antibióticos apresentaram diferença não significativa com p > 0,05. Tanto a P. aeruginosa quanto a B subtilis mostraram sensibilidade contra extratos vegetais, e os extratos vegetais mostraram diferença significativa com p<0,05. A C. zeylanicum exibiu zona de inibição máxima de 18,77±0,09 e 15,26±0,31 contra a B. subtilis e a P. aeruginosa, respectivamente. Z. jujube apresentou atividade antibacteriana mínima, com zona de inibição de 7,98±0,09 e 7,66±0,44 contra P. aeruginosa e B. subtilis, respectivamente. Tanto P. aeruginosa quanto B subtilis apresentaram sensibilidade intermediária contra E. cardamomum, com zona de inibição de 10,11 ± 0,13 e 12,13 ± 0,08, respectivamente. Conclusão: O presente estudo conclui que agentes antibacterianos devem ser usados em vez de antibióticos contra micróbios, pois o uso excessivo de antibióticos leva a efeitos colaterais e seu uso contínuo também contribui para a resistência microbiana.

Palavras-chave: *Infecções Neurocirúrgicas Pós-craniotomia. Cepas Patogênicas. Agentes Antibacterianos. Meio Ágar Nutriente. Meio Ágar Sangue. Cepas Beta-hemolíticas. Caracterização Molecular.*

1 INTRODUCTION

A craniotomy is a neurosurgical operation in which a section of skull is carefully removed to allow the access to brain and the central nervous system. During the procedure. the removed bone segment (bone flap) is set aside temporarily and then repositioned and secured back in place after the surgery is completed to ensure protection of the brain and surrounding structures (Jiménez-Martínez *et al*.. 2019; De Morais *et al*.. 2021).The central nervous system (CNS) is normally well protected against infections due to the presence of the blood–brain barrier. However. this natural defense mechanism is compromised during a craniotomy. thereby increasing the susceptibility of patients to intracranial infections (Liu *et al*.. 2024). Infection occurring after cranial surgery is a significant complication that demands prompt identification and timely management (Shi *et al*.. 2017). Post operative complications arise in approximately 13-27% of patients during the first month after surgery (Tarimah *et al*.. 2026).

Post-craniotomy CNS infections are associated with higher mortality rates and long-term neurological disabilities (Liu *et al*.. 2024). These neurological complications include post central nervous system infections (PCNSI) can lead to meningitis. subdural empyema. cerebral abscess. (De Morais *et al*.. 2021; Campioli *et al*.. 2022) and occasionally surgical site infections (Campioli *et al*.. 2022). The incidence of SSI after craniotomy ranges from 2.2 to 19.8%. The factors affecting the infection rate include skin preparation. wound contamination. the length of pre-operative hospital stay. drainage of wounds. the age of the patient. duration of surgery. and skill and technique of the surgeon (Pralea *et al*.. 2023).

Post craniotomy infections is caused by Preoperative. intraoperative and post-operative complications (Liu *et al*.. 2024). Risk factors for post-craniotomy SSIs include patient-related. surgical. and postoperative variables. Patient factors include diabetes mellitus. immunocompromised states. obesity. and prior radiation therapy. Surgical factors include prolonged operative duration (>3.5 h). multiple procedures. frontal or paranasal sinus involvement. and cerebrospinal fluid (CSF) leakage. Postoperative factors include wound complications. hematoma formation. and foreign body presence (Qiang *et al*.. 2025).

