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Towards the Statistical Modelling of Machine Learning Parameters for Sentiment Analysis of Social Media Messages

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ABSTRACT

We present the series of steps involved in the statistical modelling of machine learning parameters for the development of a sentiment analysis framework for social media messages Data collection and dataset creation is presented as the first step towards the creation of the a statistical model using machine learning. The dataset is then divided into three subsets: a training set, a validation set and a test set. The training set is used to train the statistical model, the validation set is used to estimate how well the model is trained and the test set is used to measure the performance of the model. We conclude by presenting the Model Architecture

Keywords: Statistical, Model, Machine Learning, Sentiment Analysis, Social Media

1. INTRODUCTION

Data collection and dataset creation are the first step when creating a statistical model using machine learning. The dataset is commonly divided into three subsets: a training set, a validation set and a test set. The training set is used to train the statistical model, the validation set is used to estimate how well the model is trained and the test set is used to measure the performance of the model. The proposed system consists of four modules - (1) Data pre-processing module: for pre-processing the data (2) Feature representation module: for extracting out features from pre-processed tweets (3) Sentiment classification using base classifiers: in which different base classifiers are used for sentiment analysis and finally (4) Sentiment classification using ensemble classifier.

The details regarding each module is presented below. In the first step, the problem and the objectives for the research is defined. In the second step a literature review is done. The literature study focus on reviewing related work as well as gaining knowledge about the techniques that will be used in the project. In the third step, the experiment setups and configurations will be designed and data will be collected. In the forth step, a prototype tool is developed in order to collect, prepare and analyze data. The analysis is based on mood and sentiment word lists. For the machine learning components in this project the Weka data mining tool is used. In the fifth step, the results are evaluated by measuring the accuracy of performance prediction.

The work in this research is done through five steps, as illustrated in Figure 1

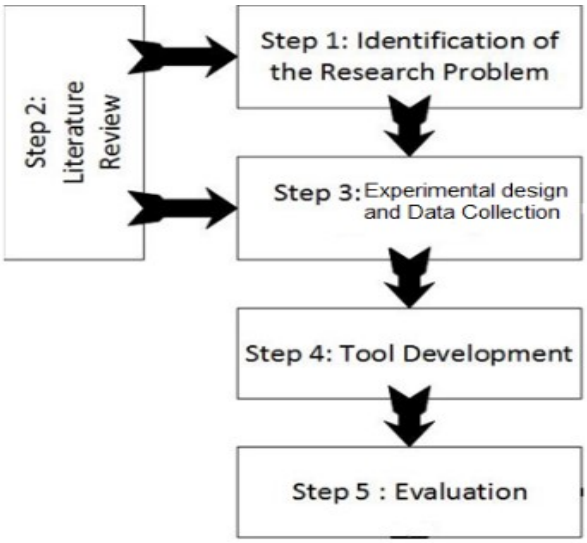

Figure 1: Proposed Methodology

2. METHODOLOGY WORKFLOW

Data Collection

Data collection and dataset creation is the first step when you want to create a statistical model using machine learning. The dataset is commonly divided into three subsets: a training set, a validation set and a test set. The training set is used to train the statistical model, the validation set is used to estimate how well the model is trained and the test set is used to measure the performance of the model.

Tweet Type	Count
Original Tweets	10189
Retweets	6287

Fig 2: Number of original and retweeted Tweets

Data Cleaning

After tweets are gathered from the social network using twitter API based on the query string hash tags, we prepared dataset for sentiment analysis.

i. Collect the tweets that are describing a particular topic from the dataset.
ii. Remove retweet entities, URL removal, markup removal, and hash tags removal. For each given set of tweets, we removed punctuation, numbers, white spaces, and unnecessary symbols

Data Balancing

If the number on instances in classification categories in a dataset are having a huge difference, the dataset is called imbalanced. To counter the issues of imbalanced data, methods such as over-sampling (creating new samples of a certain class) and under-sampling (removing instances of a class) have been proposed. Synthetic Minority Oversampling TEchnique (SMOTE) (Nitesh et al., 2017) is an over-sampling algorithm which provides more instances of the class with lower number of instances in addition to under-sampling of the class with more number of instances. In SMOTE, based on the required number of over-sampling K number of the nearest neighbor to the data point is selected and then after these steps the synthetic sample will be created:

• Take the difference of a data instance to its nearest neighbor,
• Multiply the number by a random value between 0 and 1,
• Add the new data point to the considered feature vector

Data Processing

Data pre-processing module is responsible to decrease the size of the feature set to make it suitable for learning algorithms. This is required because a tweet may contain several features as shown in a sample tweet in Figure 3.1. Following are the steps in the data pre-processing:

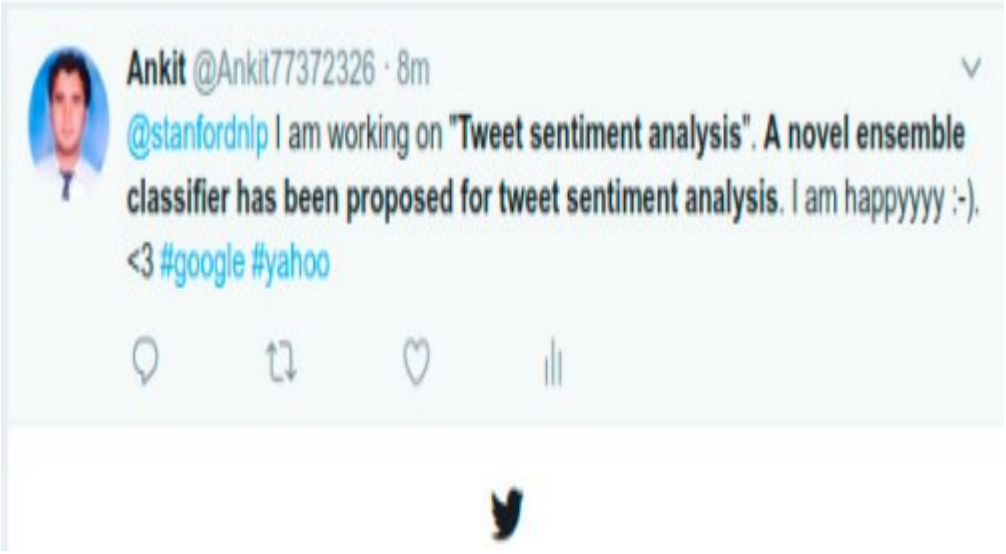

Figure 3: A sample tweet with various features

- Retweets, which starts with "RT" are eliminated.
- User names preceded by '@- and external links are eliminated.
- Hashtag '#- (used to point subjects and phrases that are currently in trending topics) is removed from the tweet.
- Emoticons1are replaced by its equivalent meaning because these can serve as a useful feature to detect sentiments.
- "Stemming" is done to reduce each word to its root word.
- Slangs are converted to words with equivalent meaning.
- Stop-words or useless words are removed from the tweet.

