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# Molecular identification, antibiotic susceptibility, and biofilm formation of airborne bacteria

Malik Asif Hussain[1], Mubashir Zafar[2], Yusuf Saleem Khan[3], Kamaleldin B. Said[1], Sadaf Anwar[4], Mohd Saeed[5], Mohammad A. Abdulhakeem[5], Mejdi Snoussi[5,6,7] and Mohd Adnan Kausar[4*]

**Abstract**

Pathogenic bacterial communities present in urban green spaces significantly affect human health, particularly for immunocompromised populations. The diverse range of pathogenic bacteria found in these areas poses substantial management challenges because of their high prevalence of antibiotic resistance, which can be life-threatening, particularly for immunocompromised individuals, including older adults and children. This study identified airborne bacterial species from 14 natural parks in the Hail region of the Kingdom of Saudi Arabia. Bacterial colonies isolated on blood agar plates were purified and characterised based on their morphological traits and their ability to secrete various virulence factors. A total of 28 distinct airborne bacterial species were isolated and purified. Antibiotic susceptibility tests revealed high resistance to fosfomycin (41.17%), ampicillin (17.64%), tetracycline (17.64%), and gentamicin (11.76%). Biofilm formation was evaluated qualitatively by slime production and quantitatively by crystal violet technique. The results revealed that 41.17% of the tested strains were non biofilm producers on polystyrene surfaces, 17.64% were weak biofilm formers, and 23.52% exhibited moderate biofilm formation. Notably, six strains exhibited strong biofilm-forming capabilities. Additionally, two bacteria from the *Arthrobacter* genus (*A. crystallopoietes* and *A. saudimassiliensis*) were identified. These findings provide valuable insights into the microbial composition of natural parks in the Hail region and highlight effective management strategies to mitigate health risks.

**Keywords** Molecular identification, Antibiotic susceptibility, Biofilm formation, Airborne bacteria

*Correspondence:
Mohd Adnan Kausar
ma.kausar@uoh.edu.sa
[1]Department of Pathology, College of Medicine, University of Ha'il, 55476 Hail, Saudi Arabia
[2]Department of Family and Community Medicine, College of Medicine, University of Ha'il, 55476 Ha'il, Saudi Arabia
[3]Department of Anatomy, College of Medicine, University of Ha'il, 55476 Hail, Saudi Arabia
[4]Department of Biochemistry, College of Medicine, University of Ha'il, 55476 Hail, Saudi Arabia
[5]Department of Biology, College of Science, University of Hail, 55476 Hail, Saudi Arabia
[6]Laboratory of Genetics, Bioaffiliationersity and Valorization of Bio-Resources (LR11ES41), Higher Institute of Biotechnology of Monastir, University of Monastir, Avenue Tahar Haddad, BP74, 5000 Monastir, Tunisia
[7]Medical and Diagnostic Research Center, University of Ha'il, 55476 Hail, Saudi Arabia

## Introduction

Exposure to biological agents, including microbes, is associated with numerous substantial public health problems, such as infectious diseases, allergies, and acute toxic effects (Kumar et al. 2021). Air, being an essential component for the survival of living organisms on Earth, serves as a critical medium for the transmission of microorganisms and their components. Diseases can spread through both direct and indirect contact between individuals, with air acting as a common indirect medium for cross-infection. Skin flakes, aerosols, and spores can remain suspended in the air for extended periods and travel over long distances passively (Schmidt 2019; Chen et al. 2020). In both rural and urban settings, bacteria constitute a major component of the microbial diversity and abundance in outdoor as bioaerosols. Human-associated airborne bacteria and diseases caused by such bacteria have been the subject of extensive research, with their infection and transmission processes comprehensively reviewed in recent studies (Ruiz-Gil et al. 2020).

The growing prevalence of antibiotic resistance poses a serious public health challenge. Research on global urban microbiomes across 33 countries have discovered antimicrobial resistance genes, emphasizing the need for public health strategies and as well as urban planning (Zhai et al. 2022; Chen et al. 2023). Numerous studies have established a significant link between respiratory infections and microorganisms originating from outdoor environments. To effectively address the rising threat of antibiotic resistance, it is crucial to deepen our understanding of antibiotic-resistant bacteria in urban settings. Among the diverse airborne bacterial populations reported in outdoor environments, the most prevalent phyla include Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes (Mhuireach et al. 2016; Parajuli et al. 2018). Rangaswamy et al. (2013) identified diverse bacterial species present in garden air that could contribute to respiratory conditions. These species primarily included *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., *Salmonella* spp., *Staphylococcus* spp., *Proteus* spp., and *Enterococcus* spp. Seasonal variations were also found to affect bacterial concentrations in urban parks, with higher levels observed during summer and autumn (Rangaswamy et al. 2013). In addition, Mhuireach and co-worker. compared bacterial species in parks as well as parking lots and identified distinct bacterial signatures in parks, with Acidobacteriaceae being particularly abundant (Mhuireach et al. 2016). Studies have reported the prevalence of antibiotic-resistant airborne bacteria in diverse environments, including urban parks and healthcare settings (Robinson et al. 2021; Sajjad et al. 2024). These findings emphasize the need for continued monitoring and assessment of airborne microbial communities.

The formation of biofilms by outdoor bacterial pathogens and the emergence of antibiotic resistance represents significant public health challenge. Biofilms contribute to antibiotic resistance by enhancing the production of efflux pumps and increasing mutation rates (Hall-Stoodley et al. 2005; Jamal et al. 2018). Emerging evidence suggests that extracellular DNA and quorum sensing also play critical roles in biofilm-mediated antibiotic tolerance, as demonstrated in urban environmental isolates (Chen et al. 2024). The close cell-to-cell contact within biofilms facilitates horizontal gene transfer, further accelerating the dissemination of antibiotic resistance genes. Biofilm-embedded bacteria exhibit high tolerance and resilience, making biofilm-associated infections particularly difficult to treat. This is evident in chronic infections caused by bacteria that form biofilms resistant to conventional antibiotic therapies, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* (do Nascimento et al. 2021; Lin et al. 2021; Li and Zhang 2022).

The identification and treatment of outdoor pathogenic bacteria are hindered by various factors, including environmental conditions, technological limitations, and antibiotic resistance development (Cox and Wright 2013). The present study investigated the antibiotic susceptibility and biofilm formation capabilities of culturable bacteria present in the air of 14 natural parks located in the Hail region of the Kingdom of Saudi Arabia.