Post craniotomy infections primarily considered nosocomial (hospital-acquired) infections. as they developed during hospitalization and shortly after discharge following a surgical procedure. The most common nosocomial pathogens are *Klebsiella pneumonia* (22.12%). followed by *Pseudomonas aeruginosa* 20.19%) and *Escherichia coli* (10.58%). Other organisms included *Candida albicans*. *Staphylococcus aureus*. and *Acinetobacter* *baumannii* (Nuñez-Lupaca *et al*.. 2025). In patients with SSI. common pathogens identified were *Staphylococcus aureus* (n = 14. 28%). including 11 methicillin-susceptible *Staphylococcus aureus* (MSSA) isolates (22%). and 3 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates (6%). followed by 8 coagulase-negative staphylococci isolates (CoNS. 16%). 8 *Cutibacterium acnes* isolates (16%). 5 *Klebsiella* spp.(10%). and 4 *Pseudomonas aeruginosa* (8%) (Campioli *et al*.. 2022) and *Bacilli* spp. may produce intracranial infections (Zhan *et al*.. 2014).

To reduce surgical site infections (SSIs). patients and caregivers should receive clear instructions on postoperative wound care. early signs of infection. and appropriate points of contact. Appropriate perioperative antibiotic prophylaxis and strict adherence to aseptic techniques in the operating room are essential to minimize contamination. Wound dressing changes must be performed by trained nursing staff. and patients should be advised to bathe with soap the day before or on the day of surgery (Sattar *et al*.. 2019).

Antibiotics interfere with the proton-driven force that mostly passes through the cell membrane of cells. killing the bacteria. They reduce bacteria's ability to generate or store energy. stop protein production. and affect the structural components of the cell wall. The different types of resistance found in *P. aeruginosa* are described as population-level events that happen through high levels of antibodies in the host and subsequent antibody selection. Multi-drug-resistant bacteria are causing a lot of medical problems worldwide (Alara and Alara. 2024; Zhang *et al*.. 2024).

Herb plants are a safe and effective natural remedy for various illnesses (Mehmood *et al*.. 2025; Shaikh *et al*.. 2025). For treating wounds or diseases. either the whole plant or a part of it is used as herbal medicine. often referred to as plant material (Balkrishna *et al*.. 2024; Cedillo-Cortezano *et al*.. 2024). Plant extracts are rich sources of natural chemical compounds with potential medical uses and have long been known for their antimicrobial properties. These extracts contain various bioactive compounds that can prevent or stop bacterial growth (Nguyen *et al*.. 2024; Sattar *et al*.. 2024).

2 METHODOLOGY

2.1 Sample collection

No experimental trials were done on patients. Only samples by using sterilized culture sticks were collected by swapping on the infection site in order to isolate bacterial strains. Samples were brought from the General Hospital. Lahore to Microbiology Laboratory. Government College University. Lahore for further analysis.

2.2 Isolation of bacterial strains

Samples were streaked on to the freshly prepared sterilized Nutrient Agar medium and inverted plates were incubated for overnight at 37ºC for growth of bacterial strains.

2.3 Isolation of pure culture

Isolated colonies on the basis of morphology. texture and odour were picked up and streaked on to Nutrient Agar medium to get pure culture of each bacterial strain. Plates were incubated for 24 hours at 37ºC (Chaudhry *et al*.. 2024).

2.4 Pathogenicity test

Isolated colonies of pure culture were picked up with sterilized inoculating loop and streaked onto the freshly prepared Blood Agar medium and incubated for overnight at 37ºC. to check whether the strains are pathogenic or not and if pathogenic then which kind of hemolysis (alpha. beta and gamma) shown by isolates (Chaudhry *et al*.. 2024).

2.5 Inoculum preparation and glycerol stock preparation

Sterilized Nutrient broth medium was poured into 15ml falcon tubes and pure culture of each pathogenic strain showing beta hemolysis (β-hemolysis) was inoculated into medium and incubated for overnight at 37ºC to get broth culture.

200µl glycerol was sterilized into Eppendorf and after incubation time. as broth culture was prepared 800µl of broth culture was poured into glycerol and vortexed few seconds for thoroughly mixing. Glycerol stock was stored at -20ºC.

2.6 Molecular characterization

Phenol-Chloroform DNA extraction method was used to isolate bacterial DNA. Gel electrophoresis confirmed the isolation of DNA. After PCR. PCR products were sent for sequencing. Nucleotide sequences were blast at NCBI to get the accession number of bacterial strains having homology with the previously published data (Prashanthi *et al*.. 2021; Mazhar. B *et al*.. 2024).

