SCREENING OF AMOXICILLIN DEGRADATION POTENTIAL BY AMOXICILLIN-RESISTANT BACTERIA

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ABSTRACT

Currently, many antibiotic types have been introduced to the world according to their mode of action, bacterial spectrum, route of administration, chemical structure, etc. Despite the emergence of novel antibiotics in recent years, amoxicillin (AMX) remains one of the most prescribed antibiotics due to its classification as a β-lactam antibiotic with superior oral absorption and a broad spectrum of activity against many gram-positive and some gram-negative bacteria. The widespread use of amoxicillin has resulted in the accumulation of the compound in the environment, eventually leading to antibiotic-resistant bacterial strains and toxicity in ecosystems. The present study aims to evaluate the AMX degradation feasibility of 55 AMX resistant bacteria isolated from the hospital wastewater samples. MT2 plate assay was used to screen the 55 bacterial strains. This assay aimed to identify bacterial strains that can grow in the presence of amoxicillin, utilizing it as their sole carbon source. The bacteria identified as AMX degraders were subjected to 96 well plate method to determine the highest degraders against environmental detection amoxicillin concentration. Eight bacterial strains were selected according to the absorbance level (>0.500): *Micrococcus luteus, Bacillus cereus* (DJ080579.1)*, Bacillus subtilis, Lactobacillus bulgaricus, Lactobacillus* sp. (DI438712.1), *Enterobacter aerogenea, Bacillus cereus* (EU678635)*,* and *Lactobacillus* sp. (HW413258.1). Furthermore, the biodegradation ability of selected 8 bacterial strains was evaluated against different concentrations of AMX based on environmental detection level. To complete that 10µg/l of overnight starved bacterial suspensions were added into the sterile antibiotic medium in triplicate at a final concentration of 2.5µg/l, 5µg/l, 7.5µg/l. The samples were incubated at 28^0C for 14 days. Analyses of degradation were performed by Enzyme-Linked Immunosorbent Assay (ELISA) at 595nm through absorbance value. Most bacterial strains show their high potential for degradation at 2.5 µg/l concentration, with considerable potential observed at 5µg/l. As a trend, the high potential to degrade amoxicillin was shown at three different concentrations by *Bacillus cereus* EU67863. Accordingly, those amoxicillin-resistant bacteria show their high potential to use biodegrading amoxicillin at the lowest concentrations (=<5µg/l).

Keywords: Antibiotic, β - lactam antibiotics, Amoxicillin, Amoxicillin Resistant Bacterial Strains, Biodegradation, ELISA

INTRODUCTION

With the expanding human population, demand for healthcare facilities has continued to become prevalent such as pharmaceutical products generally drugs and chemical-based care products. Among those products, antibiotics can be introduced as one of the most used pharmaceutical products in the world because they are used all over the world for preventing and treating human, animal, and plant infections as well as a growth promoter in animal farming. (Hutchings et al., 2020)

Antibiotics are organic compounds that are created by the secondary metabolism of living bacteria or synthesized or semi-synthesized and it used to combat microorganisms by either killing them (cytotoxic) or inhibiting their growth (cytostatic), thereby allowing the human body's immune system to eliminate them (nicolaou & rigol, 2018). (harris, 1964).

Despite the availability of newer antibiotics in recent years, amoxicillin remains one of the most prescribed antibiotics due to its classification as a β-lactam antibiotic with superior oral absorption and a broad spectrum of activity against gram-positive bacteria, as well as some gram-negative bacteria. Amoxicillin is primarily employed to treat various infections in humans, including those affecting the upper and lower respiratory tracts, skin, soft tissues, and urinary tract, and veterinary medicine to treat infections in animals and to promote growth in certain species such as cattle and fish (groups, 1945).

The widespread use of amoxicillin has resulted in the drug entering the environment through various sources such as incompletely metabolized human and animal excreta, as well as waste materials from hospitals that contain high levels of amoxicillin, feed additives used for stock breeding and aquaculture, and amoxicillin production (occurrence et al., 2012). After entering the environment, amoxicillin can be transported through various environmental compartments, including groundwater, stream water, soil, and plant roots, where it can persist for a certain duration.

