

Evaluating the effectiveness of commonly used antibiotics in Pakistan

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ABSTRACT

Antibiotic resistance and its consequences have now become a global crisis. The increasing resistance of bacteria combined with the misuse of drugs has led to an era of antibiotics that prove to be of no value to the affected individual. This raises the question of how to encounter Antimicrobial Resistance (AMR) and what reason lies behind the increasing resistance of these bacteria. This research has shown the causes of resistance due to motif combinations, identified through the literature review. The method employed was of two types, the manual type and by automation through a designed code. It exhibits that the decreasing number of motifs has shown higher resistance of bacteria. This is one of the approaches that have not been studied in detail. This would help in identifying a mutation pattern that could encounter the antibiotic effectiveness problem. The obtained results can be further validated through experimental procedures to deduce a concrete hypothesis. As a result, alternatives to the existing ineffective antibiotic can be designed in the future.

Keywords: Antibiotics, Antimicrobial resistance, Penicillin Binding Sites, Motifs

INTRODUCTION

Antimicrobial resistance (AMR) is one of the top 10 public threats in the world. According to the World Health Organization (WHO), Antimicrobial resistance (AMR) is a serious problem to confront (Prestinaci *et al.* 2015). The main notion is that multiple bacteria have developed resistance to encounter antibiotics which have led to the excessive use and/or misuse of the said drugs. This misuse is a way of welcoming side effects that may lead to fatal diseases and infections. AMR serves as a declining force in the healthcare system as it takes up a huge amount of expenditure (Penry *et al.* 2016). Moreover, the productivity of routine healthcare workers is also affected due to AMR. It is estimated that several 10 million deaths can occur by the year 2050 which can be avoided if there were to be a solution to AMR (Ventola, 2015).

Antimicrobial resistance (AMR) is not a problem that can be comprehensively eradicated. This is because it is in bacteria's nature to survive and adapt according to the environment that surrounds the organism (Rosselló-

Móra and Amann, 2015). A motif is the protein part that is conserved throughout generations. Conservation detected through motifs is easier to evaluate since it helps in determining the mutation rate. The Penicillin Binding Proteins (PBPs) of a bacterial strain with a high amount of motif combinations will have a fewer chance of mutation. Due to the higher number of motif combinations in the strain, even if a mutation occurs it will vary among any one of the pre-existing combinations. Hence, we can say mutation will not cause any change (Chatterjee *et al.* 2016). It has been observed that if the percent count of motif combinations in Penicillin Binding Proteins (PBPs) of bacterial strains is more, then there is a higher chance of stability and less chance of mutation. Therefore, the antibiotic will be more effective. Similarly, if there is less percent count of motif combinations in PBPs of

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bacterial strains then the availability of the attachment sites for the antibiotic will be less which might be due to mutation in bacteria. Therefore, it will show resistance to antibiotics and will no longer be effective (Sander *et al.* 2002). In Pakistan, there are numerous diseases that are prevalent even today. Although bacterial diseases are not as common as viral diseases in Pakistan, the intensity of bacterial diseases is much higher than that of viral infections (Davies and Davies, 2010). Different studies have been conducted to describe the situation of antibiotic resistance in Pakistan (Bilal *et al.* 2021). The aim of this research is to determine whether the commonly used antibiotics are effective against their target bacteria. Moreover, it can be deduced if the bacteria have become resistant. This research will also help in understanding the mutation pattern in the penicillin protein.

MATERIALS AND METHODS

Data Mining and Extraction: 50 bacterial strains were selected for this research. This sample size was based on the priority pathogens enlisted by WHO in Pakistan (Frieri *et al.* 2017). The resistance pattern can be identified through various methods such as the Disk Diffusion Method (DDM) a molecular identification of the resistant gene. However, in this study, antibacterial resistance was evaluated by motifs (Campoccia *et al.* 2010). The AMR of those pathogens was studied that were considered critical and high priority by WHO. Specifically, those bacterial strains were selected that were found in humans and not in any other organisms (Ventola, 2015; Massova and Mobashery, 1998]. Research articles that showed antimicrobial resistance were taken from PubMed, PakMedi Net, and Google Scholar. Research articles that were not in English, lacked data on antimicrobial resistance and showed studies in other geographical locations than Pakistan were excluded (Knöppel *et al.* 2017). Ensembl Bacteria is a part of the European Bioinformatics Institute. It contains sequences of all the related bacterial strains that were required for this research. Primarily, sequences of 50 bacterial strains were gathered to constitute a database for this study. The reason for choosing these bacteria is because according to WHO, they are enlisted as common pathogens in Pakistan.