2.7 Preparation of plant extracts

Powder (20g) of *Ziziphus jujube*. *Elettaria cardamomum* and *Cinnamomum zeylanicu* wasmixed into 100ml distilled water and for thoroughly mixing. flasks with the mixture placed into shaker for overnight. After mixing. by using Whatman filter paper. mixture was filtered and filtrate was taken as aqueous plant extract for antibacterial activity (Dzimitrowicz *et al*.. 2021).

2.8 Antibacterial activity

Antibacterial activity was done by using well diffusion method on freshly prepared sterilized Muller Hinton Agar medium (MHA). Four wells were made by using back side of a sterile blue pipette tip (9mm) and 50µl pathogenic strain was spread onto the plate. Central well was taken as control group and poured 100µl sterilized distilled water (negative control) and 100µl of three antibiotics (Erythromycin. Gentamicin and Levofloxacin) of 500mg/100ml H_2O dilution was poured in wells. Aqueous Plant extracts (*Z. jujube*. *E. cardamomum* and *C. zeylanicum*) of 100µl was poured in wells as antibacterial agents (Chaudhry *et al*.. 2024; Munir *et al*.. 2024).

2.9 Results

Off-white to pale. irregular and whitish. shiny colonies appeared on Nutrient Agar medium

Figure 1

Pure culture of (a) B. subtilis and (b) P. aeruginosa

β-hemolysis was shown by isolated strains indicating pathogenic characteristics and cause of infections. Greenish and clear zones are shown around colonies. (Fig. 2).