## Material and methods

### Sampling sites and bacterial isolation and characterization

Bacterial growth was studied in 14 parks in the Hail region of Saudi Arabia (Table 1). Airborne microorganisms were allowed to grow on blood agar Petri dishes exposed to air at the selected sites. Blood agar plates were exposed to air for 30 min at each sampling site to allow for the settling of airborne microorganisms. This duration was chosen based on preliminary studies indicating optimal colony formation without overgrowth (Redhwan et al. 2021). After exposure, the settling plates were carefully sealed without contaminating the media surface. The isolated bacteria were purified on plate count agar medium following overnight incubation at 37 °C. Pure single colonies were then characterised based on morphological and microbiological tests, following previously described protocols (Snoussi et al. 2006). After incubation, the mean number of colonies at each site was determined and expressed as colony-forming units (Redhwan et al. 2021).

All purified isolates were assessed for their ability to produce various exoenzymes, including DNAase, lipase, amylase, caseinase, and lecithinase. For these experiments, PBS agar medium was supplemented with specific substrates: Tween-80 for lipase activity, starch for

**Table 1** Morphological characteristics of 34 bacterial isolates obtained on blood agar plates isolated from 14 natural parks in Hail region

| Site of isolation | Code | Color | Size | Shape |
|---|---|---|---|---|
| Al Muntazah Al Gharbi | 17.1 | Orange | 6–7 | Circular, Convex, entire |
| | 17.2 | Black | 2–3 | Circular, Convex, entire |
| Al Muntazah Al Shargi | 10.3 | Brown | 4–5 | Circular, flat, entire |
| | 10.4 | Brown | 2 | Circular, Convex, entire |
| Al-Wadi | 15.1 | Colorless | 10 | Irregular, flat, undulate |
| | 15.4 | Pink | 6–7 | Circular, convex, entire |
| Sharaf | 26.2 | Pink | 2–3 | Circular, raised, entire |
| | 26.3 | Pinkish | 3–4 | Circular, raised, entire |
| | 26.4 | Black-gray | 7 | Circular, flat, entire |
| Al Shefa | 13.1 | Brown | 3–4 | Circular, flat, entire |
| | 13.3 | White | 5–6 | Circular, flat, entire |
| Al-Masif | 6.1 | Yellow | 2–3 | Circular, convex, entire |
| | 6.2 | Pink | 1–2 | Circular, convex, entire |
| | 6.4 | Brown | 3–4 | Circular, flat, entire |
| Al-Fajr | 3.2 | Pink | 2–3 | Circular, convex, entire |
| | 3.3 | Creamy | 5 | Circular, flat, entire |
| | 3.4 | White | 5 | Circular, convex, entire |
| | 3.5 | Black | 10 | Circular, flat, undulate |
| Al-Nysia | 4.1 | Yellow | 2–3 | Circular, convex, entire |
| | 4.2 | Gray | 1 | Circular, convex, entire |
| Al-Mahatta | 14.1 | Pink | 6–7 | Circular, convex, entire |
| Al-Nugra | 25.4 | Brown | 6–7 | Circular, flat, entire |
| | 25.5 | Pink | 6–7 | Circular, convex, entire |
| Shark Al-Mujamaa | 24.2 | Creamy | 3–4 | Irregular, flat, entire |
| | 24.3 | Yellow | 2–3 | Circular, flat, entire |
| | 24.5 | Red | 3–4 | Circular, convex, entire |
| Al-Khuraymi | 28.1 | Creamy | 5–6 | Circular, flat, entire |
| | 28.4 | Red | 1–2 | Circular, convex, entire |
| Al-Jamiyin | 16.2 | Orange | 5–6 | Circular, convex, entire |
| | 16.5 | Brown | 6–7 | Circular, convex, entire |
| | 16.6 | Red | 2–3 | Circular, convex, entire |
| | 16.11 | Dark creamy | 5 | Circular, convex, entire |
| Al-Rasf | 12.2 | Brown | 4–6 | Circular, flat, entire |
| | 12.4 | Yellowish brown | 2–3 | Circular, low convex, entire |

amylase activity, skim milk powder for caseinase activity, and egg yolk for lecithinase production. A positive result was indicated by the formation of a clear zone around the inoculation spots on Petri dishes after 72 h of incubation at 37 °C. In addition, haemolysin production was evaluated on blood agar supplemented with 5% human blood (Abdulhakeem et al. 2023).

#### Molecular identification of selected isolates

DNA extraction was performed using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). The PCR protocol consisted of the following steps: (1) an initial denaturation at 95 °C for 5 minutes, (2) 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 53 °C for 30 seconds, and extension at 72 °C for 30 seconds, and (3) a final extension at 72 °C for 2 min. The reaction mixture had a total volume of 25 µl, containing 12.5 µl of 2× SuPCR Master Mix (Sugenomics, Cat No: PCR01B0451), 1 µL of each primer targeting the V3 and V4 regions of the 16S rRNA gene (341F: 5′-CCTACGGGNGGCWGCAG-3′ and 805R: 5′-GACTACHVGGGTATCTAATCC-3′), and 8.5 µL of ddH2O. The amplified DNA products were visualised on a 1% agarose gel and subsequently purified using the HighPrep PCR Cleanup Kit (Magbio, Cat No: AC-60050). The purified products were sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific). Sequence analysis was conducted using the ABI 3730XL Genetic Analyzer.

#### Antibiotic susceptibility test

The susceptibility of identified bacteria to various antimicrobial agents was evaluated using the disc diffusion assay on Mueller–Hinton agar. The tested antibiotics (Oxoid, UK) included amikacin (AK, 30 µg), ampicillin (AMP, 10 µg), gentamicin (GEN, 10 µg), tetracycline (TET, 10 µg), kanamycin (KMN, 10 µg), fosfomycin (FOS, 50 µg), norfloxacin (NX, 10 µg), and doxycycline (DOX, 30 µg). The diameter of the inhibition zone surrounding each disk was measured using a 1-mm flat ruler after 24 h of incubation at 37 °C. Antibiotic susceptibility profiles were classified as sensitive, intermediate, or resistant based on the guidelines provided by the Clinical and Laboratory Standards Institute M45 and M100. The data were further analysed using two indices: (i) the multiple antibiotic resistance (MAR) index of the isolates and (ii) the antibiotic resistance index (ARI) of each bacterial population (Abdulhakeem et al. 2023).