Figure 3 illustrates the main steps of the data processing. The starting point is a set of tweets which was extracted via Twitter API. Based on sentiment analysis approach, a sentiment classifier will be built by learning from previously annotated subset of tweets in order to classify the rest of tweets.
The classifier to be built will be able to learn the defined sentiment: "Positive" and "Not Positive". The processing steps are divided into three main steps: Tweets' text filtering, feature extraction, and sentiment classification.

Tweets Text Filtering

As previously mentioned, tweets are informal sentences that have to pass through a filtering stage before it can be processed for the upcoming steps. Filtering is the process of cleaning
the tweets text removing all irrelevant text for the sentiment classifier learning step. The following are tweets filtering steps mentioned in the order they were performed

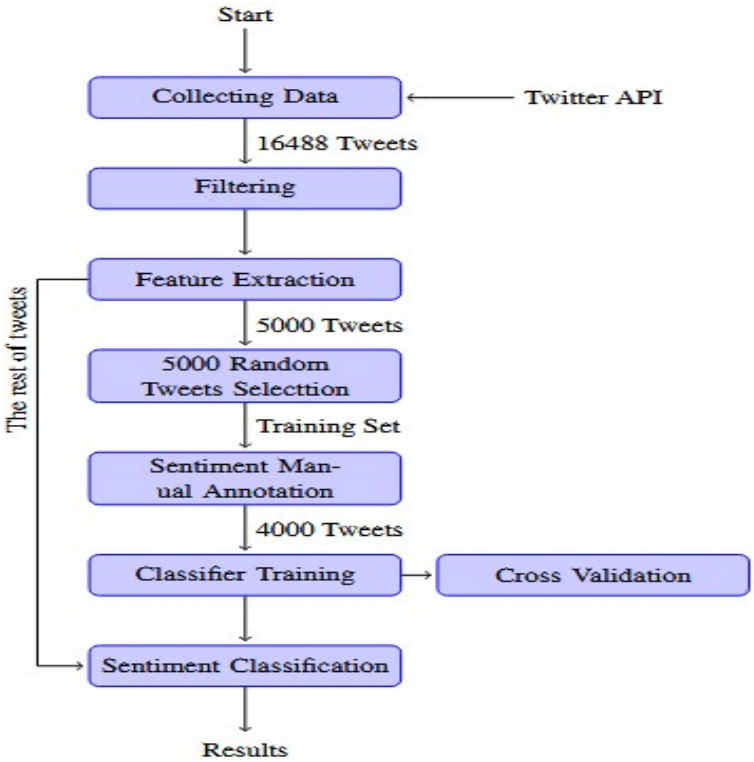

Figure 4: Data Processing Flow

i. All text is switched to lowercase including those words which are completely capitalized. Despite the fact that some users tend to emphasize specific words with capitalization, this was not the general case with the collected tweets. Many names and sentences are found completely capitalized indicating no emphasizing on the meaning. Taking the capitalization into account in such cases might lead to false results. Therefore, all text is changed to lowercase.
ii. All hyperlinks are removed. Tweets mostly contain hyperlinks to other sites and photos which does not contribute to the sentiment of the tweet.
iii. All mentioned usernames (identified by words that start with @) are removed and all hashed words with the # symbol are replaced with the word itself. These specific symbols and markups mentioning usernames or include hashed words that tag a place, name, etc. are so general to con-
tribute to a specific tweet sentiment.
iv. The "RT" text which indicates a retweet is removed.
v. Repeated lettered are filtered. Often, users emphasize words by repeating letters such as: "I am Happyyyy". Alec et al. (2019) suggest to remove out repeated letters leaving only two of them. This also guarantees that words such as "cool" with original double letters are left unaffected.
vi. Common emoticons are replaced with their semantic. Emoticons are often used in social media language to indicate the users' emotions Pak & Paroubek, 2020). The found emoticons are classified as:
vii.

Happy emoticons: ":)", ":-)", ":D", ";)", ";']", etc. which are replaced by "HAPPY FACE".

Sad emoticons: ":-(", ":(", "=(", ";(", ":[", etc. which are replaced by "SAD FACE"

viii. Negations detected in the tweet. Depending on the language, negation appears in different forms. Accordingly, the sentiment of the words appear before and after the negation are changed. For example, "I don't like exams" is changed to "I NOT do NOT Like exams".
ix. All words which do not start with a letter are removed. This eliminates all phone numbers and dates included in the tweet.
x. Extra spaces and punctuation marks are removed.
xi. All stop words and keywords (including the universities' names) are removed based on the language of the tweet

Feature Vectors

A feature vector is the way an object presented in machine learning and pattern recognition. Feature vectors are n-dimensional vectors where each vector represents an object. A numeric representation of the features (variables) will enhance statistical analysis, therefore many machine learning algorithms requires numerical features.

