Science World Journal Vol. 19(No 4) 2024 https://dx.doi.org/10.4314/swj.v19i4.39
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

Full Length Research Article

# BIOINFORMATICS-DRIVEN ANALYSIS AND ANTIBIOTIC RESISTANCE PROFILING OF *ENTEROBACTER* SPECIES ISOLATED FROM ACNE LESIONS

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**ABSTRACT**
This study characterised *Enterobacter* sp**.** isolated from individuals with acne, focusing on their biochemical, antibiotic resistance, and molecular profiles. Using standard morphological, biochemical, and molecular methods, the isolates exhibited typical *Enterobacter* traits, including rod-shaped morphology and positive to catalase and oxidase reactions. The isolates were highly resistant to beta-lactam antibiotics, particularly ampicillin (85%) and ceftazidime (76.7%); while susceptibility to ciprofloxacin remained high (87.3%). Molecular identification using 16S rRNA sequencing, confirmed two isolates as *Enterobacter hormaechei*. Phylogenetic analysis of the isolates using MEGA software indicated a close relationship with clinical strains, suggesting clinical reservoirs of resistant bacteria. RAST subsystem analysis revealed the presence of multiple resistance genes, including those for beta-lactamases (CTX-M, OXA, AmpC) and fluoroquinolone resistance (gyrA, parC). These findings highlight the multidrug-resistant nature of *Enterobacter hormaechei*, emphasizing their role in the spread of antimicrobial resistance in the clinical settings and underscoring the need for ongoing surveillance and management.

**Keywords:** Bioinformatics, Genomics, Phylogenetic analysis, *Enterobacter hormaechei*, RAST software, Antibiotic Resistance.

## INTRODUCTION

Acne vulgaris (or simply acne) is a frequent disorder of the skin diagnosed and treated by dermatologists and skin therapists alike (Rathi, 2011; Knutsen-Larson *et al*., 2012). It is a multifactorial pleomorphic disorder of the pilosebaceous follicles of the skin characterized by non-inflammatory and inflammatory lesions (Dhillon and Varshney, 2013; Patel *et al*., 2015; Nelson *et al*., 2016). Acne is also referred to as a skin disease caused by bacterial induction (Nasri *et al*., 2015; Polugari *et al*., 2016). Acne usually occurs on the face, chest, neck, upper back and upper arm of the body due to presence of largest oil glands (Zandi *et al*., 2011; Polugari *et al*., 2016; Nosa *et al*., 2023).

The genus *Enterobacter* consists of facultatively anaerobic, Gram-negative bacilli that are approximately 2 µm in length and motile due to peritrichous flagella. They belong to the family Enterobacteriaceae. Initially described in 1960, the taxonomy of the genus has undergone several changes over the past five decades (Davin-Regli *et al*., 2019).

*Enterobacter* species are found in a wide range of environmental settings, including soil and water, and are either endophytic or considered phytopathogens in various plant species. Some species are also commonly associated with bioprocessing and metabolic engineering applications (Singh *et al*., 2018). Additionally, *Enterobacter* species are naturally occurring members of the gut microbiota in both humans and animals. However, only specific subspecies/species have been implicated in hospital-acquired infections and outbreaks (Akbari *et al*., 2016).

As part of the ESKAPE group, *Enterobacter* species play a major role in antibiotic-resistant nosocomial infections (Akbari *et al*., 2016; Wu *et al*., 2018). Among the species most frequently isolated in clinical infections are *Enterobacter aerogenes*, *E. cloacae* and *E. hormaechei*, particularly among immunocompromised patients and those under medical care units (Wu *et al*., 2018). These species have demonstrated an ability to adapt to antimicrobial treatments, emerging as opportunistic pathogens. Since the mid-1990s, Europe has witnessed several hospital outbreaks involving these bacteria, driven by the widespread use of broad-spectrum antibiotics, which has contributed to the spread of resistant strains (Anastay *et al*., 2013)

Antibiotics (both oral and topical) have been used as therapeutics for treatment of acne along with the use of benzoyl peroxide, hormonal therapies and topical retinoid (Rathi, 2011; Knutsen-Larson *et al*., 2012, Lynn *et al*., 2016). Long term use of antibiotics is proving not effective owing to increasing antibiotic resistance (Zandi *et al*., 2011; Jonczyk-Matysiak *et al*., 2017). Resistance to antibiotics is a multifactorial process, which includes host characteristics, the precise nature of the reaction of the bacterium to antibiotics, environmental factors and the antibacterial usage (Kumar *et al*., 2007).