Detection of Motifs: It was hypothesized that a higher number of mutations in motifs is through randomization, if not then the mutation must be the result of positive natural selection. To check the validity of this hypothesis

the motif detection procedures were performed. The following motifs were extracted from different research papers that are known to exhibit susceptibility to bacteria and are motifs present in antibiotics.

- ♦ SXXK
- ♦ (K/H)(S/T)G
- ♦ (S/Y)XN

Manual Curation: The protein sequences of these bacteria were downloaded in the FASTA format. The motifs present in the sequence were identified manually with the help of control keys. It was found that different combinations of the motifs are present in the sequence. These combinations were compiled manually in the Excel Sheet for further analysis.

Automatic Curation: The identification of motifs done manually was further done computationally using Python. A code was generated to automate the procedure of motif identification. Through this, the results were identified. Manually were tallied and confirmed. In the future, the generated Python-based code will further aid in identifying more bacterial strains in less amount of time hence being more efficient and less time-consuming.

Statistical Analysis: Statistical analysis and visualization of data were performed using Microsoft Excel Canva, and Inkscape. Out of the 50 sampled bacteria, those bacteria isolated and carried affinity to resist their targeted drug over time. The drugs were taken out from the DrugBank and the bacteria's resistance was confirmed through the research papers. It was found that different bacterial strains had developed resistance over time. Although some of them were plant pathogens, they too had evolved to become resistant over time. The bacteria were isolated because some of them displayed resistance to Beta-lactam composed of antibiotics. Some examples are Amoxicillin, penicillin G, ampicillin, etc. As for the other bacteria, the drugs proved effective against them.

For analysis, the number of occurrences of motifs of the resistant bacterial strains was observed. The bacteria exhibited a certain count of the number of combinations of motifs. Each bacterium was analyzed according to the different penicillin types but at the end of compiling results, the total count of motifs was taken. The following equation was used for the analysis:

$$\text{No. of motif count} \propto \frac{1}{\text{Chances of mutation}}$$

The results were then compared with the number of occurrences of motifs of the non-resistant bacterial strains. Through this comparison, the statistical percentile of each bacterium was observed. Furthermore, to verify the results, five bacterial strains on average were taken for three different bacteria. These particular bacteria were isolated because they were on the critical list proposed and published by the World Health Organization (Perron *et al.* 2012). Moreover, these bacteria had been proven to become resistant over time against their respective drug which was confirmed through DrugBank and different research papers.

RESULTS

The results show variations in motif combinations in different bacterial strains. Some strains have several motif combinations in PBPs while some do not. Table 1 shows some strains that are known to provide against specific drugs.

The results found from the database were similar to

those from relevant research (Huber *et al.* 2021). Since there is a greater variation in the motif combinations in different bacterial strains. So, even if a mutation occurs it will not generate any newly mutated gene rather it will be from the pre-existing combination. On the contrary, the PBPs of bacterial strains with a low amount of motif combinations will have a high chance of mutation. Fig. 1 shows the behavior of different bacterial strains with respect to their motif combinations.

Fig. 2 shows the frequency of the highest occurring motif combinations in Penicillin Binding Protein 1. KSG is the most frequently occurring motif combination followed by YLN.

It was also observed that motif combinations in different strains such as *Neisseria gonorrhoeae*, *Staphylococcus aureus*, and *Salmonella enterica* decreased through the years as shown in Fig. 3.