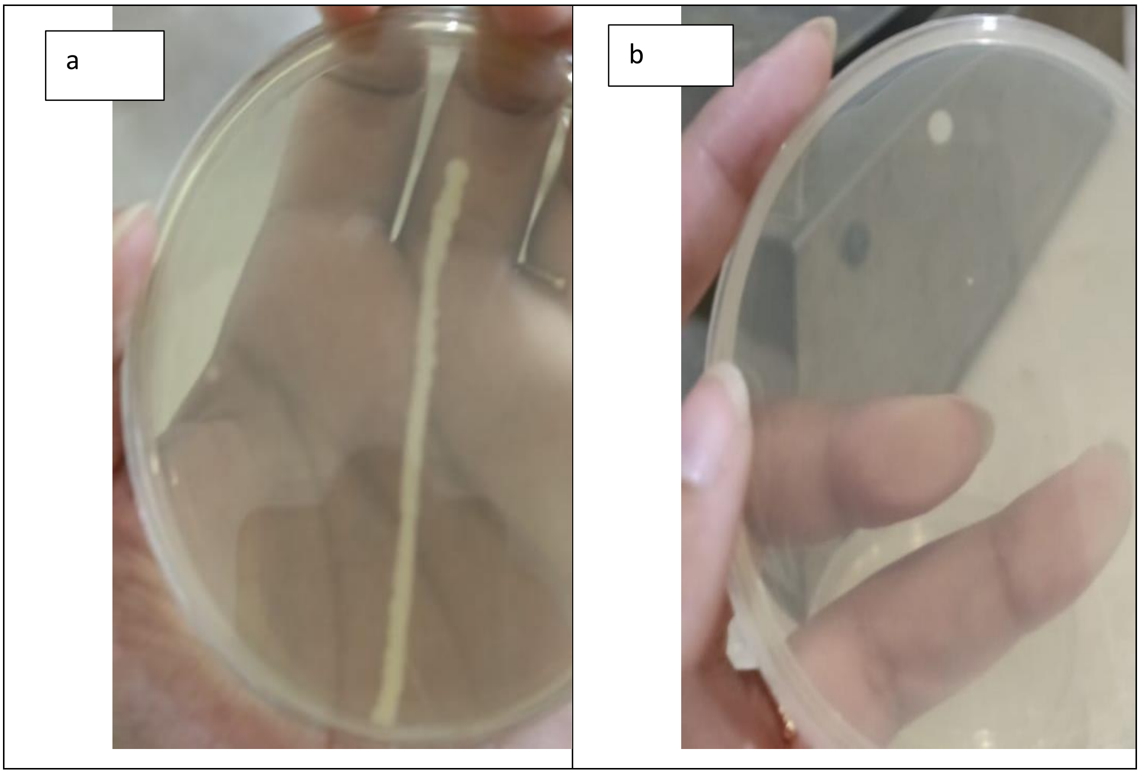


Figure 2

β-Hemolysis done by a) B. subtilis and (b) P. aeruginosa

Molecular characterization of isolated pathogenic strains was done and accession number of P. aeruginosa and B. subtilis were obtained (Table 1; Fig. 3).

