

# Screening of Antimicrobial Resistance in Bacterial Isolates from Red Sea Coast in Jeddah, Saudi Arabia

## Abstract

**Objectives:** Antibiotic resistance is a major public health problem worldwide. There is little data on the incidence, sources, and importance of antibiotic-resistant bacteria on the Red Sea coast of Jeddah. This study aims to identify drug-resistant microbes found on the Red Sea coast in Jeddah and to screen their resistance profile against different antibiotics.

**Methodology:** Forty-eight samples were obtained from different locations on the Red Sea coast in Jeddah. Antimicrobial susceptibility tests were performed against the following antibiotic discs (Oxoid): cefotaxime (CTX) (30g); meropenem (MEM) (10g); gentamicin (CN) (10g) and chloramphenicol (C) (30g).

**Results:** The isolates showed resistance to Gentamicin 10 mcg (39.47%; 15/38), Cefotaxime 30 mcg (18.42%; 7/38), Meropenem 10 mcg (0%; 0/38) and Chloramphenicol 30 mcg (5.26%; 2/38).

**Conclusion:** From this study there is a high resistance rate to cefotaxime, chloramphenicol and gentamicin 39%. This research has substantiated the existence of a substantial prevalence of resistance to various microbial agents within our environment, specifically within the Red Sea in Jeddah. We propose conducting future studies across diverse geographic regions, utilizing extensive sample sizes, to gain a deeper insight into the potential impact of environmental factors and pollution on the propagation of drug-resistant bacteria. Additionally, the identification of these bacterial strains is essential for understanding their characteristics and devising strategies to combat them effectively, thereby assisting in curtailing their proliferation.

**Keywords:** Antimicrobial resistance, Cephalosporin, Carbapenems, Red Sea, Jeddah.

## 1. Introduction

Antibiotics are strong medicines that are used for the treatment of diseases that were once fatal. Antibiotics have a wide spectrum of side effects, much like any strong drug (Mohsen et al., 2020). Antibiotic resistance represents a global health

concern. Soil, water, livestock, and plant foods are directly or indirectly exposed to antibiotics due to their agricultural use or contamination (Skandalis et al., 2021). Today, it can be said with certainty that the mass production and use of antibiotics for purposes other than medical treatment have an impact on both the environment and human health (Wien, 2020).

Antibiotics use also effect on the structure of the infectious agent, which causes bacteria to adapt or change, creating new strains that are resistant to the existing antibiotic treatment. This is a serious public health concern because the improper use of antibiotics in one patient could lead to the development of a resistant strain that spreads to other individuals who do not use antibiotics (Skandalis et al., 2021). Acute respiratory illnesses accounted for the most inappropriate antibiotic use in 2015, accounting for 50% of all outpatient antibiotic prescriptions (Habboush and Guzman, 2018).

There is a pressing necessity to enhance our comprehension of the environmental factors that contribute to the dissemination of antibiotic-resistant bacteria (ARB) and antibiotic-resistance genes (ARGs) among humans, plants, and animals, both within and between these populations. One of the primary goals of the WHO 2020 update is to enhance and broaden monitoring initiatives (Fletcher, 2015). Within the realm of the environmental sector, there exist various pathways through which antimicrobial resistance (AMR) can undergo evolution and dissemination. However, an extensive body of research has consistently indicated that water environments serve as a common medium for the transmission of AMR (Liguori et al., 2022). Aquatic environments can be exposed to ARB (antibiotic-resistant bacteria), ARGs (antibiotic resistance genes), and MGEs (mobile genetic elements) through various means, including direct contamination from aquaculture, leachates from landfills, inadequate treatment of waste streams from pharmaceutical manufacturing (Larsson et al., 2018), runoff from agricultural activities, combined sewer overflow events, and the discharge of human and animal waste. Sewage is recognized for its presence of a combination of antibiotics, antibiotic-resistant bacteria (ARB), antibiotic resistance genes (ARGs), mobile genetic elements (MGEs), and human bacterial pathogens originating from diverse residential, industrial, and clinical origins, which are received by wastewater treatment plants (WWTPs) (Haenni et al., 2022). Wastewater treatment plants (WWTPs) possess significant potential in mitigating the dissemination of antimicrobial resistance (AMR) to aquatic environments, albeit their primary design objectives do not explicitly encompass this