In addition, amoxicillin has the potential to break down the balance of microbial populations in natural ecosystems, which can contribute to the development of antibiotic-resistant strains of bacteria. On the other hand, can cause potential carcinogenic and mutagenic impacts on humans at high doses. Among these effects, generating amoxicillin-resistant bacteria (arb) is the most severe effect on both the ecosystem and humans because it can unbalance the ecosystems, make the standard treatments ineffective, and increase the risk of infection spreading(levy, 1991). In recent years, more than 1.2 million people, and potentially many more, have died directly because of infections caused by antibiotic-resistant bacteria. Arbs are the bacteria that reduce or eliminate the ability of amx to kill infectious microorganisms through genetic alterations. As a result, alternative antibiotics may need to be used to effectively treat infections caused by amoxicillin-resistant bacteria (sodhi et al., 2021). Considering all these points, it is important to establish a proper remediation method for improperly discharged amoxicillin. Therefore, to address pollution caused by amoxicillin, a range of remediation methods are employed, involving physical, chemical, and biological treatments. These methods include adsorption, oxidation, photolysis, and biodegradation. The selection of a particular method depends on various factors, including the concentration of amoxicillin, the type of contamination, and the environmental conditions, as each method has its advantages and drawbacks (l. Aaron albert aryee, 2022). While a range of methods is available to remediate amoxicillin pollution, bioremediation is a more natural and cost-effective approach, and it has become increasingly popular due to its ability to avoid some of the problems associated with other methods. Among bioremediation techniques, microbial remediation is commonly employed due to its effectiveness and efficiency in removing amoxicillin pollution from the environment. This method involves the introduction of specific bacteria or fungi into the contaminated environment to degrade it into less harmful substances. To implement bioremediation, it is crucial to identify bacteria or fungi that can survive in the amoxicillin-contaminated environment. Therefore, scientists aim to use amoxicillin-resistant bacteria for this remediation process.

This study aimed to identify the ability of amoxicillin-resistant bacteria to degrade amoxicillin, ultimately, the findings of this study can contribute to reducing the environmental impacts of amoxicillin in a sustainable and eco-friendly manner.

METHODOLOGY

I. Preparation of bacterial samples for the degradation process.

The previously isolated 55 AMX resistance bacteria were transferred into 5 ml of liquid Nutrient Broth (NB) medium and incubated overnight at 28^0C. Then centrifugation to remove the carbon source from the NB media (13g per 1000ml) was followed and the bacteria suspension was subjected to the starvation procedure, in 0.9% saline water solution. Thereafter, the turbidities of the bacteria suspension were equalized by using 0.5 McFarland standard solution. (Liyanage & Manage, 2019).

II. MT2 Plate Assay

Then the prepared bacteria were exposed to the known amount (5µg/L) of amoxicillin concentration by using biology MT2 Plates. In this process, each bacterial strain (10µl) is tested against 5 ppm of amoxicillin in triplicates. The control wells in the BIOLOG MT2 plate contained amoxicillin and sterile 0.9% saline solution in triplicates. Then the plate was wrapped with wet tissue and incubated at 28^0C. (Liyanage & Manage, 2018)

III. Antibiotic degradation Process.

In this step, firstly the 22 bacteria which have screened by MT2 plate assay were analyzed against environmental detection amoxicillin concentration (5µg/l), and 8 bacterial strains out of 22 were selected according to the absorbance level (>0.500). Then, the 8 bacterial strains that have high potency to degrade amoxicillin were introduced to three different concentrations as 2.5µg/L, 5 µg/L, and 7.5 µg/L which are selected according to the environmental detection level. Controls were prepared without bacterial suspension for each concentration level. The absorbance was measured by an ELISA plate reader at 0, 2, 4, 6, 8, 10, 12, and 14-day intervals at 595nm. The results are used to screen potential bacteria for Amoxicillin degradation (Kaur et al., 2019).

RESULTS AND DISCUSSION

I. MT2 Plate Assay

The MT2 plate assay is used for evaluating the ability of microorganisms to degrade amoxicillin through color reactions. Each of the wells in the MT2 plate contained the same concentration of a dye called tetrazolium violet, which is sensitive to the oxidation of a carbon source and bacterial respiration. (Frac et al., 2016). Under these conditions, bacterial strains that can survive with amoxicillin react and feed on the AMX (only carbon source) and produce CO_2 through their respiration. That was shown as purple color wells through the reaction of tetrazolium violet dye and CO_2. According to the Results, 22 bacterial strains were identified as resistant bacteria which have the potential to degrade amoxicillin (Taha et al., 2015)

I. Assessment of the bacteria strains which have a high potential for amoxicillin degradation.

In a study, twenty-two bacterial strains were screened by using the MT2 assay were subsequently exposed to environmental detection concentration of amoxicillin (5µg/l). Based on the experimental results, eight bacterial strains were identified which have a higher potential for amoxicillin degradation when compared to the other 14 bacterial strains. These eight bacterial strains shown in Table: 1 exhibited an absorbance value greater than 0.500 absorbance, indicating their higher efficiency in utilizing amoxicillin as the carbon source for their survival.