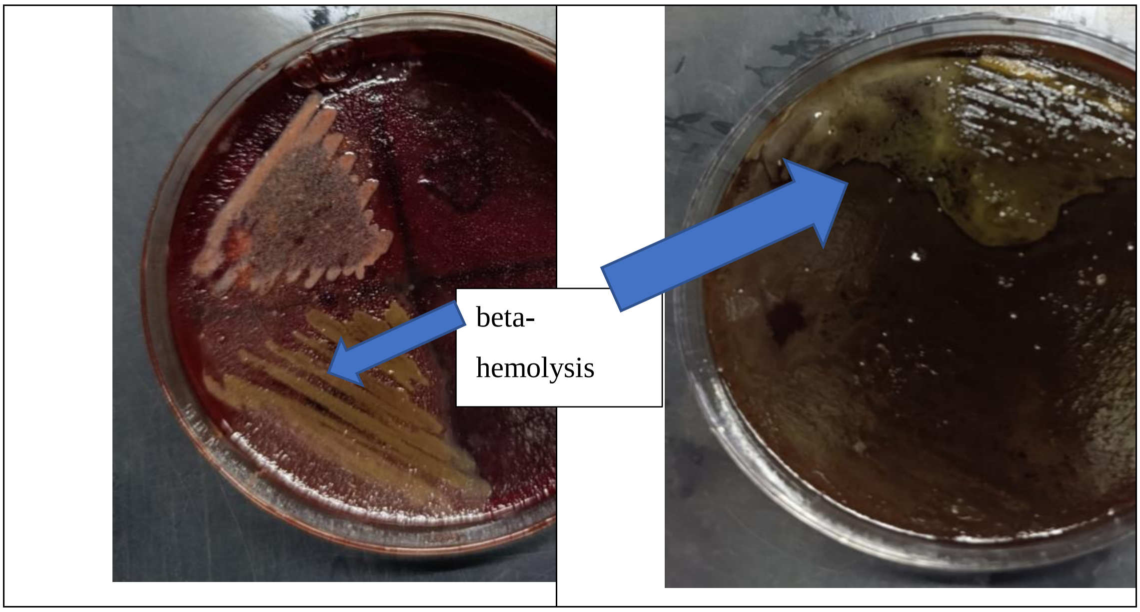


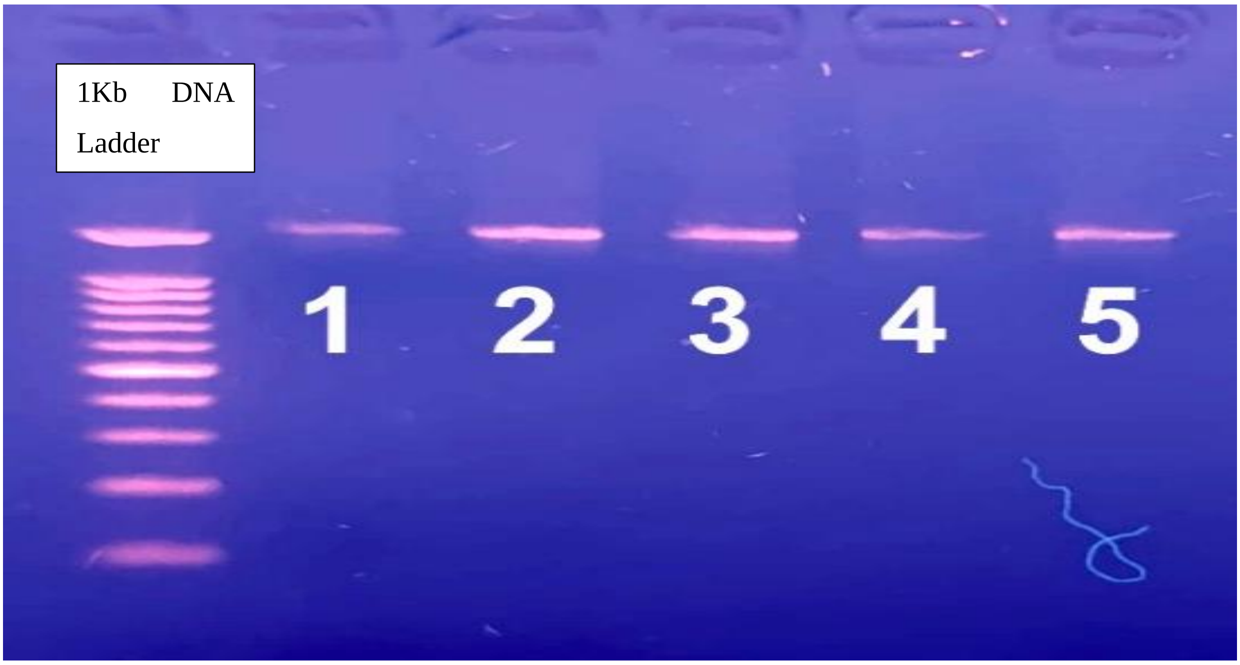
Table 1

Pathogenic strains: Accession number

Sr. No	Strain	Base pair	Accession No
1	*Pseudomonas aeruginosa*	1023 bp	PX905786
2	*Bacillus subtilis*	1026 bp	PX915596

Figure 3

Molecular characterization 1kb DNA Ladder



ONE WAY ANOVA was used as statistical analysis and for comparison Tukey's Post Hoc test was applied.

The Clinical & Laboratory Standards Institute (CLSI) guidelines were used for measurement of Zone if inhibition (ZOI) to check the sensitivity of pathogenic strains against antibacterial agents. ZOI was measured in (Mean±S.E). *P. aeruginosa* exhibited resistance against Erythromycin while *B. subtilis* showed sensitivity against Erythromycin with ZOI 21.56±0.06 (Mean±S.E). Levofloxacin exhibited maximum antibacterial activity against *P. aeruginosa* with ZOI 19.97±0.12 (Mean±S.E) 16.36±0.04 ZOI exhibited by *B. subtilis* against Levofloxacin. Gentamicin. showed moderate antibacterial activity against both *P. aeruginosa* and *B subtilis* with ZOI 15.78±0.02 and 13.51±0.07. respectively. but $p>0.05$ indicated non-significant difference showed by antibiotics (Table 2; Fig. 4).

Table 2

Sensitivity of P. aeruginosa and B. subtilis against antibiotics (ZOI) (mm): Mean±S.E of ZOI: R=Resistance

Strains	**Antibiotics**			
	Control	Erythromycin	Gentamicin	Levofloxacin
P. aeruginosa	0±0	R	15.78±0.02	19.97±0.12
B. subtilis	0±0	21.56±0.06	13.51±0.07	16.36±0.04

Both *P. aeruginosa* and *B subtilis*. showed sensitivity against plant extracts. *C. zeylanicum* exhibited maximum ZOI 18.77±0.09 and 15.26±0.31 against *B. subtilis* and *P. aeruginosa*. respectively. *Z. jujube* showed minimum but antibacterial activity with ZOI 7.98±0.09 and 7.66±0.44 against *P. aeruginosa* and *B. subtilis*. respectively. Both *P. aeruginosa* and *B subtilis* showed intermediate sensitivity against *E. cardamomum* with ZOI 10.11±0.13 and 12.13±0.08. respectively (Table.3; Fig. 5).