*Enterobacter* species are often linked to multidrug resistance (MDR), primarily because of their capacity to adapt to hospital environments and acquire mobile genetic elements that carry resistance and virulence genes (Davin-Regli *et al*., 2016). They exhibit intrinsic resistance to antibiotics such as ampicillin, amoxicillin, first-generation cephalosporins, and cefoxitin, owing to the production of a constitutive AmpC β-lactamase. Additionally, extended-spectrum β-lactamase (ESBL) production has been observed in these organisms, making treatment more challenging (Masi *et al*., 2017). The mechanisms of antibiotic resistance, the regulation of resistance genes, and their clinical implications have been the focus of extensive research (Dam *et al*., 2018). The increasing prevalence of *Enterobacter* species as a resistant pathogen is a significant public health issue, especially given the lack of new antibiotics effective against Gram-negative bacteria (Dam *et al*., 2018).

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Human Genome project is a recent example, whereby computational tools are helping such methods to record and interpret billions of basic pairs of the human DNA system by analysing huge amount of genetic data (Singh and Verma, 2021). Software tools are needed to interpret biological data, which can be developed by people associated with interdisciplinary science and technical fields. However, to achieve best use of the bioinformatics, software developers and biologists must be able to exchange and understand each other's opinion on requisite knowledge in areas such as statistics, logic and programming, genomics, genetics, and molecular biology.

Computational methods play a vital role in modern microbiological research, particularly in analysing genomic sequences and understanding the evolutionary relationships of pathogens. In this study, the Basic Local Alignment Search Tool (BLAST) was employed to compare the 16S rRNA gene sequences of isolated *Enterobacter* species against existing sequences in the NCBI database. This tool enables the identification of closely related bacterial strains by assessing sequence similarities and is essential for confirming species identification. Previous studies, such as those conducted by Cheng *et al*. (2020) and Watanabe *et al*. (2019) had successfully utilised BLAST for accurate species identification of clinical isolates, underscoring its significance in microbiological research.

Additionally, bioinformatics tools such as RAST (Rapid Annotation using Subsystem Technology) were used for functional annotation of the genomic data, identifying resistance genes such as AmpC and CTX-M, which confer resistance to beta-lactam antibiotics. These computational analyses provide crucial insights into the genetic diversity of *Enterobacter species* and their potential resistance mechanisms, supporting the development of effective treatment strategies. This study integrates microbiological techniques with advanced computational methods to elucidate the characteristics of *Enterobacter species* isolated from acne. Deploying these bioinformatics tools, we aim to contribute to genomic surveillance efforts and enhance our understanding of the molecular mechanisms underlying antibiotic resistance in this clinically significant group of pathogens. Utilising bioinformatics tools, including Basic Local Alignment tool (BLAST) for sequence alignment and MEGA for phylogenetic tree construction, we aimed to identify resistance patterns and control the spread of these pathogens among infected individuals.

## MATERIALS AND METHODS

### Collection of samples

Sterile cotton swabs were moistened with normal saline (excess of the fluid was squeezed out by pressing the swab against the side of the tube). Acne on the faces of 12 volunteered male and female individuals (Age 18 and above) were identified. Firm pressure was applied on each acne using the fingers in sterile gloves to release the pus. Sterile cotton swabs (moistened with normal saline) were used to aseptically obtain the pus. The samples were taken for analysis at Microbiology Laboratory in the Department of Biological Sciences at Glorious Vision University, Ogwa, Esan West Local Government Area of Edo State, Nigeria. It is located at latitude 6˚30 20. 16" North, and longitude 6˚12 30. 24" East.

### Bacterial isolation and biochemical characterization

The samples were inoculated on Nutrient Agar (NA) plates using the streak plate method and incubated aerobically at 37˚C for 24 h (Microbiology Society, 2016). Visible discrete colonies were subcultured to obtain pure isolates. Colonial morphology of the pure isolates was recorded, then subjected to Gram reaction and biochemical tests (which included catalase, oxidase, coagulase, indole, sugar fermentation) as outlined by Leboffe and Pierce (2008).