The mutation pattern and statistical analysis of this study show that PBPs of bacterial strains with higher motif combinations have not mutated (have not developed

Table 1: Motif combinations in different bacterial strains with their resistance status and drug name

Bacterial Strain	Motif Combinations	Resistant	Drug Name
<i>Clostridium clariflavum</i> (dsm_19732)	60	No	—
<i>Bacteroides fragilis</i> (ych46)	19	Yes	β-lactam
<i>Acinetobacter</i> (baumannii1000160)	16	Yes	β-lactam
<i>Lactobacillus farciminis</i> (gca_001485255)	35	Yes	aminoglycosides, ciprofloxacin, trimethoprim
<i>Bacillus subtilis</i> (subsp_subtilis_str168)	78	yes	β-lactam

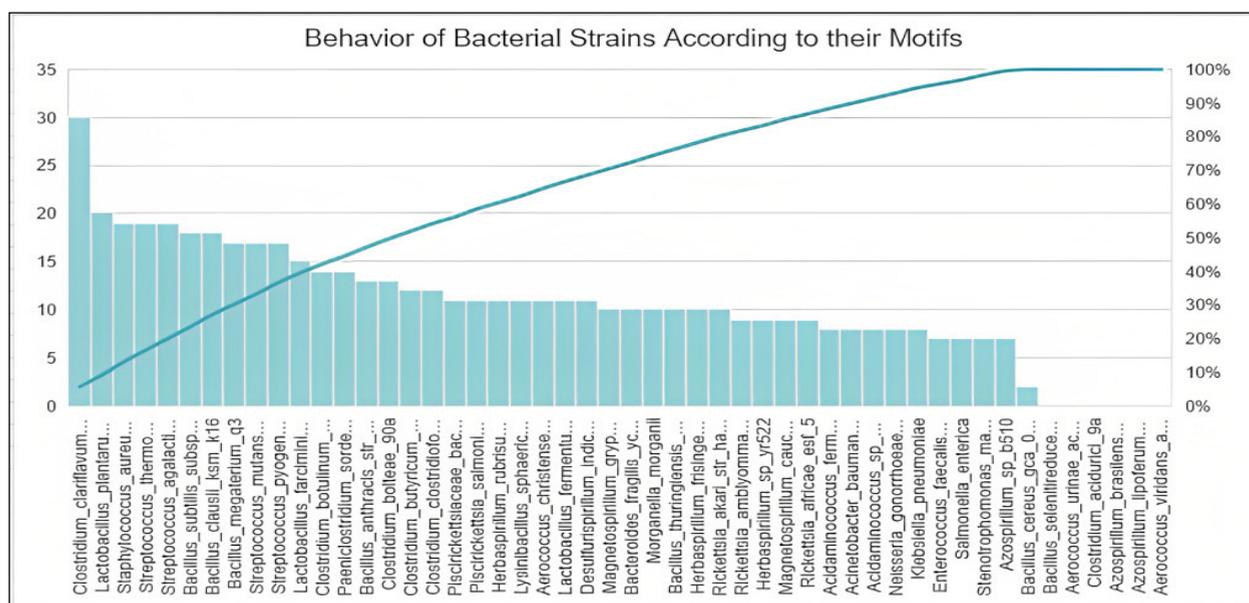


Fig. 1: Behavior of bacterial strains according to their motif combinations

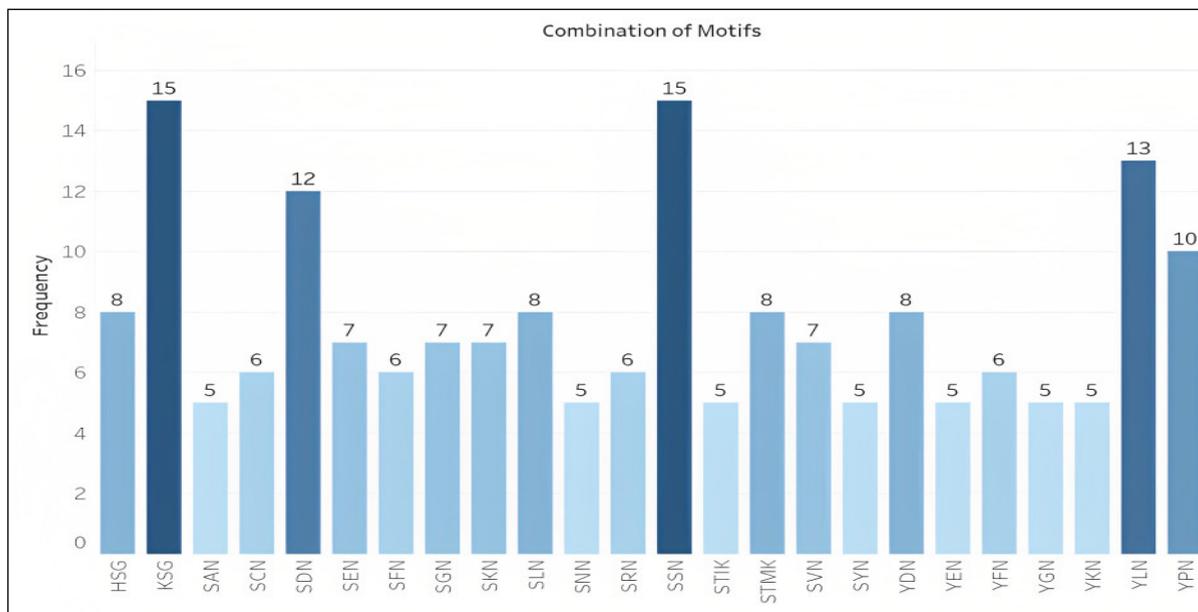


Fig. 2: Frequency of highest occurring motif combinations in Penicillin Binding Protein 1

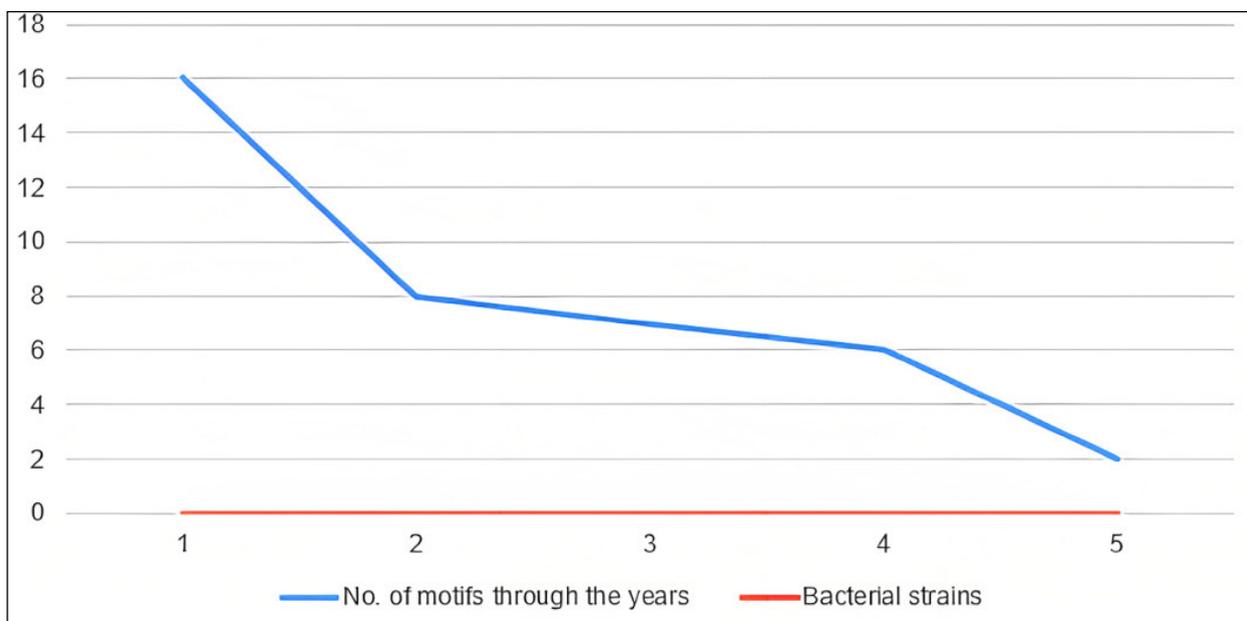


Fig. 3: Decrease in the number of motifs in bacterial strains over the years

resistance) whereas the PBPs of bacterial strains with low motif combinations have mutated and they developed resistance. These results were verified through different research papers.