#### Biofilm formation test

The ability of the isolated microorganisms to form biofilms was assessed both qualitatively (slime production) and quantitatively (crystal violet technique). For the qualitative slime assay, the capacity of isolated bacteria to produce an exopolysaccharide layer (slime production) was evaluated on Congo red agar. Pigmented colonies were classified as slime producers, whereas unpigmented colonies were considered nonproducers.

For quantitative estimation, biofilm formation was evaluated using 96-well polystyrene microtiter plates, following the protocol described by Toledo-Arana et al. (2001). All bacterial strains were pre-enriched in brain infusion broth supplemented with 0.25% (w/v) glucose. The bacteria were cultured in the microtiter plates overnight at 37 °C, after which adhering bacteria were stained with 1% crystal violet for 15 min. The dye was then solubilised using an ethanol–acetone solution (80:20 v/v), and optical density (OD) was measured spectrophotometrically

at 595 nm (OD595). Based on these readings, bacteria were classified as nonbiofilm forming (–), weak biofilm forming (+), medium biofilm forming (++), or strong biofilm forming (+++). All experiments were performed in triplicate (Toledo-Arana et al. 2001).

## Results

### Bacterial isolation and characterization

The purification and characterisation of 34 bacterial isolates in this study focused on their physical traits and ability to secrete various virulence factors (Table 1). The number of colonies on blood agar ranged from 18 to more than 50 colonies per sample, with diverse morphotypes observed in nearly all samples. Representative growth plates from three parks are displayed in Fig. 1.

Table 1 below presents the data related to the sites of placing culture plates and characteristics of bacterial colonies grown.

All tested microorganisms demonstrated the ability to produce various exoenzymes but at different frequencies (Table 2). All isolates were capable of producing caseinase, lipase, and gelatinase. Eleven strains could hydrolyse DNA on DNA agar medium, whereas one strain isolated from Al Muntazah Al Gharbi (Strain 17.2) was unable to utilise starch as its sole carbon source (Table 2). Furthermore, 27 of the tested strains could not degrade human red blood cells on blood agar. However, five strains isolated from four natural parks of Hail region of Saudi Arabia (Al-Wadi, Al Shefa, Al Jamiyin, and Al-Rasf) exhibited complete haemolytic activity. A detailed enzyme profiling of the isolates are provides in Table 2.

### Bacterial identification

Our analysis identified 34 bacterial isolates based on 16S rRNA sequence results. These strains were classified into 14 genera: *Kocuria* (n = 9), *Bacillus* (n = 5), *Pseudomonas* (n = 2), *Planococcus* (n = 4), *Exiguobacterium* (n = 3), *Arthrobacter* (n = 3), *Mesobacillus* (n = 2), *Planomicrobium* (n = 1), *Sporosarcina* (n = 1), *Metaplanococcus* (n = 1), *Agrococcus* (n = 1), *Staphylococcus* (n = 1), *Peribacillus* (n = 1), and *Brachybacterium* (n = 1). Details of the isolated bacteria and their accession numbers across the various sites are provided in Table 3.

### Antibiotic susceptibility testing

The results of antibiotic susceptibility testing revealed that all tested bacteria exhibited high resistance to fosfomycin (41.17%), ampicillin (17.64%), tetracycline (17.64%), and gentamicin (11.76%). By contrast, all isolates were sensitive to doxycycline and kanamycin. The multiple ARI (MARI) ranged from 0 to 0.411, whereas the ARI ranged from 0 to 0.625. Antibiotic sensitivity data are summarised in Table 4.

### Biofilm formation

Figure 2 and Table 5 summarise the morphotypes observed on Congo red agar and the biofilm-forming capabilities of the tested strains, as assessed using the crystal violet staining technique in 96-well polystyrene plates. Six morphotypes were identified on Congo red agar, including black and nearly black colonies, which were interpreted as slime-producing strains, and red, pink with a red centre, and orange colonies, which were considered non-slime producers. Slime-producing strains accounted for 38.23% of the total tested strains, whereas non-slime producers represented 61.76%. In addition, biofilm formation analysis revealed that 41.17% of the tested strains were non-biofilm producers on polystyrene surfaces, 17.64% were weak biofilm formers, and 23.52% were moderate biofilm formers. Notably, six strains demonstrated strong biofilm-forming capabilities. The findings of this analysis are detailed in Table 5.

Figure 2 below depicts the growth of bacteria on congo red agar.

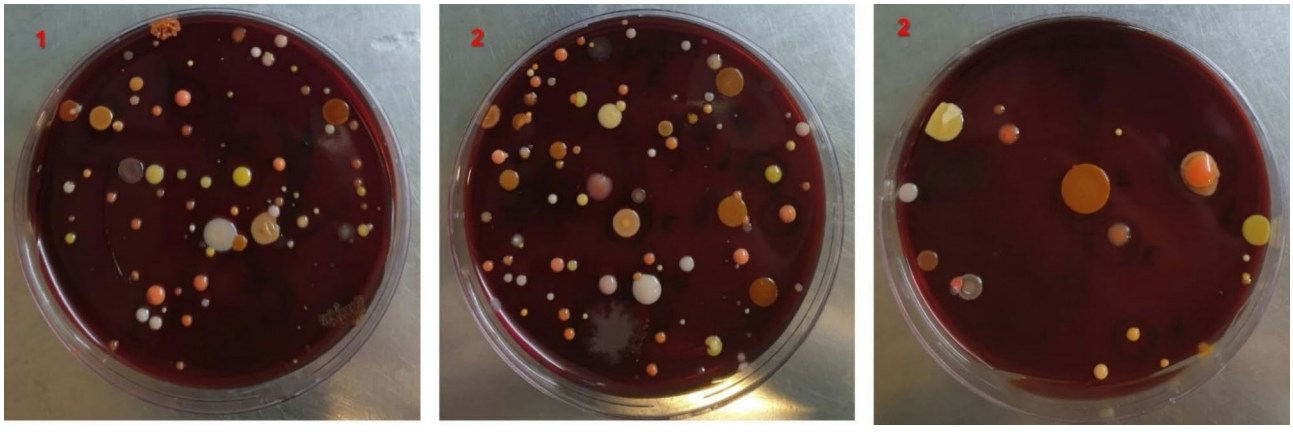


**Fig. 1** Morphological characteristics of bacterial colonies isolated on blood agar from (1) Al-Masif, (2) Al-Fajr, and (3) Al-Mahatta natural parks from Hail region