Feature selection

The process of selecting a subset of features that, should be used to construct the model is called feature selection. In machine learning and statistics, the process is also called variable selection. There

are various ways to do feature selection. As an example, information gain IG specify the most important features following the formula:

$$p(c|x) - \frac{p(c)p(x|c)}{p(x)} \quad (1)$$

where:
T is set of training example,
a is the index of a feature
H() function is an entropy (Entropy is a measure of the randomness of a variable and it measures the level of impurity in a group of examples,

Features Selection and Extraction
An important part of the sentiment analysis process is features selection. Features are the sentence properties that are analyzed in an attempt to correlate it to the tweet sentiment (i.e. "Positive" or "Not Positive"). A feature can be the fact that the tweet contains a word, emoticon, a combination of words, etc. The selection of the features is very important as they act as the input for the classifier in the next step. In the features extraction step, they take part in forming the unigrams and bigrams features of the tweets. This leads to a better performance for the classifier (Alec, 2019)

Sentiment Classification
Different classifiers are been presented in the literature for Twitter sentiment analysis. Supervised classifiers are the focus. They require a training set to be prepared forehead. The training set have to be annotated. Pak and Paroubek 2020 did this automatically base on the fact that all tweets in their dataset contains emoticons. They labelled each tweet based on the emoticon sentiment to be either "Positive" or "Negative". For the training set, it got annotated manually by different people based on their own feeling whether the tweet indicate a "Positive" or "Not Positive" sentiment. Automatic labelling was not possible at this stage as the tweets lack a common feature for sentiment labelling. Several classifiers can be used when it comes to the tweets sentiment analysis. Mainly, three common classifiers in the field of machine learning have been used in the literature: Naive Bayes classifier, Support Vector

Machines (SVM), and Maximum Entropy. Naive Bayes and SVM have been compared by Pak and Paroubek 2020 and Alex et al. 2019; Naive Bayes has performed better. Theoretically, Maximum Entropy performs better than Naive Bayes as it handles feature overlap better. However, in practice, Naive Bayes showed better performance on a variety of problems (Alec et al., 2019). Naive Bayes classifier is adapted by this paper's approach. It is a common method for text categorization. It appeared often for solving the problem of determining the category or class of documents that belongs to using word frequencies as the features. In machine learning, Naive Bayes classifier belongs to the family of probabilistic classifiers based on applying Bayes' theorem with the assumption that features are conditionally independence from each other given a specific class

$$P(s|f) - \frac{P(s).P(f|s)}{P(f)} \quad (2)$$

Equation 1 shows the basic formula of Naive theorem where s is the sentiment class (i.e. "Positive" or "Not Positive") and f is a specific feature. This equation computes the probability of having a tweet with the sentiment s when it contains the feature f . It is calculated based on the probability of having a specific sentiment, probability of the feature existence in all tweets, and the probability of finding the feature in the tweets that belongs to that specific sentiment.

Feature Representation

This module is responsible to extract features from preprocessed tweets. In this paper, Bag-of-Words technique (Han & Kamber, 2016) is used to convert training tweets into numeric representation. Bag-of-Words(BOW) learns a vocabulary of known words from all of the tweets (Yoon et al., 2018). After learning vocabulary, BOW describes the presence of known words within a tweet. For example, consider the following three tweets:

Tweet1: "yesterday is past"
Tweet2: "today is present"
Tweet3: "tomorrow is future".

The vocabulary is {yesterday, is, past, today, present, tomorrow, future}

Now, the above tweets are represented as:
tweet1 vector: [1 1 1 0 0 0 0] tweet2 vector: [0 1 0 1 1 0 0] tweet3 vector: [0 1 0 0 0 1 1]

The parameter values of the BOW are tuned as:
analyzer = "word", ngram range = (1, 2), max features = 4000

3. MACHINE LEARNING

Machine learning is a field of computer science which studies and explores ways of making algorithms find patterns or learn how to do certain tasks. In this thesis machine learning is used to predict the performance of a company. Figure 3.2 shows the workflow for the machine learning process we have used in this thesis.

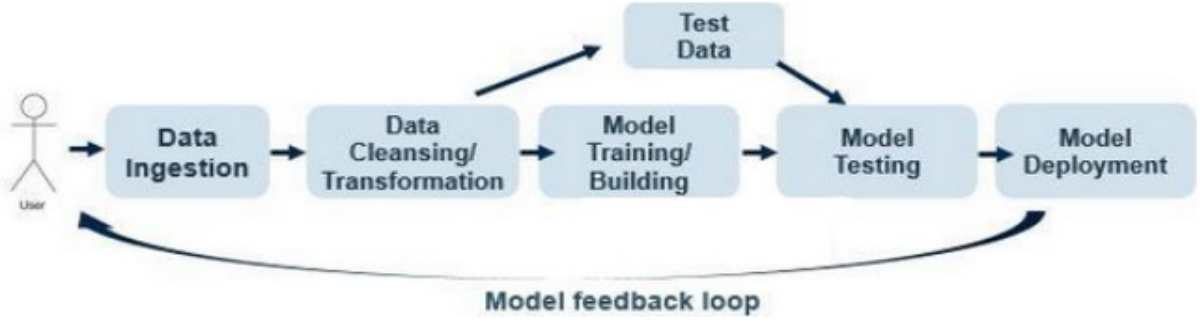

Figure 5: Machine learning workflow (Carol et al., 2015)

A. Supervised Learning: In supervised learning the computer receives a set of inputs and their related outputs from a teacher. The goal is to find a general mapping model from input to output.

B. Unsupervised Learning: In unsupervised learning, the computer find structures in the input data without having any input from a teacher.

C. Reinforcement Learning: In reinforcement learning the computer inter acts with an environment to achieve the goal without any help from a teacher

Classification Algorithms (Base Classifiers)
A classification algorithm task is to pick the right identified categories in data, for the new observations, the classifier estimates categories for new data based on the model parameters that are learned from the training data. Different classification algorithms use different classifier methods and variables and therefore a number of classification algorithms can be applied on the data in order to find the most suitable and efficient algorithm (Karina et al., 2020).

A. Naive Bays is a probabilistic classifier that uses Bayes theory with the assumption that the features are independent (occurrence of one feature. when training model time is important Naive Bays is useful.

$$p(c|x) = \frac{p(c)p(x|c)}{p(x)} \qquad (3)$$

B. AdaBoost (Freund & Schapire, 2016) stands for adaptive boosting and it assumes that finding many weak models are easier than finding one accurate model. Boosting is an approach to create predictions rules with high accuracy using a combination of weak models and rules that have low accuracy in prediction. Boosting generates a sequence of base models and then decides a final estimate of the target variable based on aggregating the estimates of the base models. AdaBoost generates a numbers of weak classifiers and a final estimate of the target variable is chosen based on aggregating the estimates made by the base models. Similar to the random forest algorithm, AdaBoost also have a variable importance estimation but in a different way. In AdaBoost the more informative variables are used more often, and the less informative features are barely used.