### Antibiotic Susceptibility Testing

All the confirmed bacterial isolates were subjected to antibiotic susceptibility testing using agar disk diffusion method. Bacterial inocula were prepared by suspending 24 hours old bacterial culture in 3 ml sterile normal saline and the turbidity was adjusted to 0.5 McFarland standards (Moges *et al*., 2014). Each inoculum suspension was spread evenly on a Mueller-Hinton Agar plate surface with a sterile swab stick. Antibiotics (which included Tetracycline, Ciprofloxacin, Chloramphenicol, Ampicillin, Ceftazidime, Trimethoprim-sulfamethoxazole and Gentamicin) were aseptically placed on the seeded MHA plates. The plates were incubated at 37°C for 24 hr. The zones of inhibition were measured to the nearest millimeter (mm) and compared with the susceptibility breakpoints of Clinical and Laboratory Standards Institute (CLSI, 2019).

### Genomics analysis of *Enterobacter* species

DNA extraction Genomic DNA was extracted from the cultures using Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, Catalogue No. D6005)

### Amplification of 16S rRNA gene

The 16S target region was amplified using OneTag® Quick-Load 2X Master Mix (NEB, Catalogue No. M0486) with the primers presented in Table 1.

**Table 1:** 16S Primer sequences

| Name of Primer | Target | Sequence (5' to 3') | References |
|---|---|---|---|
| 16S-27F | 16S rRNA sequence | AGAGTTTGATCMTGGCTCAG | (Frank *et al*., 2008) |
| 16S-1492R | 16S rRNA sequence | CGGTTACCTTGTTACGACTT | (Vanhee *et al*., 2024) |

### Detection of amplified product by agarose gel electrophoresis

The PCR products were electrophoresed on an agarose gel and the gel bands were extracted using the Zymoclean™ Gel DNA Recovery Kit (Zymo Research, Catalog No. D4001). The extracted DNA fragments were sequenced in both the forward and reverse directions using the Brilliant Dye™ Terminator Cycle Sequencing Kit V3.1 (Nimagen, Catalog No. BRD3-100/1000). Following sequencing, the fragments were purified using the ZR-96 DNA Sequencing Clean-up Kit™ (Zymo Research, Catalog No. D4050). The purified fragments were then analyzed using the ABI 3500xl Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific) for each reaction in all samples.

### Bioinformatics analysis

Determination of the 16S rRNA gene sequence was done using the BLAST- NCBI database to evaluate the closely related bacterial strains. The gene sequence was submitted to NCBI database with

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the ascension number (NR) and a total of fourteen sequences were subjected to multiple sequence alignment using Clustal W program. For the phylogenetic analysis, MEGA 11 software was utilised, which enabled the construction of evolutionary trees to understand the genetic relationships between the isolates. The RAST server was used to annotate genomic sequences and identify resistance genes such as AmpC and CTX-M. The 16S rRNA sequences from the *Enterobacter* isolates were aligned using Clustal W, for the detection of conserved regions and mutations associated with antibiotic resistance. Phylogenetic trees were constructed using the Maximum Likelihood method in MEGA.

## RESULTS

**Table 2:** Cultural and Morphological Characteristics of Bacterial Isolates Facial Acne

| Isolate code | Shape | Colour | Elevation | Surface | Margin | Texture |
|---|---|---|---|---|---|---|
| NB1 | Rod | Nonpigmented | Flat | Rough | Irregular | opaque |
| NB5 | Rod | Nonpigmented | Flat | Rough | Irregular | opaque |

**Table 3:** Biochemical Characteristics of Bacterial Isolates from Facial Acne

| Isolate Code | Gram Reaction | Cellular Morphology | Catalase | Oxidase | Methyl Red | Voges Proskauer | Coagulase | Indole | Glucose | Lactose | Maltose | Sucrose | Mannitol | Inference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NB1 | -ve | R | +ve | -ve | +ve | +ve | -ve | -ve | A | +ve | +ve | AG | +ve | *Enterobacter* sp. |
| NB5 | -ve | R | +ve | -ve | +ve | +ve | -ve | -ve | A | +ve | +ve | A | +ve | *Enterobacter* sp. |