**Table 2** Exoenzyme profiling of 34 bacterial isolates from 14 natural parks in Hail region

| Site of isolation | Code | Amylase | DNase | Lipase | Caseinase | Gelatinase | Haemolysis |
|---|---|---|---|---|---|---|---|
| Al Muntazah Al Gharbi | 17.1 | + | + | + | + | + | α-Haemolysis |
| | 17.2 | – | – | + | + | + | α-Haemolysis |
| Al Muntazah Al Shargi | 10.3 | + | + | + | + | + | α-Haemolysis |
| | 10.4 | + | – | + | + | + | α-Haemolysis |
| Al-Wadi | 15.1 | + | + | + | + | + | γ-Haemolysis |
| | 15.4 | + | – | + | + | + | β-Haemolysis |
| Sharaf | 26.2 | + | – | + | + | + | α-Haemolysis |
| | 26.3 | + | – | + | + | + | α-Haemolysis |
| | 26.4 | + | + | + | + | + | α-Haemolysis |
| Al Shefa | 13.1 | + | – | + | + | + | β-Haemolysis |
| | 13.3 | + | + | + | + | + | β-Haemolysis |
| Al-Masif | 6.1 | + | – | + | + | + | γ-Haemolysis |
| | 6.2 | + | – | + | + | + | α-Haemolysis |
| | 6.4 | + | + | + | + | + | α-Haemolysis |
| Al-Fajr | 3.2 | + | + | + | + | + | α-Haemolysis |
| | 3.3 | + | – | + | + | + | α-Haemolysis |
| | 3.4 | + | – | + | + | + | α-Haemolysis |
| | 3.5 | + | + | + | + | + | α-Haemolysis |
| Al-Nysia | 4.1 | + | – | + | + | + | α-Haemolysis |
| | 4.2 | + | – | + | + | + | α-Haemolysis |
| Al-Mahatta | 14.1 | + | – | + | + | + | α-Haemolysis |
| Al-Nugra | 25.4 | + | + | + | + | + | α-Haemolysis |
| | 25.5 | + | – | + | + | + | α-Haemolysis |
| Shark Al-Mujamaa | 24.2 | + | – | + | + | + | α-Haemolysis |
| | 24.3 | + | – | + | + | + | α-Haemolysis |
| | 24.5 | + | – | + | + | + | α-Haemolysis |
| Al-Khuraymi | 28.1 | + | – | + | + | + | α-Haemolysis |
| | 28.4 | + | – | + | + | + | α-Haemolysis |
| Al-jamiyin | 16.2 | + | – | + | + | + | β-Haemolysis |
| | 16.5 | + | + | + | + | + | α-Haemolysis |
| | 16.6 | + | – | + | + | + | α-Haemolysis |
| | 16.11 | + | – | + | + | + | α-Haemolysis |
| Al-Rasf | 12.2 | + | – | + | + | + | β-Haemolysis |
| | 12.4 | + | + | + | + | + | α-Haemolysis |

Figure 3 shows the 96-well polystyrene plate analysis for biofilm estimation.

## Discussion

Air is a vital medium for the transfer of pathogenic bacteria, which can have significant detrimental effects on public health. Considering the profound effect of pathogenic microorganisms in outdoor environments, numerous studies have employed metagenomic, proteomic, and metabolomic techniques to identify and quantify microbial constituents in the environment (Dybwad et al. 2012; Singh et al. 2020). However, data accurately describing the concentration of microbiological material in the environment remain limited. In Saudi Arabia, the microbial composition of air exhibits seasonal variations influenced by factors such as wind speed, humidity, and temperature (Al Salameen et al. 2020; Sajjad et al. 2024). This study identified culturable bacteria in the air of 14 natural parks in the Hail region of the Kingdom of Saudi Arabia and assessed their antibiotic susceptibility and biofilm formation capabilities. Twenty-eight different airborne bacterial species were isolated, predominantly belonging to the genera *Kocuria*, *Bacillus*, and *Exiguobacterium*. The complete list of identified bacterial species is provided in Table 3.

Previous studies have explored the isolation and microbial diversity of airborne pathogens across various locations, including shopping malls, urban and nonurban sites, underground subway stations, hospitals, and office buildings (Kim et al. 2011; Dybwad et al. 2014; Zhai et al. 2018; Ziaee et al. 2018; Gohli et al. 2019). In urban areas of Hangzhou (southeastern China), the dominant bacterial genera identified were *Micrococcus*, *Staphylococcus*, *Kocuria*, and *Pseudomonas* (Fang et al. 2016). Similarly, Goudarzi et al. reported *Streptomyces*, *Bacillus*, *Kocuria*, *Corynebacterium*, and *Paenibacillus* as the dominant