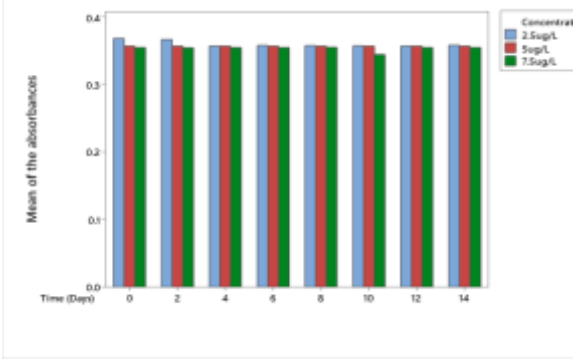
Table 1: Selected 8 bacterial strains.

Bacteria Name	Accession No:
Micrococcus luteus	DQ192164.1
Bacillus cereus	DJ080579.1
Bacillus subtilis	AB615253.1
Lactobacillus bulgaricus	AF429677.1
Lactobacillus sp.	DI438712.1
Enterobacter aerogenea	KY398209.1
Bacillus cereus	EU678635
Lactobacillus sp.	HW413258.1

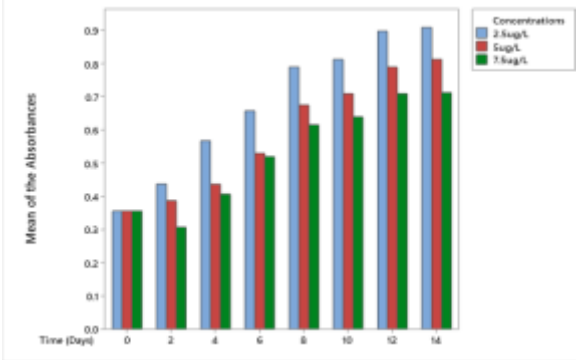
Furthermore, these 8 bacterial strains were evaluated against three different concentrations of amoxicillin (2.5 µg/L, 5 µg/L, and 7.5 µg/L) based on the detection level in the environmental samples (5 µg/L). Concentrations surrounding the detection level are the most effective values for assessing the degradation potential of amoxicillin, Otherwise, it may not be possible to accurately evaluate their ability to degrade amoxicillin in environmental samples(Yang et al., 2020). Under these conditions, the degradation potential of each bacterial strain was assessed by analyzing the absorbance values with the use of an enzyme-linked immunosorbent assay (ELISA) plate reader at a wavelength of 595nm. The experimental measurements were conducted over a period of 14 days to observe the time-dependent changes in the degradation potential of the bacterial strains.

Figures 01 represent the trend of amoxicillin degradation by 8 amoxicillin resistant bacterial strains. The presented data in the form of a graph depicts a positive correlation between the absorbance values, which represent the microbial suspension, and time. These are shown that the microbial population was increasing over time. Moreover, since no external food supplement was added, it can be inferred that the microorganisms were utilizing amoxicillin as the sole source of nutrition.

The results indicate that most of these ARB strains exhibit their optimal potential at a concentration of 2.5 µg/l. remarkably, this value is below the environmental detection level. Furthermore, bacteria strains that can degrade contaminants at lower concentrations than the environmental detection level can also be useful for developing early warning systems to detect contamination before it reaches harmful level. Additionally, all the bacterial strains represent considerable potential at environmental detection concentration. That may also help to prevent the emergence of antibiotic-resistant bacteria. As a trend, the high potential to degrade amoxicillin was shown at three different concentrations by *Bacillus cereus* EU67863.