function (Kormos et al., 2022). There is a growing concern over the need for specific interventions in the pretreatment of hospital sewage before to released, or alternatively, for the treatment of wastewater containing high levels of clinically significant antimicrobial resistance (AMR) forms (Serwecińska, 2020). The main objective of this study is to identify drug-resistant bacteria from the Red Sea in Jeddah and to screen their resistance profile against different antibiotics.

## 2. Methodology

### 2.1. Water sample collection procedure

Water samples were collected from different locations on the Red Sea coast of Jeddah. Samples were collected at a distance of 10 m from the water's edge and a depth of 20 cm from the water surface (Figure 1).

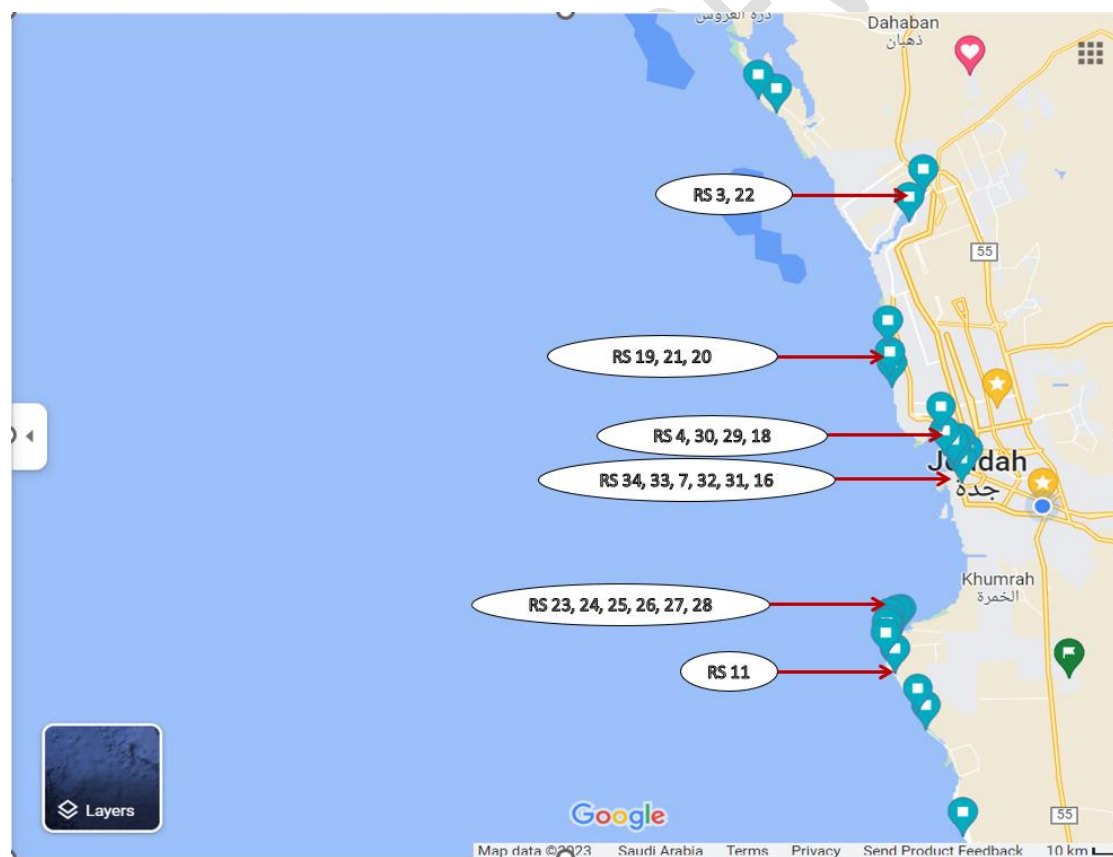


Figure 1. Map showing sample collection sites.