+ve = Positive, -ve = Negative, A = Acid Production, G = Gas Production, AG = Acid and Gas Production, R= Rod

**Table 4:** Antibiotic Resistance Profiles of the *Enterobacter* sp.

| Classes of Antibiotics used | Antibiotics | Resistant (%) | Susceptible (%) | Intermediate (%) |
|---|---|---|---|---|
| **Beta-Lactam** | AP | 85 | 2 | 13 |
| | CAZ | 76.7 | 1.2 | 22.1 |
| **Aminoglycosides** | AK | 6.1 | 62.5 | 31.4 |
| | GM | 11.2 | 5.9 | 82.9 |
| | T | 70.8 | 1.74 | 27.46 |
| **Fluoroquinolone** | CIP | 1.1 | 87.3 | 11.6 |
| **Anti-metabolites** | TS | 76.8 | 12.3 | 10.9 |
| **Phenicol** | C | 38.9 | 39.5 | 21.6 |

Legend: AP = Ampicillin, CAZ = Ceftazidime, , GM = Gentamicin, CIP = Ciprofloxacin, T= Tetracycline, TS = Co-trimoxazole and C = Chloramphenicol

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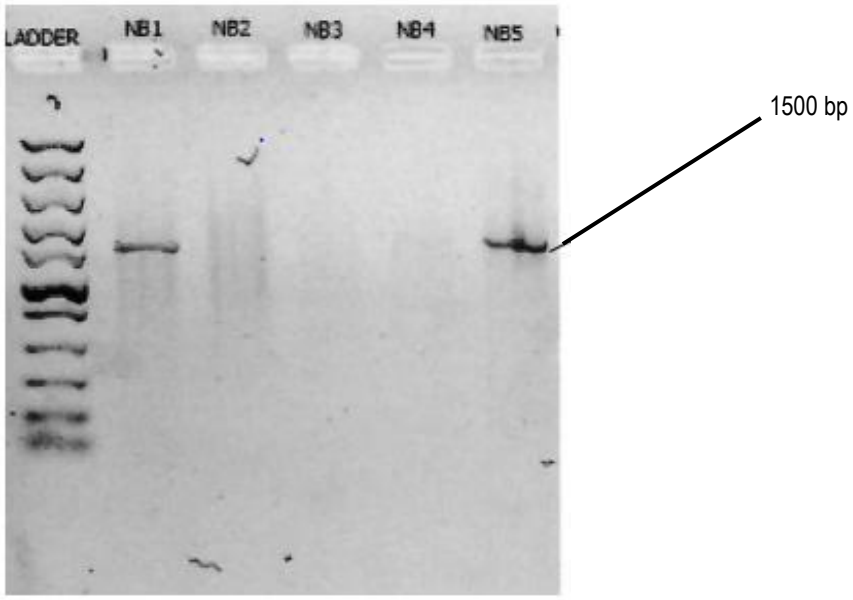


**Figure 1:** Agarose gel electrophoretogram of PCR products of 16s rRNA of *Enterobacter* sp. Keys: Ladder = DNA marker; Lanes NB1 and NB5 = positive (1500bp); Lanes NB2, NB3, and NB4 = negative

Table 5 presents the BLAST results for the 16S rRNA molecular sequencing of the bacterial isolates NB1 and NB5. Both isolates were identified as *Enterobacter hormaechei,* with high percentage identities, confirming their classification

**Table 5:** Results for 16S rRNA sequencing using BLAST

| Isolate code | GenBank Accession | Percentage ID | Predicted Organism |
|---|---|---|---|
| NB1 | MT705739.1 | 83.97 | *Enterobacter hormaechei* |
| NB5 | MT539386.1 | 97.24 | *Enterobacter hormaechei* |

**Phylogenetic Analysis**

Figure 2 illustrates the phylogenetic relationship of *Enterobacter hormaechei* strain kamsi.L1 (NB1) based on a partial sequence of its 16S ribosomal RNA gene. It presents a phylogenetic tree where different branches represent various bacterial strains or species, with branch lengths indicating genetic divergence. The placement of strain kamsi.L1 signifies its genetic relatedness to other *Enterobacter hormaechei* strains and potentially other *Enterobacter* species.