a



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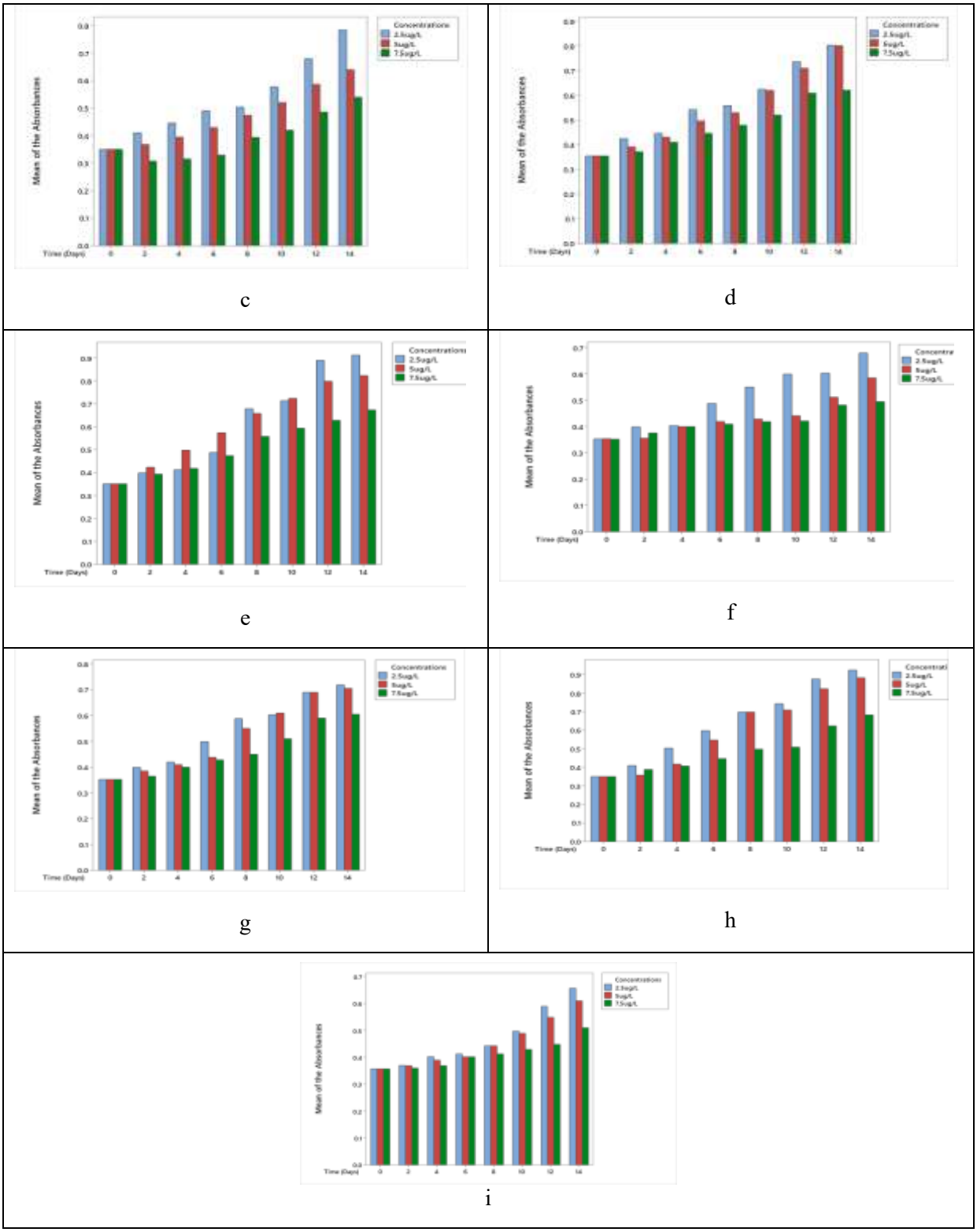

Figure 1: Trend of selected bacterial strains in biodegradation (a-Control, b- *Micrococcus luteus,* c- *Bacillus cereus* (DJ080579.1), d- *Bacillus subtilis*, e- *Lactobacillus bulgaricus*, f- *Lactobacillus* sp. (DI438712.1), g- *Enterobacter aerogenea*, h- *Bacillus cereus* (EU678635), i- *Lactobacillus* sp. (HW413258.1))

CONCLUSIONS/RECOMMENDATIONS

This study examines the efficacy of using amoxicillin resistant bacteria for bioremediation purposes. Especially, the potential for the 8 bacterial strains to degrade amoxicillin was evaluated. Results indicate that amoxicillin resistant bacteria display high potential for degrading amoxicillin at concentrations as low as 2.5µg/l (below detection levels), with considerable potential observed at 5µg/l. This result would be useful for developing early warning systems to detect contamination before it reaches harmful level. As a recommendation, it is important to investigate the environmental condition in which these bacterial strains exhibit their optimum growth, and their degradation mechanisms before using for a field application.

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