### 2.2. Sample handling procedures

Ideally, samples were grown in heart reperfusion broth (ISO) (Himedia, India) if not additionally processed, cooled at  $-4^{\circ}\text{C}$ .

### 2.3 Bacterial isolation and identification

Urinary tract infections chromogenic agar (UTIC) was used to culture the samples, and the plates were incubated at 37°C for 24 hours. Dry colonies appear in various shapes and colors. Color sub-growths were planted in colonies on Mueller-Hinton agar, and the plates were incubated at 37°C for 24 hours.

### 2.4. Antibiotic susceptibility testing

All study isolates were tested for antibiotic susceptibility using the Kirby-Bauer disc-diffusion method against the following antibiotic discs (Oxoid): cefotaxime (CTX) (30g); meropenem (MEM) (10g); gentamicin (CN) (10g) and chloramphenicol (C) (30g). The tests were carried out using the CLSI's approved procedure, and the results were evaluated using CLSI recommendations (FR, 2010).

## 3. Results

### 3.1. Antimicrobial susceptibility

The obtained isolates that were isolated from sea water showed resistance to Gentamicin 10 mcg (39.47%; 15/38), Cefotaxime 30 mcg (18.42%; 7/38), Meropenem 10 mcg (0%; 0/38) and Chloramphenicol 30 mcg (5.26%; 2/38) (Table 1).

**Table 1. Antimicrobial resistance pattern against different antibiotics**

ID	Gentamicin (CN-10)	Cefotaxime (CTX-30)	Meropenem (MEM-10)	Chloramphenicol (C-30)
RS- 3	S	S	S	S
RS- 4-1	S	S	S	S
RS- 16R	S	S	S	S
RS- 19R	S	S	S	S
RS- 20R	S	S	S	S
RS- 21W	S	S	S	S
RS- 21R	S	R	S	S
RS- 21G	S	R	S	S
RS- 24	S	S	S	S
RS- 25R	S	S	S	S
RS- 25W	S	S	S	S

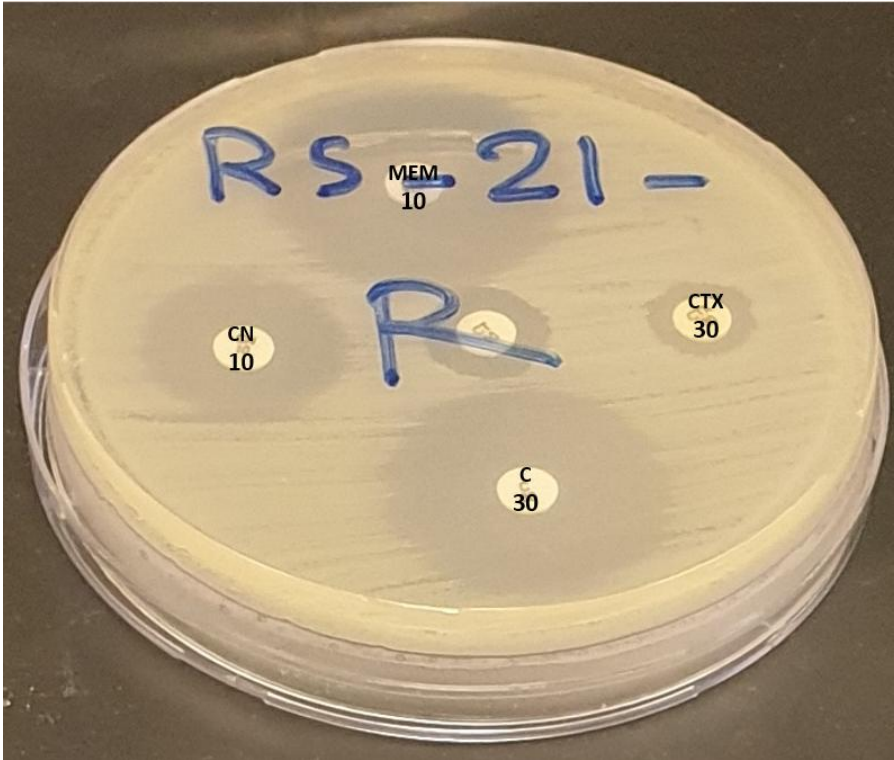
RS- 26W	S	S	S	S
RS- 27R	S	R	S	S
RS- 28W	S	S	S	S
RS- 29G	S	R	S	S
RS- 30	S	S	S	R
RS- 31G	S	R	S	R
RS- 32G	R	R	S	S
RS- 32R	S	R	S	S
RS- 33R	S	S	S	S
RS- 33W	S	S	S	S
RS- 34R	S	S	S	S
RS- 34G	S	R	S	S