**Table 3** 16SrRNA identification and accession number of the tested isolates

| Site of isolation | Code | Bacteria name | Accession number |
|---|---|---|---|
| Al Muntazah Al Gharbi | 17.1 | *Pseudomonas stutzeri* | OR121006 |
| | 17.2 | *Pseudomonas songnenensis* | OR398896 |
| Al Muntazah Al Shargi | 10.3 | *Planococcus ruber* | OR121007 |
| | 10.4 | *Bacillus firmus* | OR398897 |
| Al-Wadi | 15.1 | *Bacillus cereus* | OR176533 |
| | 15.4 | *Kocuria polaris* | OR398898 |
| Sharaf | 26.2 | *Mesobacillus jeotgali* | OR398899 |
| | 26.3 | *Exiguobacterium aestuarii* | OR121008 |
| | 26.4 | *Planococcus massiliensis* | OR121009 |
| Al Shefa | 13.1 | *Exiguobacterium aurantiacum* | OR398900 |
| | 13.3 | *Arthrobacter saudimassiliensis* | OR121010 |
| Al-Masif | 6.1 | *Bacillus licheniformis* | OR121011 |
| | 6.2 | *Kocuria rosea* | OR176534 |
| | 6.4 | *Bacillus cereus* | OR121013 |
| Al-Fajr | 3.2 | *Sporosarcina aquimarina* | OR121012 |
| | 3.3 | *Planomicrobium okeanokoites* | OR121014 |
| | 3.4 | *Arthrobacter crystallopoietes* | OR121015 |
| | 3.5 | *Bacillus gottheilii* | OR121016 |
| Al-Nysia | 4.1 | *Kocuria carniphila* | OR121017 |
| | 4.2 | *Arthrobacter crystallopoietes* | OR398901 |
| Al-Mahatta | 14.1 | *Kocuria himachalensis* | OR121018 |
| Al-Nugra | 25.4 | *Metaplanococcus flavidus* | OR121019 |
| | 25.5 | *Kocuria rosea* | OR121020 |
| Shark Al-Mujamaa | 24.2 | *Kocuria carniphila* | OR121021 |
| | 24.3 | *Agrococcus lahaulensis* | OR121022 |
| | 24.5 | *Kocuria flava* | OR176532 |
| Al-Khuraymi | 28.1 | *Staphylococcus equorum* | OR121023 |
| | 28.4 | *Kocuria turfanensis* | OR121024 |
| Al-Jamiyin | 16.2 | *Exiguobacterium mexicanum* | OR121025 |
| | 16.5 | *Planococcus koreensis* | OR121026 |
| | 16.6 | *Kocuria flava* | OR121027 |
| | 16.11 | *Peribacillus simplex* | OR121028 |
| Al-Rasf | 12.2 | *Exiguobacterium aurantiacum* | OR121029 |
| | 12.4 | *Brachybacterium paraconglomeratum* | OR121030 |

genera isolated using culture-based techniques from Ahvaz (Iran). In the United States, *Streptophyta*, *Bacillus*, *Corynebacterium*, *Pseudomonas*, and *Acinetobacter* were identified as the main genera in retail stores using pyrosequencing techniques (Hoisington et al. 2016). In addition, *Bacillus*, *Micrococcus*, and *Staphylococcus* were reported as dominant genera in underground subway stations through culture-based methods and partial 16S sequencing (Hoisington et al. 2016). The composition of airborne microorganisms depends on factors such as vertical stratification and vegetation complexity in the studied environment (Mhuireach et al. 2016; Robinson et al. 2021; Lu et al. 2024). For example, bacterial composition and diversity in the air are strongly correlated with those found in the soil and phyllosphere (Lu et al. 2024). Furthermore, the same authors observed higher bacterial diversity in the phyllosphere of trees compared to grasses, broadleaf plants, and conifers (Lu et al. 2024).

We identified six species of *Kocuria*: *Kocuria carniphila*, *Kocuria flava*, *Kocuria himachalensis*, *Kocuria polaris*, *Kocuria rosea*, and *Kocuria turfanensis*. Madsen et al. (2023) identified *Kocuria carniphila*, *K. palustris*, and *K. rhizophila* in indoor air samples from homes (Madsen et al. 2023). These Gram-positive, nonencapsulated, non-spore-forming, and catalase-positive bacteria are classified as risk class 2 pathogens and have been implicated in various human complications, including bacteraemia, infective endocarditis, endophthalmitis, peritonitis, skin and soft tissue infections (Ziogou et al. 2024). Notably, *K. marina* and *K. rosea* have been specifically linked to cases of peritonitis (Dotis et al. 2015; Ziogou et al. 2024).

Three species of *Exiguobacterium* were identified: *Exiguobacterium aestuarii*, *Exiguobacterium aurantiacum*, and *Exiguobacterium mexicanum*. Members of the *Exiguobacterium* genus are predominantly isolated from diverse cold and hot biotopes (Vishnivetskaya et al. 2009) and are associated with poorly understood human infections, including skin infections, wounds, and cerebrospinal fluid infections (Tena et al. 2014). We also identified four *Bacillus* strains: *Bacillus cereus*, *Bacillus firmus*, *Bacillus gottheilii*, and *Bacillus licheniformis*. Among these, *B. cereus* is well-documented as an environmental organism that causes various gastric ailments. Moreover, three *Planococcus* species were identified: *Planococcus koreensis*, *Planococcus massiliensis*, and *Planococcus ruber*. The *Planococcus* genus, part of the *Planococcaceae* family, comprises 14 genera, namely *Planococcus*, *Bhargavaea*, *Chryseomicrobium*, *Filibacter*, *Indiicoccus*, *Jeotgalibacillus*, *Kurthi*, *Marinibacillus*, *Paenisporosarcina*, *Planomicrobium*, *Psychrobacillus*, *Savagea*, *Sporosarcina*, and *Ureibacillus* (Adeolu et al. 2016). Members of the *Planococcus* genus are valued for their production of biosurfactants and carotenoids (Waghmode et al. 2020; Moyo et al. 2022). Furthermore, we identified additional microorganisms from the *Planococcaceae* family, including *Mesobacillus jeotgali*, *Peribacillus simplex*, *Planomicrobium okeanokoites*, and *Sporosarcina aquimarina* (Gupta and Patel 2019).

In this study, *Brachybacterium paraconglomeratum*, a member of the *Brachybacterium* genus, was isolated from Al-Rasf natural park. This bacterium, which belongs to the phylum Actinobacteria, was first described by Takeuchi and co workers in 1995 (Takeuchi et al. 1995). The colonies exhibited circular, entire, low convex, smooth, opaque, and yellowish-brown pigmentation (Mandragutti and Sudhakar 2023). In addition, we identified two bacteria belonging to the *Arthrobacter* genus: *Arthrobacter crystallopoietes* and *Arthrobacter saudimassiliensis*.