C. Cross validation (Sylvain, (2019) creates a training set and a test set by partitioning the original data with the goal to train and evaluate the model. In k-fold cross validation the original data will be divided into k number of subsamples. One subsample is selected as test dataset and the rest (k − 1) number of subsamples are used as training set for the model. The same process will be repeated for k number of times (folds) and each subsample will be used at least once as test set and then the results will be averaged or combined to make the best estimation

4. SENTIMENT ANALYSIS

Sentiment analysis is a method that analyzes how opinions, reactions, impressions, emotions and perspectives are expressed in a language. Its algorithms can extract evaluative information from large text databases and summarize it (Maynard & Funk, (2016). In order to analyze the opinion of people and customers, sentiment analysis appears as the main tool in different contexts.

As an example, sentiment analysis has been used to measure customers satisfaction via statements they comment on a specific product they bought or a service they were delivered. It also appeared in the extent of detecting different opinions regarding political events such as elections. Sentiment analysis methods are; well developed in the domain of blogs and product reviews. Researchers have been working on detecting sentiment in text via presenting different algorithms for detecting semantic orientation. In favour of producing meaningful information from tweets, sentiment analysis used. Different features selection techniques are been investigated, establishing a comparison between different one such as n-grams, part of speech, lexicons, etc. Besides, different classifiers with their learning performance been tested in different contexts (Alec et al., 2019). This paper applies the existing developed approaches in sentiment analysis to microblogging platforms data such as Twitter in order to explore complimentary resources for university evaluation and comparison

Twitter Sentiment Analysis for Evaluating Student Performance

This project suggests that social media content is a vital source for collecting feedback and reactions on the daily events and activities that relates to universities. To prove this hypothesis, a case study is established which evaluates the reactions and feedback from the social media data that is related to the university

Defining Tweets Sentiment

This project approach classifies the sentiment of each tweet to be either "Positive" or "Not Positive". These, known as two-way sentiment classification (Agarwal et al., 2016). A "Positive" tweet refers to text that indicates a positive statement regarding an event such as a lecture, class or activity that relates to one of the TU9. A "Not Positive" tweet can be either a negative statement regarding an event, or a neutral one, such as an announcement or advertisement regarding an event in the university. Adapting two-way classification can be considered as a limitation. Nevertheless, it is easier to process in the classifier learning step. The same approach was adapted by Alec et al. (2019) who consider that the "Not Positive" tweets are actually "Negative" ones, ignoring the neutral nature of some tweets. Pak and Paroubek proved that adapting a three way classification leads to bad performance which can be avoided by the two way classification (Alec et al., 2019)

Tweets Sentiment Analysis Challenges

Dealing with social media as a source of information - especially microblogging platforms such as Twitter - adds extra difficulties to the sentiment analysis process (Kouloumpis et al., 2016). Tweets are plain text written in an informal manner and its processing face challenges such as:

a) **Length:** Tweets have a limited text length, which is 140 characters. This forces users to start using some common and uncommon abbreviations and phrases. As an example, abbreviations such as OMG5, WTH6, DKDC7, TY8, etc. appears often in twitter
b) **Informality:** Twitter is mostly used as a non-formal communication medium. This leads to many informal statements which probably contain errors such as misspellings, unstructured sentences and slang. Informality may also infer sarcasm, which adds an extra layer of difficulty in guessing the right sentiment of each tweet.
c) **Credibility:** This paper's approach of gathering the tweets is based on a list of keywords. This does not guarantee the credibility of who and what tweets are generated on Twitter. This leaves the possibility that one anonymous user has generated all the content about a specific university with different usernames, rather than the students.

d) Data availability: collecting the right data is always a challenge, but having enough data is another critical issue. The target data for this study is very specific, which can be problematic for collecting data over a six-month period of time. It is significant to note that more data leads to more trusted results

5. SENTIMENT CLASSIFICATION USING *ENSEMBLE CLASSIFIER*

Base classifiers have been widely used to solve the task of sentiment analysis.

Naive Bayes (NB)

This is a probabilistic classification technique. This classifier performs well when applied to large datasets (Han & Kamber, 2016). NB classifier computes posterior probability by using the formula

$$\text{posterior probability} = \frac{\text{likelihood} \times \text{prior probability}}{\text{evidence}}$$

Equivalently,

$$P(\text{Classi}|z) = \frac{p(z|\text{Classi}) \times P(\text{Classi})}{p(z)}$$

Where z represents the feature vector and Classi represents the i th class. NB classifier makes an assumption that features are conditionally independent. Smoothing techniques are used to eliminate undesirable effects.

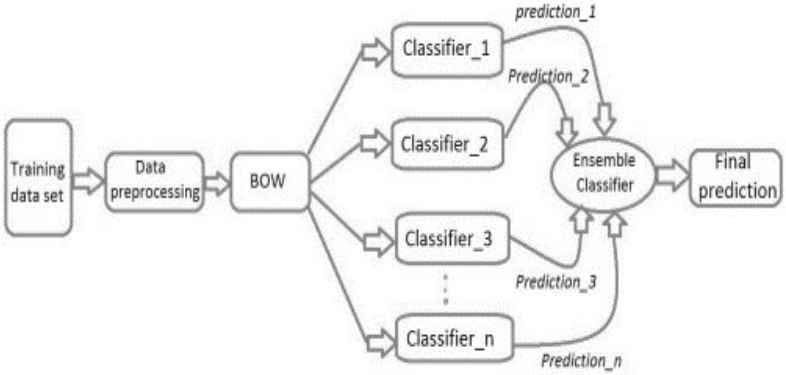

Fig. 6: An Overview of Tweet Sentiment Classification Approach Using Ensemble Classifier

Random Forest (RF)

RF is an ensemble method. Every classifier in the Random Forest is a decision tree classifier. RF classifier builds a set of decision trees from the training dataset (Jianqiang, 2016). After collecting votes from the different decision trees, it decides the final label or class of the test object. The parameter values of the RF classifiers are tuned as: n estimators = 150, max depth = 30. 3.3.3. Support Vector Machine(SVM) This model requires training data to train the model. It is also called a probabilistic classifier (Pang et al., 2017). SVM uses a nonlinear mapping whose aim is to find large margin between different classes.