S= Sensitive, R= Resistant. RS= Red Sea

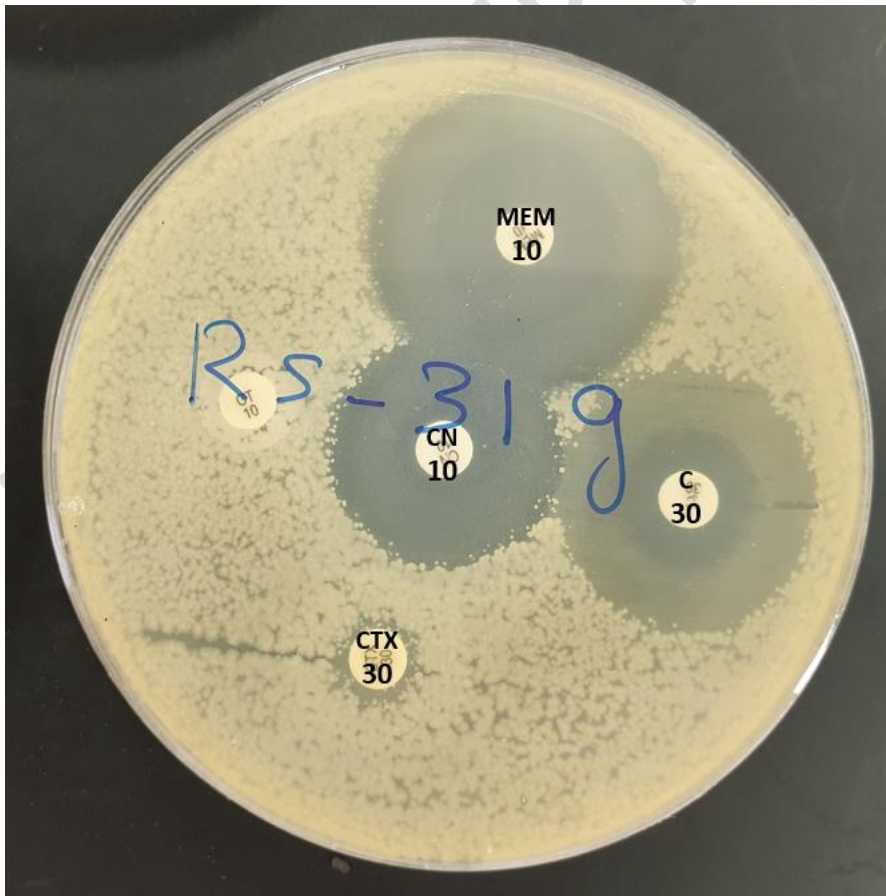
The following pictures (Figures 2A, B, C and D) show the extent and strength of bacteria's resistance to antibiotic tablets.