Figure 3 depicts the phylogenetic relationship of *Enterobacter hormaechei* strain EN-314T (NB5) based on a partial sequence of its 16S ribosomal RNA gene. It shows a phylogenetic tree with branches representing various bacterial strains, where branch lengths indicate genetic divergence. The position of strain EN-314T highlights its genetic similarities and differences with other *Enterobacter hormaechei* strains and possibly related species.

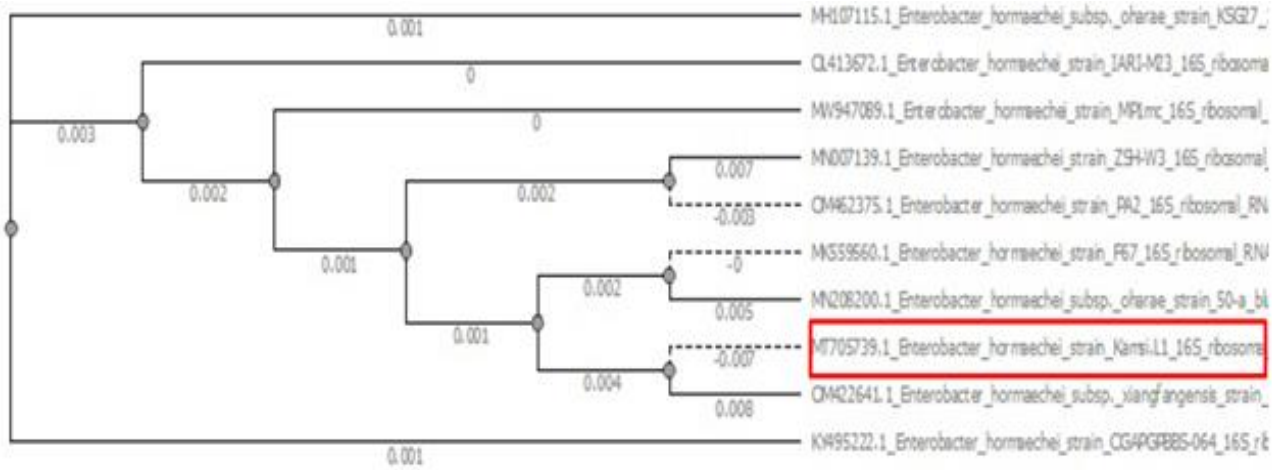


**Figure 2:** The phylogenic relationship of *Enterobacter hormaechei* strain kamsi.L1 (NB1) 16S ribosomal RNA gene

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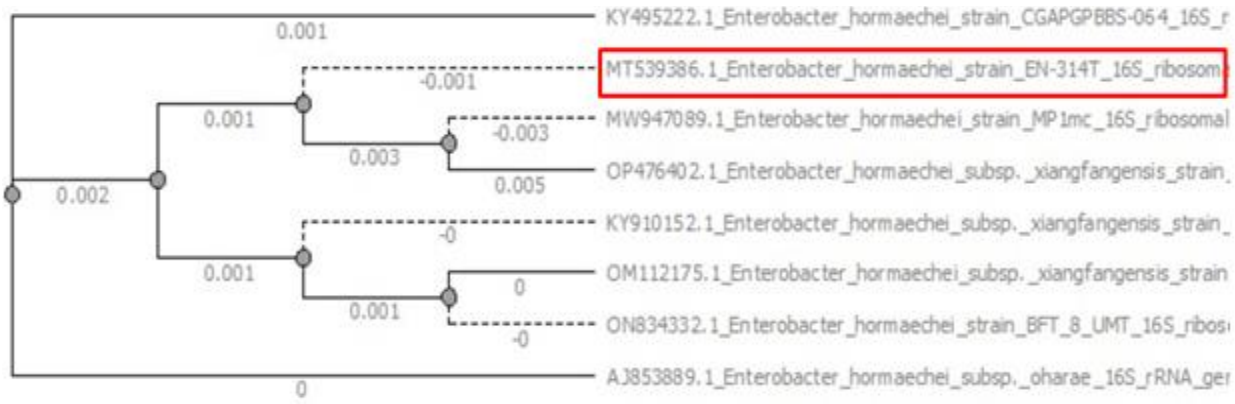