Although training time of SVM can be slow but it is highly accurate. SVMs attempt to find a decision boundary which maximizes the separation gap between the classes. Unlike Naive Bayes classifier, SVM makes no class conditional independence assumption. SVM yields good result for the task of the Twitter SA problem. The parameter values of the SVM classifiers are tuned as: C = 0.1, kernel = linear.

Logistic Regression (LR)

This is a regression model that is used for classification purpose. LR is generally used to relate a single categorical dependent variable to one or more independent variables (Onan et al., 2016). LR attempts to find a hyper-plane which maximizes the separation gap between the classes. The parameter values of the LR classifiers are tuned as: C = .01, max iter = 100. 3.4. Proposed Ensemble Classifier Ensemble classifier aggregates multiple base classifiers in order to obtain a robust classifier (Prusa et al., 2015). Generally ensemble classifiers have been used to enhance the performance and accuracy of base learning techniques. Figure 2 shows an overview of tweet sentiment analysis approach using the ensemble classifier. Base learners like NB, RF, SVM, and LR are used in ensemble classifier

Algorithm 1: Proposed ensemble algorithm to calculate the Sentiment score of a tweet

1 Function Calculate Sentiment score (Test tweet);
Input : Test tweet
Output: Sentiment score
2 foreach Tweeti in Test tweet do
3 Positive counti = 0
4 Negative counti = 0
5 foreach classifier ci in classifier ensemble do
6 if ci predict Positive then
7 Positive counti += 1;
8 end
9 else
10 Negative counti += 1;
11 end
12 end

$$\text{Probability(Positive}_i\text{)} = \frac{\text{Positive count}}{\text{Positive count}_i + \text{Negative count}_i}$$

$$\text{Probability(Negative}_i\text{)} = \frac{\text{Negative count}_i}{\text{Positive count}_i + \text{Negative count}}$$

13 end
14 foreach classifier ci in classifier ensemble do

$$Weight_{c_i} = \frac{acc_{c_i}}{\sum_{j=1}^{n} acc_{c_j}}$$

// Where accci is the accuracy of i th classifier, j denotes the no. of learning classifiers in the ensemble classifier and acccj represents to the accuracy of j th learning classifier.

15 end
16 foreach Tweeti in Test tweet do
17 Positive scorei = 0
18 Negative scorei = 0
19 foreach classifier ci in classifier ensemble do
20 if ci predict Positive then
21 Positive scorei += Weightci * Probability(Positivei);
22 end

23 else
24 Negative scorei += Weightci * Probability(Negativei);
25 end
26 end
27 return Positive scorei, Negative scorei
28 end

Algorithm 1 calculates sentiment score of the tweet. The system was trained using the training data. The T est tweet is a set of tweets that was used to test the system. Each base classifier in ensemble classifier determines the sentiment (Positive/Negative) of each tweet in T est tweet. In addition, the classification report of each base classifier was calculated on the testing data (T est tweet). The next step is to calculate the probability of each tweet being positive and negative. After assigning this probability, we assign the weight to each classifier in the ensemble technique based on the accuracy of each classifier. Finally, the algorithm calculates the positive and negative score of the tweet based on the prediction of each classifier.

Algorithm 2 predicts the sentiment of the tweet. The inputs to this algorithm are the positive score and negative score of the tweet. If the positive score of the tweet is more than its negative score, then the sentiment of that tweet is taken as positive. And, if the negative score of the tweet is more than positive score then the sentiment of that tweet is taken as negative. Finally, If the positive score and the negative score of a tweet are equal then the system calculates the cosine similarity of that tweet with all other tweets in the testing data and identifies the most similar tweet. Then it calculates the positive and negative score of the identified tweet. Now if positive score is more than negative score then tweet is positive otherwise it is taken as negative.

Algorithm 2: Proposed ensemble algorithm to predict the sentiment of a tweet
1 function SentimentPredictor (Tweeti, Positive scorei, Negative scorei);
Input : Tweeti, Positive scorei, Negative scorei
Output: S entiment
2 if Positive scorei > Negative scorei then
3 Sentiment = “Positive”;
4 else
5 if Negative scorei > Positive scorei then
6 Sentiment = “Negative”;
7 else
8 Calculate cosine similarity of Tweeti with all other tweets in test data using distance calculation formula.
9 Find the most similar tweet of Tweeti in Test tweet, say Tweetj.
10 calculate Positive scorej and Negative scorej of Tweetj using Algorithm 1.
11 if Positive scorej >= Negative scorej then
12 Sentiment = “Positive”;
13 else
14 Sentiment = “Negative”;
15 end
16 end
17 end
18 Return Sentiment

Distance calculation "Cosine similarity" measures the similarity of a pair of tweets. Cosine similarity can be computed by using this formula:

$$\cos(\text{tweet1}, \text{tweet2}) = \frac{\text{tweet1} \cdot \text{tweet2}}{||\text{tweet1}|| \cdot ||\text{tweet2}||}$$

Where tweet1 and tweet2 represent vectors and output value 1 represents high similarity

5.1 Architecture

Figure 7 shows the general framework of our proposed approach. The different components involved in this framework are explained in the subsections.