B



C



D

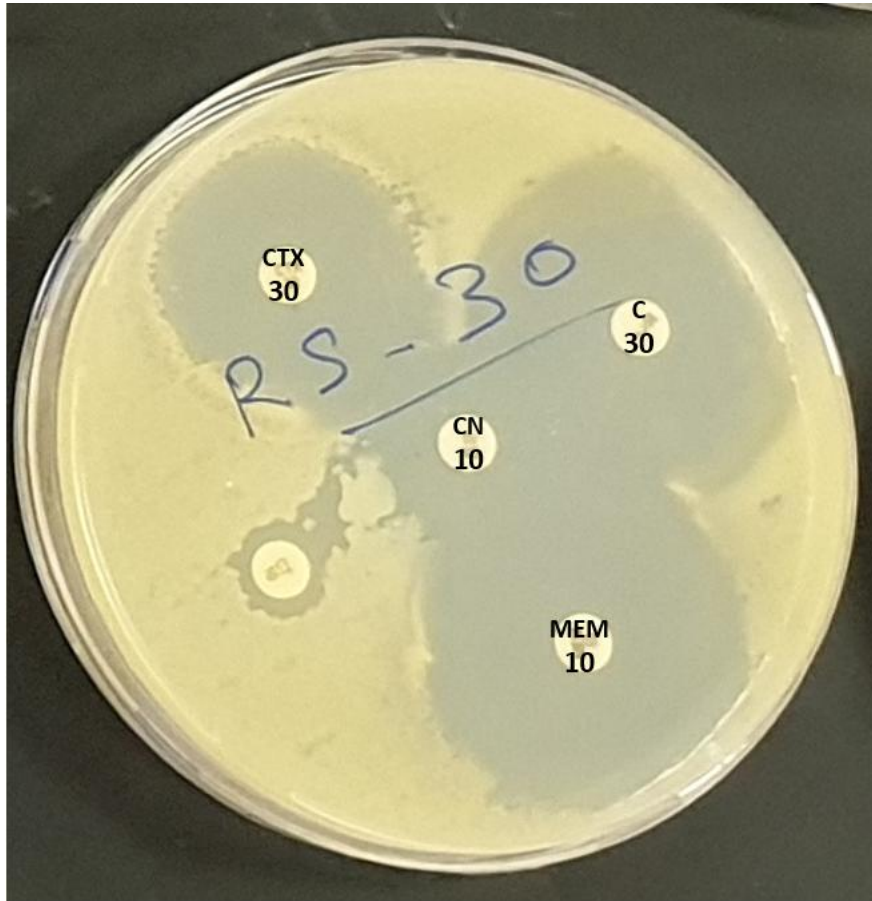


Figure 2. Showing the disc diffusion test of different antimicrobial agents, against bacterial strains identified from Red Sea. A: Showing resistance to CN10, CTX-30, C-30, B: Showing resistance to CTX-30, C: showing resistance to C-30, CTX-30 and D: Showing resistance to C-30 (Chloramphenicol).

#### 4. Discussion

Antibiotic resistance is a significant public health challenge in all countries globally. The proliferation of antibiotic-resistant bacteria (ARB) in the environment is associated with the excessive and improper utilization of antibiotics, as well as their unregulated discharge into waste systems throughout many regions, including aquatic ecosystems. Consequently, aquatic habitats, particularly coastal waters, are acknowledged as reservoirs and conduits for the dissemination of antibiotic resistance (Gambino et al., 2022). The observed occurrence of antibiotic resistance in marine waters can be attributed to the extensive use of antibiotics in human and animal sectors, with the subsequent introduction of these compounds into the sea via

wastewater (Kraemer et al., 2019). Consequently, marine waters have the potential to serve as a reservoir of antibiotic resistance, impacting both wild marine creatures and those cultivated in mariculture facilities, such as molluscs, sea bass, sea bream, and salmon. Moreover, this antibiotic resistance can potentially pose a risk to human health (Gambino et al., 2022). Antibiotic resistance can be acquired via the ingestion of edible marine organisms, such as fish and molluscs, or through direct exposure to seawater. The data presented indicate that water samples obtained, which are subject to human activities, bathing places, and proximity to residential centers, exhibit the presence of antibiotic resistance genes (Leonard et al., 2015). Furthermore, findings suggest that these genes are more prevalent in locations characterized by higher levels of pollution. The samples contained *Vibrio* and *Aeromonas*, which are Gram-negative bacteria (Zdanowicz et al., 2020).