**Figure 3:** The phylogenic relationship of *Enterobacter hormaechei* strain EN-314T (NB5) 16S ribosomal RNA gene

**The Subsystem Features of *Enterobacter* sp. using RAST Software**

Figure 4 illustrates the subsystem features of *Enterobacter* sp., emphasizing its multidrug resistance capabilities. The figure provides a visual representation of the various genetic elements and pathways associated with the organism's resistance mechanisms. The subsystems include genes responsible for the production of enzymes that degrade antibiotics, such as beta-lactamases, as well as those involved in efflux pumps that expel antimicrobial agents from the bacterial cell. Additionally, the figure depict regulatory pathways that control the expression of these resistance genes.

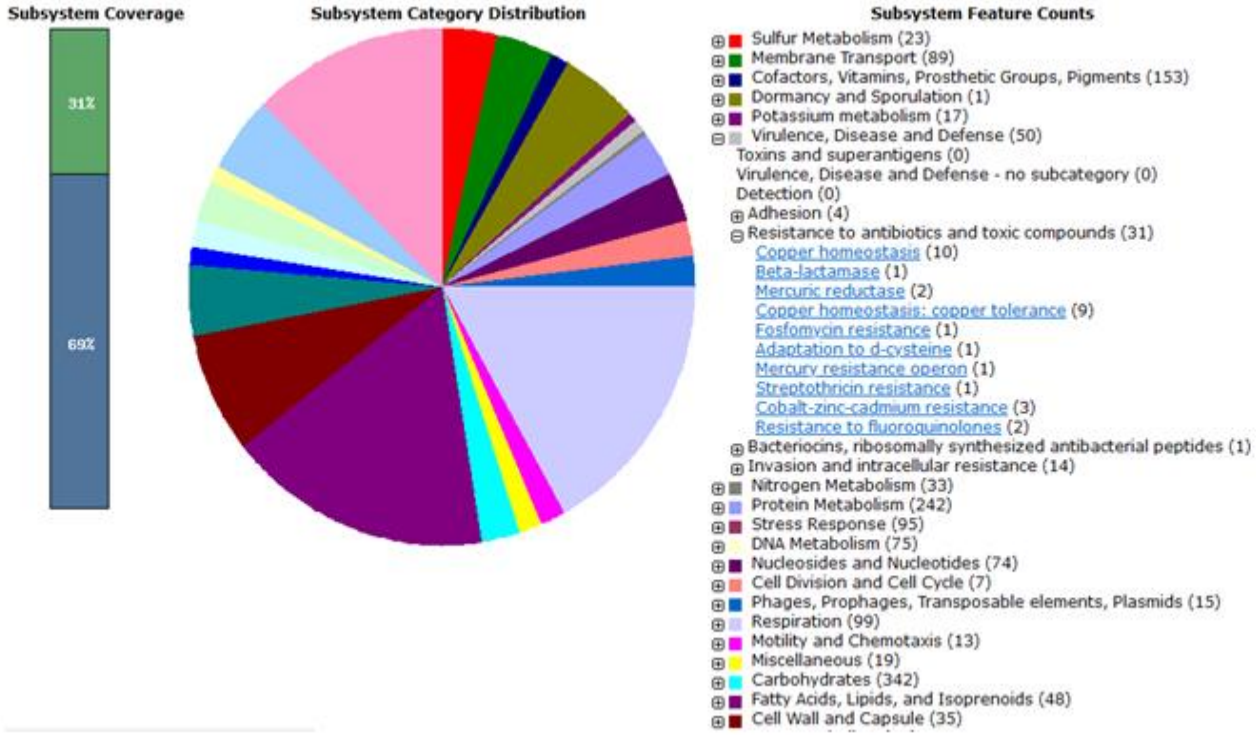


**Figure 4:** Subsystem features of *Enterobacter* sp. indicating its multidrug resistance.

Table 6, which lists the subsystem features of *Enterobacter* sp.'s resistance to Beta-Lactam antibiotics using the RAST software, highlights various beta-lactamase genes associated with antibiotic resistance mechanisms. These genes encode enzymes that degrade beta-lactam antibiotics, making them ineffective. Key genes include BLc, which encodes Beta-lactamase class C, and CTX, which represents the CTX-M-16 enzyme, an extended-spectrum beta-lactamase (ESBL). Other important genes include BLR, a negative regulator of beta-lactamase expression; BlaR and BlaI, which control beta-lactamase expression; and OXA, which encodes an enzyme that provides resistance to carbapenems and oxacillin.