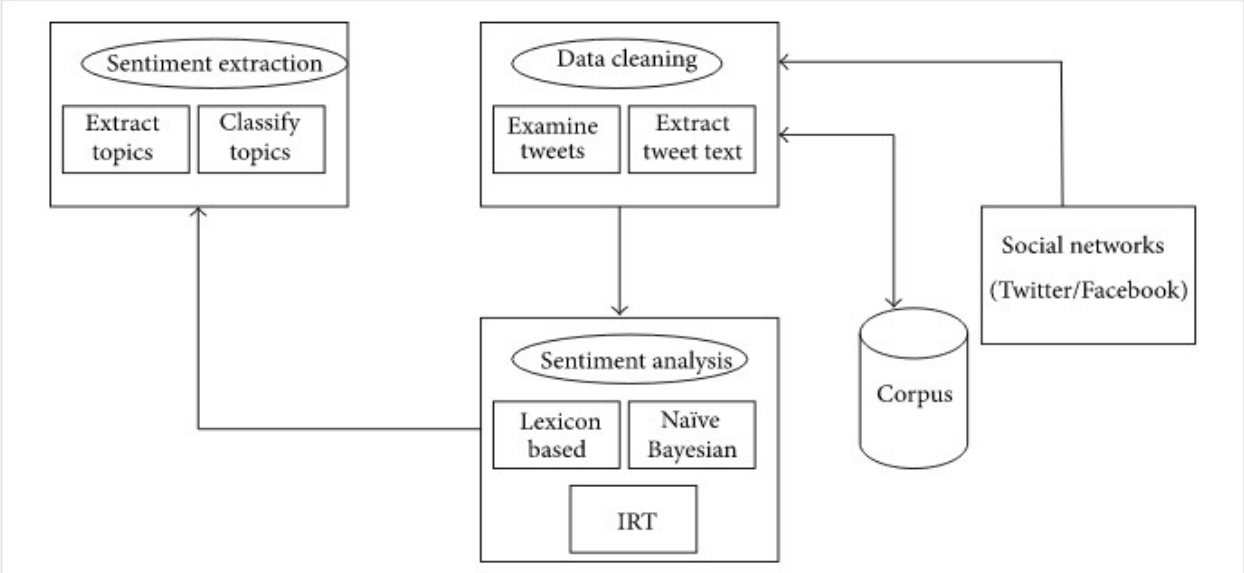

Figure 7: Model Architecture

6. CONCLUSION & Future WORKS

In this paper we provided insights into the series and sequence of steps involved in the statistical modelling of machine learning parameters for the development of a sentiment analysis framework for social media messages. Future works will seek to implement the model

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Antibacterial Effect Of Bacillus species Biomolecules (Metabolites, Silver Nanoparticles, And Functionalized Silver Nanoparticles) Against Multi-Drug Resistance Listeria species

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ABSTRACT

The study investigated the anti-listeria activity of *Bacillus subtilis* (BsaM); *Bacillus licheniformis* (BlbM and *Bacillus megaterium* (BmcM) against seven *List*eria species. Metabolites from *Bacillus* spp. were used for silver nanoparticles (BsaSNPs, BlbSNPs, and BmcSNPs) biosynthesis. The Silver Nanoparticles (SNPs) characterization was done using visual observation, UV-Visible Spectrophotometric, FTIR, SEM, and EDXRF. The anti-listeria synergistic potential of the biosynthesized SNPs and functionalized SNPs against Multidrug resistance (MDR) *Listeria* strains was evaluated. The BsaM, BlbM, and BmcM had a varied anti-listeria effect against the *Listeria* spp. 71.43 %, 100 %, and 5.71 % of the *Listeria* spp. were susceptible to the BsaM, BlbM, and BmcM metabolites respectively. The metabolite bio-actively reduced $AgNO_3$ for BsaSNPs, BlbSNPs, and BmcSNPs production. Surface Plasma Resonance (SPR) peaks of 600 nm, 400, and 600 nm, and 600 nm were recorded for BsaSNPs, BlbSNPs, and BmcSNPs. The shape of the SNPs were aggregated, rods, and crystalline. The functional groups present in the nanoparticles were carboxylic acid, amino acid alcohol, esters, and aldehydes and Ag had the highest intensity. BsaSNPs, BlbSNPs, and BmcSNPs had the highest anti-listeria potential of 20.0mm, 16.0mm, and 22.0mm against *Listeria innocua*LA22A and *Listeria* ivanoviiLA6. Functionalized BsaSNPss, BlbSNPs, and BmcSNPs had the highest anti-listeria activity compared to the metabolites and commercial antibiotics. CiprofloxacinBsaSNPs, ErythromycinFBlbSNPs, and ErythromycinFBmcSNPS had the highest antagonistic activity (28, 26, and 27 mm) against *Listeria ivanovii*LA6 respectively. The *Bacillus* strain's metabolites, the SNPs, and functionalized SNPs exhibit antimicrobial activities against Multidrug resistance *Listeria* species. Functionalization improved the anti-listerial ability of the antibiotics.

Keywords: *Bacillus* species, Anti-listeria activity, SNPs, MDR *Listeria* species, Functionalization.

1. INTRODUCTION

Antibiotic resistance (ATR) poses serious and dangerous menace to food security, populace health and global development. Geometrical increase in ATR and occurrence and prevalence of new emerging and re-emerging infections is of great public health concern (WHO, 2018). The emergence and re-emergence of Multi-Drug Resistant (MDR) are posing a big challenge to global community health (Halawani, 2016; Kamani and Lim, 2013). Recent researches have raised a red flag on the increasing zoonotic, nosocomial, and community-acquired infections due to the appearance of MDR pathogenic microorganisms which currently defy antibiotic therapies. Antimicrobial resistance as a result of improper utilization of antibiotics and the formation of resistant genes is now one of the major threats to human health (Rai et al., 2014; Halawani, 2016). Antimicrobial resistance (AMR) is a threat to the prophylaxis and treatment of different microbial infections [1]. Listeriosis is an important emerging zoonotic disease common in humans after consumption of contaminated animal food products (Van de Venter, 1999). Listeria infection is a disease caused by *Listeria* species and *Listeria monocytogen* is an etiological agent of Listeriosis in humans and some mammals (Garedew, 2015). Cases of listeriosis from ready–to–eat (RTE) food have been reported worldwide (SU, 2018).

As a result of the dangerous increase in ATR and the spread of new emerging and re-emerging infections globally growing number of infections are difficult to treat due to the ineffectiveness of antibiotics which causes longer hospitalization, expensive medication, and higher morbidity and mortality rate (WHO, 2018). Hence a need to gear research towards the development of new and broad spectrum antimicrobials to combat the ATR problems. Some species from the genus Bacillus were reported as a producer of secondary metabolites that have antimicrobial activity against diseases causing microorganisms (Katz and Demain 1977). The use of antimicrobial producing *Bacillus* spp. or their bioactive metabolites may be a supplementary means to synthetic chemicals for human protection (Leifert et al. 1995; Berger et al. 1996). Their antibiotics have a broad spectrum of antimicrobial, anti-viral, anti-ameobocytic, and anti-mycoplasma activities against microbial infections (Steller et al., 1999).