**Table 4** Antibiotic susceptibility patterns, MARI, and ARI calculation for all identified microorganisms

| Site of isolation | Code | AK | GEN | AMP | TET | DOX | KMN | NX | FOS | (ARI) |
|---|---|---|---|---|---|---|---|---|---|---|
| Al Muntazah Al Gharbi | 17.1 | S | S | R | S | S | S | S | I | 1/8 = 0.125 |
| | 17.2 | S | S | S | S | S | S | S | S | 0/8 = 0 |
| Al Muntazah Al Shargi | 10.3 | S | S | S | I | S | S | S | R | 1/8 = 0.125 |
| | 10.4 | S | S | S | I | S | S | I | S | 0/8 = 0 |
| Al-Wadi | 15.1 | S | S | R | R | S | S | S | I | 1/8 = 0.125 |
| | 15.4 | S | R | S | I | S | S | S | I | 1/8 = 0.125 |
| Sharaf | 26.2 | S | S | S | I | S | S | I | R | 1/8 = 0.125 |
| | 26.3 | S | S | S | I | S | S | S | R | 1/8 = 0.125 |
| | 26.4 | S | S | S | I | S | S | S | S | 0/8 = 0 |
| Al Shefa | 13.1 | S | S | S | I | S | S | S | S | 0/8 = 0 |
| | 13.3 | S | S | S | S | S | S | S | R | 1/8 = 0.125 |
| Al-Masif | 6.1 | S | S | R | I | S | S | S | I | 1/8 = 0.125 |
| | 6.2 | I | I | S | I | S | S | I | R | 1/8 = 0.125 |
| | 6.4 | S | S | S | R | S | S | S | I | 1/8 = 0.125 |
| Al-Fajr | 3.2 | S | R | S | S | S | S | S | S | 1/8 = 0.125 |
| | 3.3 | S | S | S | S | S | S | S | R | 1/8 = 0.125 |
| | 3.4 | S | S | S | I | S | S | S | S | 0/8 = 0 |
| | 3.5 | S | S | S | S | S | S | S | R | 1/8 = 0.125 |
| Al-Nysia | 4.1 | S | S | S | S | S | S | S | S | 0/8 = 0 |
| | 4.2 | S | I | S | S | S | S | S | R | 1/8 = 0.125 |
| Al-Mahatta | 14.1 | R | R | S | R | S | S | R | R | 5/8 = 0.625 |
| Al-Nugra | 25.4 | S | S | S | S | S | S | S | S | 0/8 = 0 |
| | 25.5 | S | S | R | S | S | S | S | S | 1/8 = 0.125 |
| Shark Al-Mujamaa | 24.2 | S | S | S | I | S | S | I | R | 1/8 = 0.125 |
| | 24.3 | S | S | S | I | S | S | S | S | 0/8 = 0 |
| | 24.5 | S | I | S | S | S | S | S | I | 0/8 = 0 |
| Al-Khuraymi | 28.1 | S | S | S | S | S | S | S | S | 0/8 = 0 |
| | 28.4 | S | R | S | R | S | S | R | R | 4/8 = 0.5 |
| Al-jamiyin | 16.2 | S | S | S | S | S | S | S | I | 0/8 = 0 |
| | 16.5 | S | S | S | S | S | S | S | S | 0/8 = 0 |
| | 16.6 | S | S | S | S | S | S | I | R | 1/8 = 0.125 |
| | 16.11 | S | S | R | S | S | S | S | S | 1/8 = 0.125 |
| Al-Rasf | 12.2 | S | S | R | R | S | S | S | R | 3/8 = 0.375 |
| | 12.4 | S | S | R | R | S | S | S | R | 3/8 = 0.375 |
| MARI | | 0.029 | 0.1176 | 0.176 | 0.176 | 0 | 0 | 0.058 | 0.411 | |

Notably, the *A. saudimassiliensis* strain was initially identified in air samples from the Makkah region (Saudi Arabia) during the Hajj season of 2012 (Papadioti et al. 2017). Furthermore, the strains *Bacillus licheniformis*, *Bacillus cereus*, *Staphylococcus equorum*, *Exiguobacterium aurantiacum*, and *Pseudomonas stutzeri* identified in this study were also previously reported as airborne microorganisms in the Makkah region (Angelakis et al. 2014). The same authors emphasised that *Bacillus* (94 strains, 45%) and *Staphylococcus* (55 strains, 26%) were the predominant genera identified from the Makkah region using the MALDI-TOF technique.

Our results demonstrated that nearly all tested bacteria exhibited high resistance to the antibiotics evaluated, particularly fosfomycin, ampicillin, tetracycline, and gentamicin. The rapid emergence of antibiotic-resistant pathogenic microbes is a significant global public health concern. One critical survival strategy for bacteria, both in the environment and within hosts, is biofilm formation, which is implicated in numerous health conditions. The potential of airborne bacteria, particularly antibiotic-resistant bacteria, to produce biofilms is a serious risk to human health. Biofilms enable bacteria to survive under unfavourable conditions and exchange resistance genes, and thus it is more probable that immunocompromised patients will be infected (Dufour et al. 2012; Jamal et al. 2018). Strong biofilm formation and multi-drug resistance have also been observed in the study conducted by *Ababneh* et al. (2022). The presence of strong biofilm formers, such as *Kocuria carniphila* and *Planococcus massiliensis*, in urban green spaces suggests that these environments may serve as reservoirs for persistent pathogens. Public health strategies should consider these findings to mitigate exposure risks, particularly in densely