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**Table 6:** Subsystem features of *Enterobacter* sp. Resistance to Beta Lactam Antibiotics Using RAST Software

| Beta-lactamase Genes | Full name |
|---|---|
| **BLc** | Beta-lactamase class C and other penicillin binding proteins |
| **BLI** | Metal-dependent hydrolases of the beta-lactamase superfamily I |
| **BLR** | Negative regulator of beta-lactamase expression |
| **BI** | Beta-lactamase |
| **BLII** | Metal-dependent hydrolases of the beta-lactamase superfamily II |
| **BLIII** | Metal-dependent hydrolases of the beta-lactamase superfamily III |
| **BLA** | Beta-lactamase class A |
| **CTX** | Beta-lactamase CTX-M-16 |
| **BlaR** | Regulatory protein BlaR1 |
| **BlaI** | Beta-lactamase repressor BlaI |
| **BLD** | Beta-lactamase class D |
| **OXA** | Beta-lactamase OXA-18 precursor (EC 3.5.2.6) |
| **AmpS** | Beta-lactamase AmpS |
| **PSE2** | Beta-lactamase PSE-2 precursor (EC 3.5.2.6) |
| **YbxI** | Probable beta-lactamase ybxI precursor (EC 3.5.2.6) |
| **CS** | Beta-lactamase (Cephalosporinase) |

Table 7 outlines the subsystem features of *Enterobacter* sp.'s resistance to fluoroquinolones, highlighting specific genes that contribute to this resistance. The parC gene encodes for Topoisomerase IV subunit A, while parE encodes for Topoisomerase IV subunit B. Both enzymes play critical roles in DNA replication, and mutations in these genes can reduce susceptibility to fluoroquinolones, which target and inhibit their function. The gyrA gene encodes for DNA gyrase subunit A, and gyrB encodes for DNA gyrase subunit B; mutations in these genes are also commonly associated with fluoroquinolone resistance as they alter the binding sites for these antibiotics. Additionally, the presence of the Lde gene, which encodes an efflux pump, indicates a mechanism of resistance that allows the bacteria to actively expel fluoroquinolones, further reducing their effectiveness.

**Table 7:** Subsystem features of *Enterobacter* sp. Resistance to fluoroquinolones using RAST Software

| Genes | Full name |
|---|---|
| parC | Topoisomerase IV subunit A (EC 5.99.1.-) |
| Pare | Topoisomerase IV subunit B (EC 5.99.1.-) |
| gyrA | DNA gyrase subunit A (EC 5.99.1.3) |
| gyrB | DNA gyrase subunit B (EC 5.99.1.3) |
| Lde | Efflux pump Lde |

## DISCUSSION

The findings of this study provide an in-depth characterisation of *Enterobacter* sp**.** isolated from facial acne, demonstrating their morphological, biochemical, molecular, and antibiotic resistance properties. This is consistent with previously documented characteristics of *Enterobacter* sp**.**, particularly *Enterobacter hormaechei*, which is increasingly recognized as both an environmental contaminant and a nosocomial pathogen (Jacoby, 2018). The results also highlight the multidrug-resistant nature of these isolates, which aligns with trends observed in clinical isolates from other studies in the region (Igbinosa *et al*., 2017).

The morphological assessment of *Enterobacter* sp**.** in this study shows rod-shaped, non-pigmented bacteria with rough surfaces and irregular margins. These characteristics are consistent with findings from several studies on *Enterobacter* sp**.**, including research by Nwachukwu *et al*. (2019), which documented similar colonial morphology for *Enterobacter* species isolated from water sources. Furthermore, the biochemical tests demonstrated that the isolates were catalase-positive and oxidase-negative, which are standard biochemical traits of *Enterobacter* species (Davidson *et al*., 2019). The ability of the isolates to ferment glucose, lactose, and mannitol with acid and gas production further supports their classification as *Enterobacter hormaechei*. These findings are consistent with molecular studies showing similar biochemical profiles in clinical *Enterobacter* isolates (Awoniyi *et al*., 2021).