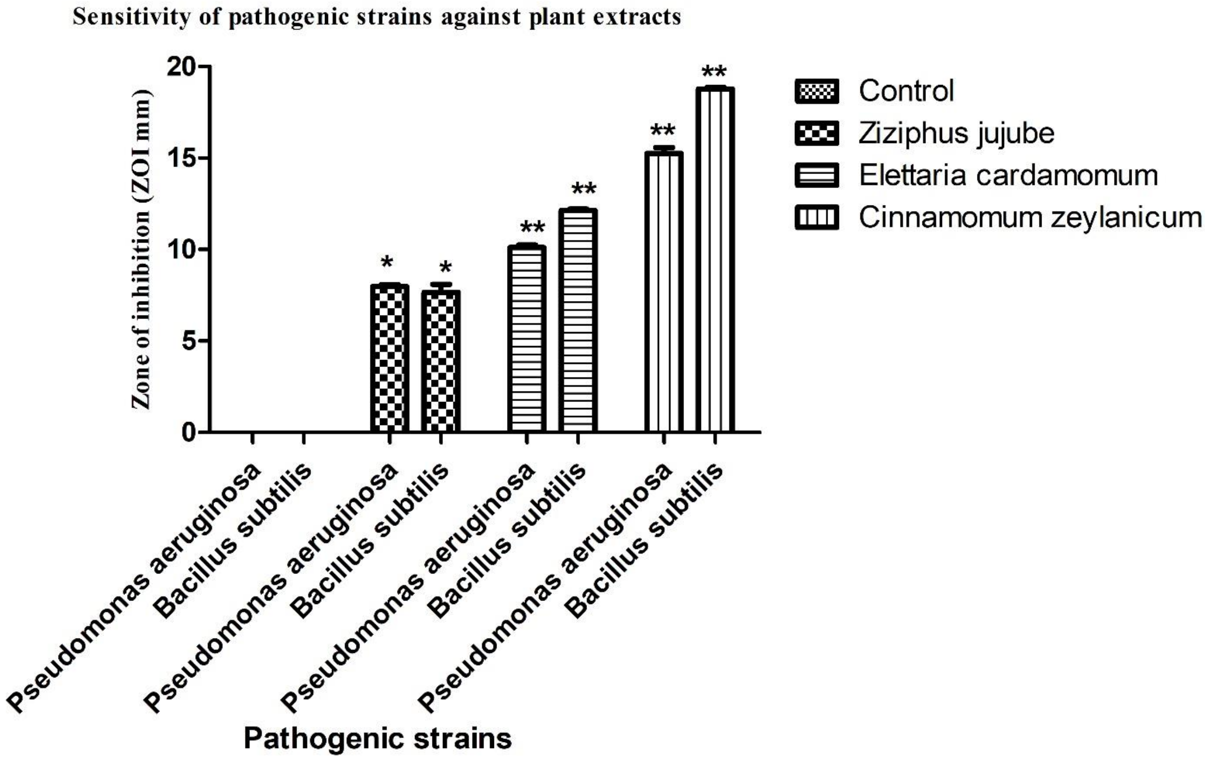
Table 3

Sensitivity of P. aeruginosa and B. subtilis against Plant extracts (ZOI) (mm): Mean±S.E of ZOI:

Strains	**Plant extracts**			
	Control	*Ziziphus jujube*	*Elettaria cardamomum*	*Cinnamomum zeylanicum*
P. aeruginosa	0±0	7.98±0.09	10.11±0.13	15.26±0.31
B. subtilis	0±0	7.66±0.44	12.13±0.08	18.77±0.09