Bioactive metabolites from Bacillus strains have a broad use in the medicine and pharmaceutical industry as antimicrobials and bio-control agents in the treatment of plants, animals, and human diseases (Leifert et al., 1995). Nanoparticles have found a wide application in various fields of science and biomedical (Herrera et al., 2001). As a result of increasing drug resistance and growing concern regarding the over-prescription of antibiotics, has led to an emerging interest in the use of silver nanoparticles as antimicrobial agents. Unlike antibiotics, silver appears to be immune to resistance (Rai et al., 2009). The strong bactericidal potential of silver and its nanoparticles on MDR strains is of great help in tackling Antimicrobial resistance (ATR) (Rai et al., 2009).

The prevalence of microbial resistance to available antibiotics is at an alarming rate most especially in developing countries. As a result of this, therapeutic options have appeared more limited than ever. The emergence and re-occurrence of Multi drug resistance disease causing microorganisms have made research interest gear towards the development of efficient and safe antimicrobials. There's a dearth of information on the anti-listeria potential of SNPs against MDR *Listeria* strains. Functionalization of the existing antibiotics with nanoparticles will be of added advantages to improve their efficacy and effectiveness, hence a necessity for this research. The research aimed at evaluation of *in-vitro* anti-listeria potential of *Bacillus* species metabolites, its SNPs, and antibiotics functionalized SNPs against MDR *Listeria* strains.

2. MATERIALS AND METHODS

2.1 Culture collection

Cultures of *Bacillus subtilis, Bacillus licheniformis*, and *Bacillus megaterium* were gotten from the Microbial biotechnology Unit culture collection in the Department of Microbiology, University of Ibadan, Ibadan, Nigeria. Multi-Drug Resistant (MDR) *Listeria* species (*Listeria* sp. LB18, *Listeria ivanovii* LA6, *Listeria ivanovii* LA20A, *Listeria* spp. LA16, *Listeria innocua* LA22A, *Listeria monocytogenes* LA1X1, and *Listeria innocua* LB21X) were obtained from the Environmental Unit Culture Collection in the Department of Microbiology, University of Ibadan, Ibadan.

2.2 Production of metabolites from the Bacillus species and antagonistic activity against the test pathogens

Bioactive metabolites were produced using *Bacillus* spp. The pure culture of the isolates was inoculated in sterile Nutrient broth (NB). The broths were kept at 35°C for 72 hrs. The supernatant obtained from the centrifuged (5000 rpm for 20 minutes) fermentation broth was used as bioactive metabolites (BsaM, BlbM, and BmcM). The anti-listeria potential of the BsaM, BlbM, and BmcM against the MDR *Listeria* species was done using Agar Well Diffusion method (Balashanmugam et al., 2013).

Cell suspension of the isolate was prepared according to McFarland standard. An aliquot of the suspension was spread on the surface of Mueller Hinton agar plates with a sterile cotton swab. Wells were bored on the dried inoculated plates using a sterile cork borer (6 mm). The wells was filled with 100 µL of the bioactive metabolites and incubated at 37°C for 24hrs. Zones of inhibition (ZOI) around the well were recorded.

2.3 Green synthesis and characterization of SNPs using BsaM, BlbM, and BmcM

SNPs were produced by adding 1mL of the BsaM, BlbM, and BmcM to 10mL of 10mM $AgNO_3$ solution respectively. The reaction mixture was incubated in the dark at room temperature for 3 days for biological reduction of $AgNO_3$ for silver nanoparticles synthesis. After 24hrs incubation, the colourless solution turned darkish brown which indicates BsaSNPS, BlbSNPs. and BmcSNPs formation (Balashanmugam et al., 2013). Visual observation of the green produced nanoparticles was done by checking the reaction mixture for changes in colour to darkish brown which indicates SNPs formation in comparison to the control. The reduction of Ag^+ to Ag^0 was observed using UV-Visible spectrophotometer (a Lambda 25-Perkin Elmer, Waltham, MA, USA). 1 mL of each of the samples was withdrawn after 24hrs, 48hrs and 72hrs and absorbance was measured at wavelengths of 200-1000nm with a resolution of 0.5 nm.

The functional groups present in the SNPs were determined using FTIR spectroscopy (Shimadzu). The dried samples and KBr pellets were pressed into a mold. The spectra were taken at a wave range of 500-4000cm^{-1} and a resolution of 4cm^{-1}. The size, shape, and morphology of the nanoparticles were determined by subjecting the SNPs aqueous solution to Scanning Electron Microscopy (SEM).
The purity of the nanoparticles was determined and metal components distribution within the biosynthesized SNPs was determined using EDX.

2.4 Anti-listeria activity of the biosynthesized SNPs against the MDR Listeria species

Anti-listeria potential of the biosynthesized SNPs against the MDR *Listeria* species was evaluated using the Agar Diffusion method as previously described.

2.5 Functionalization of the SNPs with some antibiotics and snergistic effect of the functionalized nanoparticles

BsaSNPS, BlbSNPs. and BmcSNPs were functionalized with some antibiotics (Chlortetracycline (30µg), Amoxicillin (10µg), Colistin (10µg), Oxytetracycline (30µg), Gentamycin (10µg), Erythromycin (15µg). and Ciprofloxacin (5µg)) respectively. For the production of functionalized nanoparticles, 0.1 mL of antibiotic solution was mixed with 2 mL of the biosynthesized silver nanoparticles. The reaction mixture was incubated at 35°C for 24 hrs for proper adhesion of the antibiotics to the surface of the nanoparticles.

The anti-listeria potential of nanoparticles, the functionalized BsaSNPS, BlbSNPs, and BmcSNPs, and antibiotics alone against the MDR Listeria spp. was evaluated using Agar Well Diffusion method. The lawn of the indicator strain was spread plated on Mueller Hinton agar. The holes (6 mm) were filled with SNPs, antibiotics only and functionalized samples. The plates were kept at 37°C for 24 hrs and zone of clearance around the wells were recorded. Clear zone around the well indicates antibacterial activity (Begum et al., 2009).

2.6 Determination of Minimum Inhibitory Concentration (MIC) of the biosynthesized SNPs

The MIC of the nanoparticles was determined using the Agar Well Diffusion method (Begum et al., 2009). Two fold dilutions of the nanoparticles was done using distilled water to prepared different concentrations (100%, 50%, 25%, 12.5%, 6.25%, and 3.125%) of the SNPs. Cell suspension of *Listeria* spp. was spread plated on Mueller Hinton agar. Sterile cork-borer was used to punch holes (6 mm) in the agar. The hole was filled with 100µL of the synthesized SNPs (BsaSNPS, BlbSNPs. and BmcSNPs). The lowest dilution of the SNPs (BsaSNPS, BlbSNPs. and BmcSNPs) at which zones of inhibition were recorded against the test microorganisms was taken as the MIC for each SNPs. The positive control was ciprofloxacin.