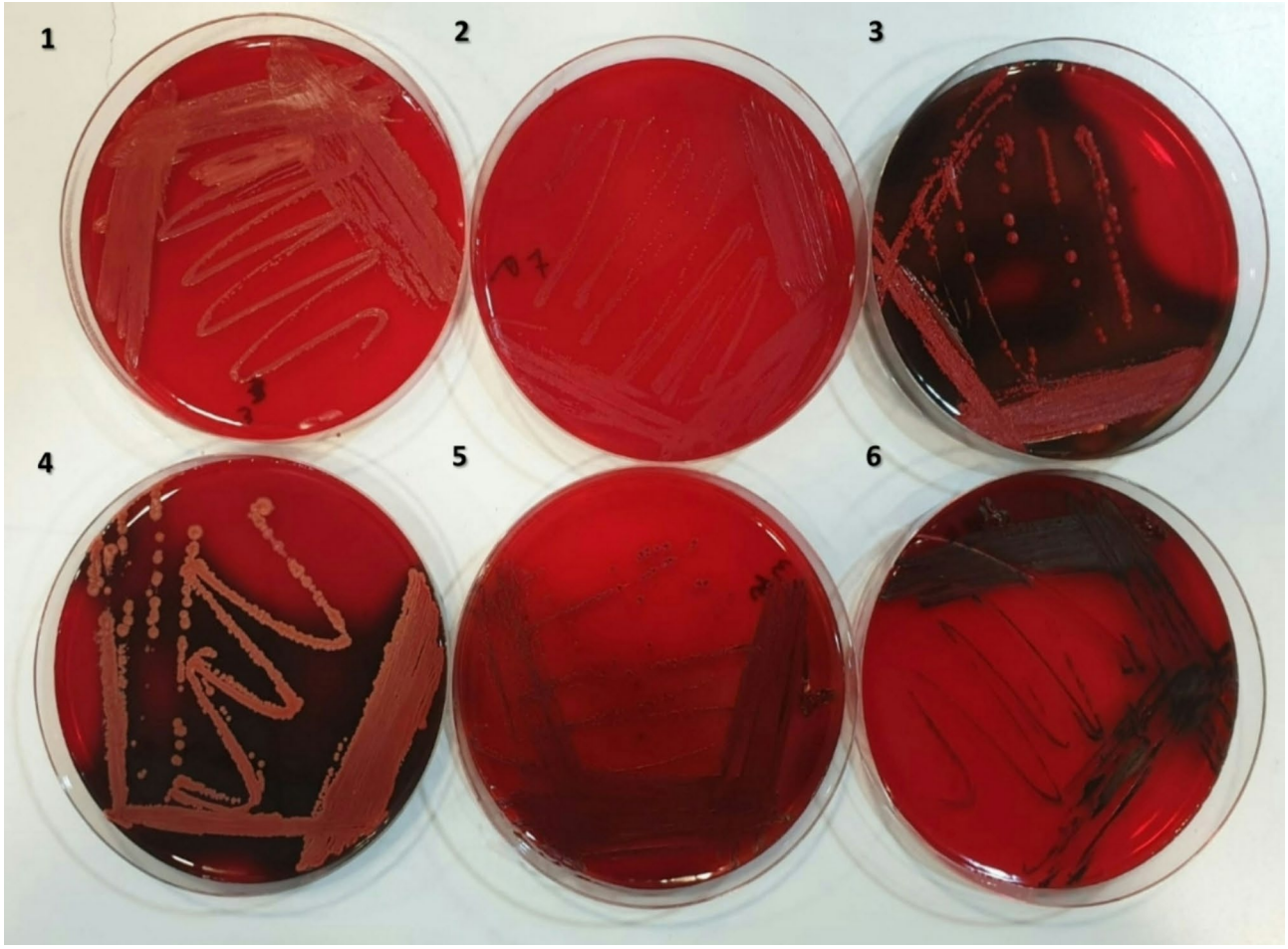


**Fig. 2** Morphotypes obtained on Congo red agar plates based on the color of the growing colonies. Legend: 1: Pink colony; 2: Pink-red colony; 3: Red colony; 4: Orange colony; 5: Almost-black colony; and 6: Black colony

populated areas. The biofilm lifestyle enhances bacterial survival and persistence in nearly all cases (Dufour et al. 2012; Peterson and Kaur 2018). In this study, the ARI reached 0.625, whereas the multiple MARI ranged from 0 to 0.411. Slime production analysis identified six morphotypes based on colony colour observed on Congo red agar, with 38.23% of the tested strains classified as slime producers. Furthermore, biofilm formation assays on polystyrene surfaces revealed that 41.17% of the tested strains were non-biofilm formers.

This study identified diverse bacterial species, including *Brachybacterium paraconglomeratum*, *Arthrobacter crystallopoietes*, and *Arthrobacter saudimassiliensis*, isolated from natural parks in the Hail region. These isolates displayed varied morphological characteristics and exoenzyme production, highlighting their potential pathogenicity and implications for human health. The findings revealed high levels of antibiotic resistance among the tested bacteria, particularly to fosfomycin, ampicillin, tetracycline, and gentamicin. The determination of the ARI and MARI provided further insights into the resistance profiles of these isolates. In addition, biofilm formation assays indicated that the bacteria exhibited varying capacities to form biofilms on polystyrene surfaces. This study offers valuable insights into the microbial composition of natural parks in the region, contributing to a better understanding of the potential health risks posed by these microorganisms. These findings are especially crucial for improving the diagnosis and management of diseases caused by these bacteria, particularly in the context of infectious disease. Such data and knowledge about environmental bacteria in a specific region could help to develop health policies to prevent population from health risks posed by such microbes. To advance these efforts, future studies should involve whole-genome sequencing of the key isolates, e.g., *Arthrobacter crystallopoietes* and *Pseudomonas stutzeri*, to determine their resistance mechanisms and ecological functions. *A. crystallopoietes* was selected for its exclusive dominance of local airborne microbiota, while *P. stutzeri* is of interest as a multidrug-resistant opportunistic pathogen of clinical interest. Similarly, studies on robust biofilm producer species such

**Table 5** Biofilm profiling of isolated strains from different sites

| Site of isolation | Code | Slime production | | Biofilm on polystyrene |
|---|---|---|---|---|
| | | Color on CRA | Interpretation | Interpretation |
| Al Muntazah Al Gharbi | 17.1 | Pink | Slime negative | (–); Non biofilm forming |
| | 17.2 | Red | Slime negative | (–); Non biofilm forming |
| Al Muntazah Al Shargi | 10.3 | Orange | Slime negative | (++); Medium biofilm forming |
| | 10.4 | Black | Slime positive | (+++); Strong biofilm forming |
| Al-Wadi | 15.1 | Orange | Slime negative | (+); Weak biofilm forming |
| | 15.4 | Black | Slime positive | (+++); Strong biofilm forming |
| Sharaf | 26.2 | Red | Slime negative | (–); Non biofilm forming |
| | 26.3 | Black | Slime positive | (–); Non biofilm forming |
| | 26.4 | Almost black | Slime positive | (+); Weak biofilm forming |
| Al Shefa | 13.1 | Black | Slime positive | (++); Medium biofilm forming |
| | 13.3 | Red | Slime negative | (+); Weak biofilm forming |
| Al-Masif | 6.1 | Red | Slime negative | (++); Medium biofilm forming |
| | 6.2 | Almost black | Slime positive | (++); Medium biofilm forming |
| | 6.4 | Red | Slime negative | (++); Medium biofilm forming |
| Al-Fajr | 3.2 | Pink with red center | Slime negative | (–); Non biofilm forming |
| | 3.3 | Orange | Slime negative | (–); Non biofilm forming |
| | 3.4 | Red | Slime negative | (–); Non biofilm forming |
| | 3.5 | Almost black | Slime positive | (–); Non biofilm forming |
| Al-Nysia | 4.1 | Orange | Slime negative | (+++); Strong biofilm forming |
| | 4.2 | Red | Slime negative | (+++); Strong biofilm forming |
| Al-Mahatta | 14.1 | Black | Slime positive | (++); Medium biofilm forming |
| Al-Nugra | 25.4 | Red | Slime negative | (–); Non biofilm forming |
| | 25.5 | Black | Slime positive | (–); Non biofilm forming |
| Shark Al-Mujamaa | 24.2 | Almost black | Slime positive | (+); Weak biofilm forming |
| | 24.3 | Orange | Slime negative | (+); Weak biofilm forming |
| | 24.5 | Red | Slime negative | (+++); Strong biofilm forming |
| Al-Khuraymi | 28.1 | Almost black | Slime positive | (–); Non biofilm forming |
| | 28.4 | Red | Slime negative | (+++); Strong biofilm forming |
| Al-jamiyin | 16.2 | Almost black | Slime positive | (++); Medium biofilm forming |
| | 16.5 | Orange | Slime negative | (+); Weak biofilm forming |
| | 16.6 | Red | Slime negative | (++); Medium biofilm forming |
| | 16.11 | Orange | Slime negative | (–); Non biofilm forming |
| Al-Rasf | 12.2 | Red | Slime negative | (–); Non biofilm forming |
| | 12.4 | Almost black | Slime positive | (–); Non biofilm forming |