Figure 5

*Sensitivity of pathogenic strains (P. aeruginosa and B. subtilis) against plant extracts. * indicating significant difference between control and antibacterial agents (Plant extracts). P<0.05 set as significant and in this study P value 0.0013 indicating significant difference with F value 48.84 and degree of freedom (df) 7. Analysis was done by Prism 5 software and ONE WAY ANOVA followed by "Tukey's Multiple Comparison Test".*



3 DISCUSSION

Craniotomy carries a risk of postoperative intracranial infection. This complication is commonly linked to factors such as prolonged operative time. infratentorial procedures. cerebrospinal fluid leakage. placement of drainage tubes. inappropriate or excessive antibiotic use. administration of glucocorticoids. advanced age. diabetes. and the presence of other systemic infections. Identifying and understanding these risk factors is essential for lowering the rate of central nervous system (CNS) infections following craniotomy (Wang *et al.*. 2020). Among patients who experienced post-craniotomy intracranial infections. Gram-positive bacteria were the most commonly detected pathogens in CSF cultures. particularly *S. epidermidis* and *S. aureus*. Gram-negative organisms. including *Acinetobacter*. *Klebsiella*. and *P. aeruginosa*. were less frequent. and only a small proportion of cases showed mixed bacterial infections (Zhu *et al*.. 2024). The incidence of meningitis following craniotomy was 8.9%. with Gram-negative *bacilli* identified as the predominant causative organisms (Zhou *et al*.. 2022; Baloch *et al*.. 2025).

In the present study. beta hemolytic bacterial strains were isolated from clinical specimens collected from post-craniotomy. Beta-hemolytic activity is commonly associated with pathogens such as *P. aeruginosa. S. aureus. B. subtilis*. These organisms are known to colonize surgical wounds and hospital environments. thereby increasing the risk of post-operative infections (Kourbeti *et al*.. 2015). Molecular characterization revealed the presence of both *P. aeruginosa* and *B. subtilis*. Similar findings have been reported in neurosurgical site infections where *P. aeruginosa* and *B. subtilis* are frequently implicated pathogens (Zhou *et al*.. 2022; Zhu *et al*.. 2024). This study assessed the antibiotic susceptibility and antimicrobial potential of plant extracts against two pathogenic strains—*P. aeruginosa* and *B. subtilis*. The findings have implications for both clinical management and future alternative therapies.

The antibiotic sensitivity data (Table 2) demonstrate markedly different resistance profiles between the two strains. *P. aeruginosa* exhibited complete resistance to erythromycin. while showing measurable susceptibility to gentamicin and levofloxacin. *P. aeruginosa* remains a significant source of infection in Western countries. largely due to its strong inherent resistance to many antimicrobial agents (Zafar *et al*.. 2025). This natural resistance is attributed to its highly selective outer membrane. which limits drug entry. along with additional mechanisms such as energy-driven multidrug efflux systems and chromosomally encoded periplasmic β-lactamase production. Because of these built-in defenses. the organism can also rapidly develop mutation-based resistance to multiple antibiotic classes (Subedi *et al*.. 2018). Previous studies examined the phenotypic and genotypic profiles of clinical *P. aeruginosa* isolates obtained from patients with wound and burn infections in Baghdad. indicate high prevalence of virulent determinants (Siddique *et al.* 2025). The overall multidrug resistance (MDR) rate was about 59%. differing from other reports. For instance. a study documented a higher MDR rate of 78%

in Iran. whereas lower rates (10.9%–25.9%) in the Arabian Gulf region (Alnaji *et al*.. 2026).