DISCUSSION

Antibiotic resistance is a problem that has been surfacing the whole world, but it is a serious issue in countries that have poor infrastructure in the clinical domain. Such poor hygienic conditions pose a serious threat to these countries

which has caused an increase in AMR (Dai *et al.* 2020). This study is to analyze the bacteria that have become resistant to antibiotics that are common in Pakistan. In research studies, Urinary Tract Infection (UTI) is among the most reported clinical disease while E.coli was in the most number of studies for showing higher resistance to the first generation of antibiotics (Rafalskiy *et al.* 2019). Such patterns of antibiotic resistance were also shown in Bangladesh and Africa because of the same trend of excessive antibiotics being used in developing countries.

The data from this research showed the increasing trend of Penicillin resistant bacteria in the region.

This study is a molecular study that may help in studying the antibiotic resistance that has evolved due to the combination of motifs present in the bacterial sequences (Revazishvili *et al.* 2006). Further analysis can help in evaluating the alternative formula and treatment options for these resistant strains.

From the results, it is concluded that the percentage of motifs affects the efficiency of the bacteria. The efficiency of the resistance of bacteria is inversely proportional to the number of combinations of motifs. An increasing number of motifs results in lower resistance to bacteria and decreasing number of motifs results in higher resistance to bacteria.

Out of 403 combinations, the bacteria chosen were calculated to have not more than 20% of the total combinations. Mostly, all the bacteria exhibited resistance except for a few that were taken to be exceptions in the study. This lower percentage suspected all the bacteria to be resistant which was further validated through research papers (Sander *et al.* 2002). The reason that not every bacterium could have all the present combinations was that there needs to exist diversity for the theory of evolution to remain valid. In light of this, it was assumed that the higher number of motifs would have a lower chance of getting mutation hence the mutation would be random. It would also be referred to as randomization (Thomas *et al.* 2019). On the other hand, if there are a lower number of motifs, there would be a higher chance of mutation. And the resulting mutation would be based on natural selection.

For specific results and further validation, a small sample size was chosen out of our fifty bacterial strains. Three bacteria were chosen that were on the high-priority list according to WHO (Laura J.V. Piddock, 2006). Comparing the original sequence with the mutated sequence resulted in a decrease in motif combinations throughout the years. This decrease in the motif count helped in finding the mutation that occurred. The study helped in providing concrete evidence that bacterial strains are evolving and developing mutation with time.

This study focuses on the antibiotic resistance exhibited in Pakistan, but the consequences of the AMR have reached the global platform. Being geographically important, Pakistan joins borders with China, Afghanistan, Iran, and India and is quite close to Uzbekistan. The fact is

that resistant species can be spread to different regions through human contact, water, or even animal species (Sah *et al.* 2021; Barbosa *et al.* 2005).

One limitation of the research is that the data obtained was from the general database of the country. There was no concrete data on different provinces regarding antibiotic resistance. The dataset that was taken was too small but the visible pattern proved that this could be a potential possibility. For verification, a larger dataset can be taken as well as a larger dataset for this research primarily. This would make the analysis a bit difficult. However, with the code, the method can be made easier, and the results would be much more concrete and authentic. This could further help in proposing the solution to this existing and increasing problem.

In a broader aspect, the resistance to antibiotics in Pakistan is quite high to which the public and the healthcare community must pay attention. For the public, it is important to note that the necessary awareness is made regarding the usage of antibiotics and their required dosage. The act of prescribing medicines to oneself should be forbidden entirely. The necessary guidelines for Pakistan's antimicrobial resistance network must be made concrete to encounter such increasing behavior of antibiotic resistance in the country (Ganguly *et al.* 2011). On the other hand, this increase in antibiotic resistance can also be controlled by the healthcare community. Necessary measures like personal hygiene, awareness programs, and training programs for healthcare workers must be enforced and implemented.

CONCLUSION

The present study summarizes the evaluated data on antibiotic resistance in Pakistan and provides significant outcomes. It indicates that is very high in Pakistan, not only that, there are considerable gaps in surveillance because of the lack of practical experimentation through vet lab. Also, the number of bacterial strains was insufficient to calculate the motif pattern. Some of the data that was observed did not have any relevant sources questioning the authenticity of the required information. Therefore, there is a need for regularization of surveillance practices on national and regional levels. Changing patterns of motifs should also be observed for studying antibacterial resistance, accompanied by specific actions to overcome the dangers associated with Antimicrobial resistance (AMR).

SUPPLEMENTARY MATERIAL

All identified motif combinations are presented in the supplementary file.

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