The antibiotic resistance profiles of *Enterobacter* sp**.** isolates reveal a significant resistance to beta-lactam antibiotics, particularly ampicillin (85%) and ceftazidime (76.7%), which aligns with numerous studies highlighting the prevalence of beta-lactamase-producing *Enterobacter* species in both clinical and environmental samples (Hernando-Amado *et al*., 2019). The high resistance to beta-lactams can be attributed to the presence of beta-lactamase genes, as identified in the RAST analysis, including AmpC, CTX-M, and OXA types, which confer resistance to penicillins and cephalosporins (Shaikh *et al*., 2015).

The resistance to co-trimoxazole (76.8%) and tetracycline (70.8%) observed in this study is consistent with previous findings in Nigeria, where environmental *Enterobacter* isolates were found to exhibit high resistance rates to these antibiotics (Akpan *et al*., 2019). The global overuse of these antibiotics in agriculture and human medicine has likely contributed to the selection of resistant strains in clinical samples (Laxminarayan *et al*., 2020). On the contrary, the high susceptibility to ciprofloxacin (87.3%) suggests that fluoroquinolones remain an effective treatment option for

*Enterobacter* infections, although resistance has been emerging (Hawkey *et al*., 2018).

Molecular analysis through 16S rRNA sequencing confirmed the identity of the isolates as *Enterobacter hormaechei* with high percentage identity, supporting their classification based on biochemical and morphological characteristics. The use of 16S rRNA sequencing for bacterial identification is well-established, and this technique has been widely applied in previous studies to characterize clinical and environmental isolates of *Enterobacter* sp**.** (Chen *et al*., 2019). The phylogenetic analysis demonstrated close relationships between the isolates and other *E. hormaechei* strains from GenBank, which further substantiates the identification.

Interestingly, the high identity score (97.24%) for isolate NB5 matches findings from similar studies, where *E. hormaechei* was commonly isolated from both clinical and environmental samples (Peirano *et al.,* 2018). This close genetic relationship between environmental and clinical strains suggests a potential for environmental sources to act as reservoirs of antibiotic-resistant pathogens that could contribute to human infections. Studies such as those by Hernandez *et al*. (2019) have demonstrated that environmental *Enterobacter* species can acquire resistance genes and transfer them to clinical isolates, which underscores the public health implications of environmental contamination.

The RAST analysis revealed multiple resistance genes associated with beta-lactamase production, including AmpC, CTX-M, and OXA, which are well-known to mediate resistance to beta-lactam antibiotics (Shaikh *et al*., 2015). These findings align with recent research documenting the prevalence of these genes in environmental *Enterobacter* isolates (Blaak *et al.,* 2015). The detection of CTX-M-16 is particularly notable, as this gene is associated with extended-spectrum beta-lactamase (ESBL) production, a significant concern in both clinical and environmental microbiology due to its ability to hydrolyze a broad spectrum of beta-lactams, including third-generation cephalosporins (Bush and Bradford, 2020).

Furthermore, the detection of fluoroquinolone resistance genes such as gyrA and parC in the isolates suggests that these bacteria have the potential to develop resistance to fluoroquinolones, despite the current high susceptibility to ciprofloxacin observed in the antibiotic resistance profile (Kaur *et al*., 2019). The presence of efflux pump genes, such as *Lde*, which contributes to multidrug resistance by actively expelling antibiotics from bacterial cells, also supports the multidrug-resistant nature of the isolates. This observation is consistent with studies reporting that efflux pumps play a significant role in the resistance mechanisms of environmental and clinical *Enterobacter* species (Li *et al*., 2015).

**Conclusion**

This study highlights the multidrug-resistant nature of *Enterobacter hormaechei* isolated from environmental samples. The high levels of resistance to beta-lactams and other antibiotics underline the public health risks associated with the dissemination of antibiotic-resistant bacteria in the environment. The detection of resistance genes through RAST analysis further corroborates the phenotypic resistance patterns, and the close phylogenetic relationship between environmental and clinical strains suggests that these sources may act as reservoirs of resistant pathogens. These findings underscore the importance of continuous surveillance and the development of strategies to mitigate the spread of antibiotic resistance in both clinical and environmental settings.

**Conflict of Interests**

Authors declare that there is no conflict of interest

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