P. aeruginosa showed complete susceptibility to gentamicin. an aminoglycoside. with a resistance rate of 0% (Popa *et al*.. 2026). Levofloxacin is a fluoroquinolone antibiotic with broad-spectrum activity that acts by inhibiting DNA gyrase and topoisomerase IV. thereby disrupting bacterial DNA replication. Although its overall clinical use is somewhat restricted. it remains an important first-line option for treating chronic *P. aeruginosa* infections (Pitchiah *et al*.. 2026). In contrast. *B. subtilis* displayed susceptibility to all antibiotics tested. especially erythromycin. In align with reported study. the minimum inhibitory concentrations (MICs) of eight antibiotics were evaluated for 85 strains—*Bacillus subtilis* subsp. *subtilis* (29). *Bacillus licheniformis* (38). and *Bacillus sonorensis* (18)—isolated from Sudanese bread starter cultures. All isolates were susceptible to tetracycline (8 mg/L). vancomycin (4 mg/L). and gentamicin (4 mg/L). but showed resistance to streptomycin (Mbhele *et al*.. 2021). *B. subtilis* no resistance was shown against Levofloxacin and Nitrofurantoin (Harba and Jawhar. 2025; Ishfaq *et al*.. 2025).

The antimicrobial screening of plant extracts (Table 3) revealed that *C. zeylanicum* had the strongest activity against both bacterial strains. followed by *E. cardamomum* and *Z. jujube*. Notably. B. subtilis was slightly more susceptible than *P. aeruginosa* to all tested extracts. These findings are consistent with literature showing that plant phytochemicals like tannins. flavonoids. and phenolic compounds can disrupt bacterial cell membranes. interfere with quorum sensing. or inhibit enzyme activity (Kalhoro *et al*.. 2025)

P. aeruginosa and *B. subtilis* both show inhibition in mg of ethanolic extract of *E. cardamomum* (mg mL-1) of pathogenic strains (Moulai-Hacene *et al*.. 2020). The reported studies evaluate the antibacterial properties and chemical composition of *Z. jujuba* seed extract. The extract was prepared using a 50% aqueous-ethanol solution and tested against six bacterial strains by determining the minimum inhibitory concentration (MIC). Results indicated that the extract effectively inhibited the growth of both Gram-positive and Gram-negative bacteria. The tested strains included Gram-positive *B. subtilis* and *Staphylococcus aureus*. as well as Gram-negative *E. coli*. *P. aeruginosa*. *K.*

pneumoniae. and *Listeria monocytogenes* (Rajaei *et al*.. 2021). Antibacterial substances present in the aqueous extract of *C. zeylanicum* (Dhakar and Agarwal. 2025).

This study highlights distinct susceptibility profiles of *P. aeruginosa* and *B. subtilis*. demonstrating the importance of antibiotic selection in post-craniotomy infection management. While conventional antibiotics remain the cornerstone of treatment. the antimicrobial potential of plant extracts. particularly *C. zeylanicum.* encourages exploration of natural compounds. These findings contribute to a growing body of evidence promoting integrated antimicrobial strategies to improve surgical outcomes.

4 CONCLUSION

Sometimes. antibiotics can't tell the difference between harmful and harmless bacteria. and they end up affecting the normal microflora in the body. Some harmful strains develop resistance to the antibiotics given. which then harm other sensitive and good bacteria. So. in this study. other factors like plant extracts were used to see how effective they are against *P. aeruginosa* and *B. subtilis*. It was observed that *P. aeruginosa* shows resistance to some antibiotics. but the harmful strains were somewhat sensitive to the plant extracts. The study concluded that to reduce and control the growth of harmful bacteria. there should be more use of alternative antibacterial treatments instead of antibiotics.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All procedures performed in studies involving human participants were in accordance with the ethical standards of institutional and/or research committee and with the 1975 Declaration of Helsinki. as revised in 2013.

TRIAL

No human trial done.

CONSENT FOR PUBLICATION

All authors approved the manuscript. There is no conflict of interest among authors.

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DATA AVAILABILITY

The sequences are deposited in NCBI. and Accession numbers are provided. Accession numbers of bacterial strains of *P. aeruginosa* and *B. subtilis* are given in Table 1.

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Authors' Contribution

All authors contributed equally to the development of this article.

Data availability

All datasets relevant to this study's findings are fully available within the article.

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