The presence of resistance to  $\beta$ -lactam antibiotics was commonly seen in seawater, as well as in many fish and wild marine species. This phenomenon potentially contributes to the dissemination of antibiotic resistance (Alduina et al., 2020). The existence of antibiotic resistance genes (ARGs) within soil and environmental bacteria poses a potential risk to human well-being. Additionally, the process of horizontal gene transfer plays a significant role in the dissemination of resistance traits among pathogenic bacteria (Tao et al., 2022).

In our study, the sensitivity of isolated samples to Gentamicin (CN) was shown to be 39.47 %, this is close to what was found in (El Shafay et al., 2016) study, where the results showed a sensitivity of 30%. While there is incompatibility in the meropenem (MEM) sensitivity test, as our study showed 0% and that there is no resistance to bacteria, and in (El Shafay et al., 2016) study there is 30 % resistance, and there is also incompatibility in the chloramphenicol (C) sensitivity test, as our study showed 5 % of resistant samples, and in (El Shafay et al., 2016) study there is a resistance of 60 %, and there is also incompatibility in the cefotaxime (CTX) sensitivity test, as our study showed 18.42 % of resistant samples, and in (El Shafay et al., 2016) study there is a resistance of 97.1 %

The observed disparities in the outcomes could potentially be attributed to the potential exposure of the sampling location to biological or chemical contaminants, or alternatively, to variations in the methodologies employed for sample collection and preparation. The researchers (El Shafay et al., 2016) obtained microorganisms through

the utilization of seaweed and afterward employed solvents to process the collected samples.

The investigation done by (Tacão et al., 2022), which focused on samples gathered from the Lis River, yielded results that were incongruent with the findings of our own study. The sensitivity test for cefotaxime (CTX) and chloramphenicol (C) yielded a cefotaxime (CTX) sensitivity rate of 98% and a chloramphenicol (C) sensitivity rate of 25%. Regarding the Gentamicin (CN) sensitivity test, the obtained yield of 31% closely aligns with our own findings. In the (Tacão et al., 2022) investigation, samples were obtained from the river water, including locations proximal to areas characterized by pollution and the discharge of sewage. This disparity in findings may potentially be attributed to these factors.

Nevertheless, it is worth mentioning that our findings in the G sensitivity test exhibited a certain degree of agreement with the results reported in the two studies. Consequently, this prompts us to contemplate the undertaking of comprehensive investigations aimed at identifying the specific bacterial strains and their corresponding genetic elements that possess the ability to withstand antibiotics belonging to the cefotaxime (CTX), chloramphenicol (C), and meropenem (MEM) categories.

## **5. Conclusion**

The results of our study indicate that a large number of antimicrobial resistance isolates conferring resistance to the antibiotics cefotaxime (CTX), chloramphenicol (C), and meropenem (MEM). Furthermore, it was observed that surface seawater showed higher prevalence of AMR bacteria in areas with higher population density.

This study offered verification of the dissemination of diverse forms of resistance to different microbiological agents within the Red Sea environment in Jeddah. A comprehensive and elucidating investigation can be conducted to examine the various categories of unique resistance genes found in the region. This analysis aims to enhance our understanding of the hazards inherent in this area, allowing for proactive measures to be taken in order to mitigate their potential exacerbation.

Future research endeavors should aim to broaden the scope beyond the examination of pathogenic bacteria exclusively. It is imperative to explore the intricate interplay between pathogenic bacteria and commensal bacteria, while also delving into the study of resistance antibiotics using a substantial number of samples.

In order to enhance comprehension of the influence of pollution and environmental factors, particularly seawater, on the transmission of drug-resistant genes within bacterial populations, it is imperative to conduct a comprehensive analysis.

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