as *Kocuria carniphila* and *Planococcus massiliensis* can reveal their survival strategies in urban ecosystems, thus informing targeted interventions towards minimizing public health risk.

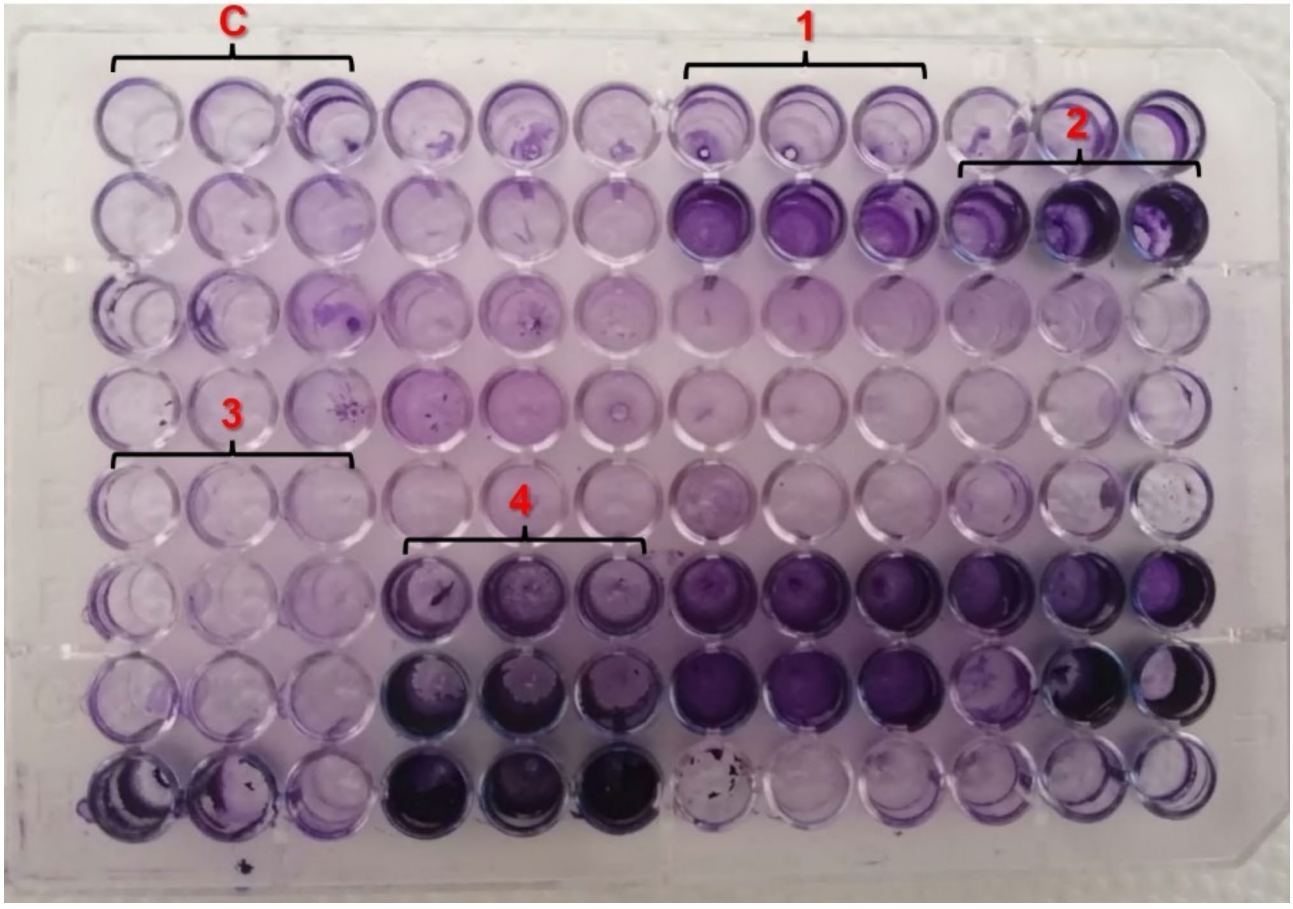


**Fig. 3** Biofilm formation on 96-well polystyrene plates assessed via crystal violet staining. Legend: C: Control wells (sterile medium); 1: Weak biofilm (strain 26.4, $OD_{595}$ = 0.3); 2: Strong biofilm (strain 4.1, $OD_{595}$ = 1.8); 3: Non-biofilm forming (strain 17.1, $OD_{595}$ = 0.1); 4: Moderate biofilm (strain 10.3, $OD_{595}$ = 0.7). Arrows indicate representative wells for each category

#### Author contributions

Conceptualization: MAK, MS; Methodology: MAA, MS; Formal analysis and investigation: MAH, MZ, YSK, KBS, SA and MS; Writing—original draft preparation: MS; Writing—review & editing MAH, MZ, YSK, KBS, SA and MS; Funding acquisition: MAH; Resources: MAK; Supervision: MAK.

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#### Data availability

All data generated or analysed during this study are included in this article.

## Declarations

#### Competing interests

All the authors declared there is no any potential competing interests.

#### Ethical approval

This article complies with ethical standards and does not contain any studies with human participants or animal performed by any of the authors.

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## Publisher's note