3. RESULTS

3.1 Production of bioactive metabolites and anti-listeria potential of Bacillus species against MDR Listeria species

Productions of bioactive metabolites (BsaM, BlbM, and BmcM) were done using the Bacillus spp. (Bsa, Blb, and Bsc). Table 1 shows the anti-listeria potential of the metabolites against the MDR *Listeria* spp.. The metabolites from the *Bacillus* species exhibited varied anti-listeria activity against the 7 Multidrug resistance *Listeria* spp. The anti-listeria activity of BsaM, BlbM, and BmcM ranged from 5.0 – 16.0 mm, 10.0 – 20.0 mm, and 6.0 – 14.0 mm. The highest antagonistic activity was against *Listeria ivanovii*LA20A follow in order by *Listeria innocua* respectively. 71.43 %, 100 %, and 5.71 % of the *Listeria* spp. were susceptible to the BsaM, BlbM, and BmcM metabolites respectively. Comparatively, BlbSNPs metabolite had the highest antagonistic potential against the *Listeria* species.

Table-1: Antibacterial activity of *Bacillus subtilis* (BsaM); *Bacillus licheniformis* (BlbM) and *Bacillus megaterium* (BmcM) metabolites against some MDR *Listeria* species

Listeria species	Antibacterial activity (mm) of the metabolites		
	BasM	BlsM	BmcM
Listeria sp. LB18	0	11.0	7.0
Listeria ivanovii LA6	16.0	18.0	14.0
Listeria ivanovii LA20A	9.0	20.0	8.0
Listeria spp. LA16	0	13.0	11.0
Listeria innocua LA22A	10.0	15.0	13.0
Listeria monocytogenes LA1X1	5.0	14.0	6.0
Listeria innocua LB21X	8.0	10.0	0

The ability of the metabolites of *Bacillus* species to inhibit the test isolates may be due to the presence of bioactive compounds present in the metabolites. Antagonistic activity of *Bacillus* species against some of the test pathogens agrees with the report of Ashim et al. (2002) on the antimicrobial activity of *B. megaterium* against some pathogens. Similarly, Silambarasan and Abraham, (2012) reported the antimicrobial activity of *Bacillus* sp. against some pathogens.

3.2. *Characterization of the greenly synthesized SNPs*

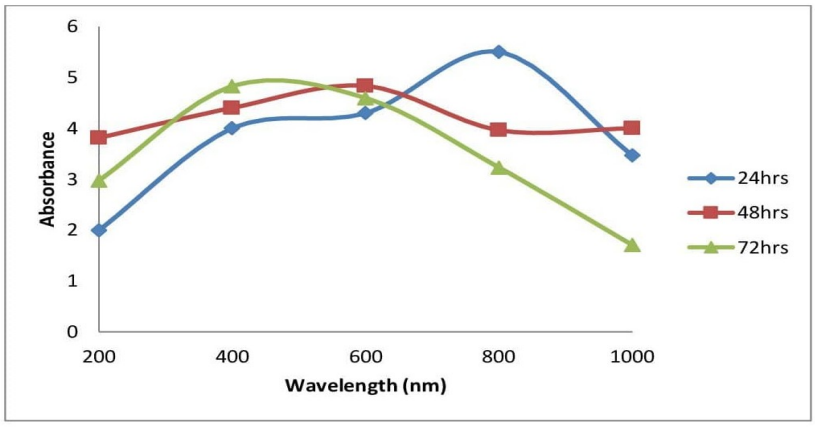
3.2.1 Visual Detection

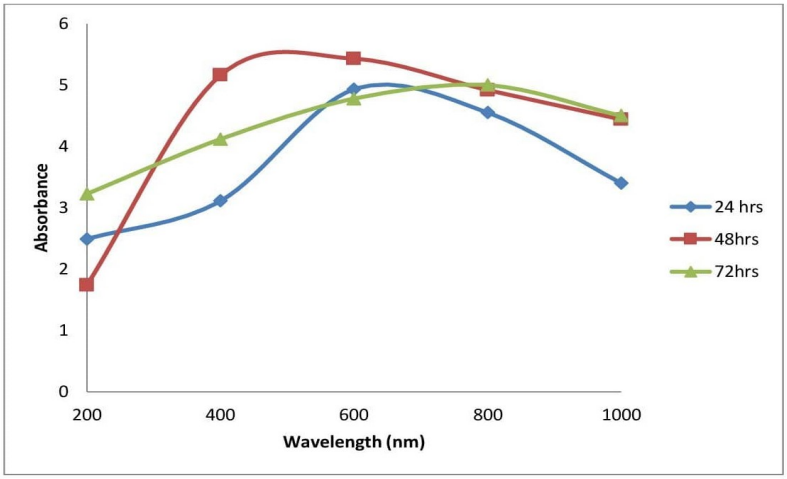
The visual observation of the BsaSNPs, BlbSNPs, and BmcSNPs was shown in Figure 1 a-c. The reaction mixture turned darkish-brown after keeping for 2 hrs respectively which indicate the formation of SNPs.

Figure-1: Visual characterization of (a) *Bacillus subtilis* (BsaSNPs), (b) *Bacillus licheniformis* (BlbSNPs) and (c) *Bacillus megaterium* (BmcSNPs) produced at 72 hrs

3.2.2. *UV –visible determination of the BsaSNPs, BlbSNPs, and BmcSNPs*

The UV –visible spectra of the BsaSNPs, BlbSNPs, and BmcSNPs at different incubation times are shown in Figure 2 a-c. BsaSNPs had a broad spectrum range between 400nm to 800nm at 24 -72 hrs. Surface Plasmon Resonance (SPR) peak was observed at 600nm. BlbSNPs had an SPR peak at 400nm, 600 nm, and 800 nm at 24, 48, and 72 hrs respectively. BmcSNPs had an SRP of 600 nm, 600 nm and 800 nm at 24, 48, and 72 hrs respectively.





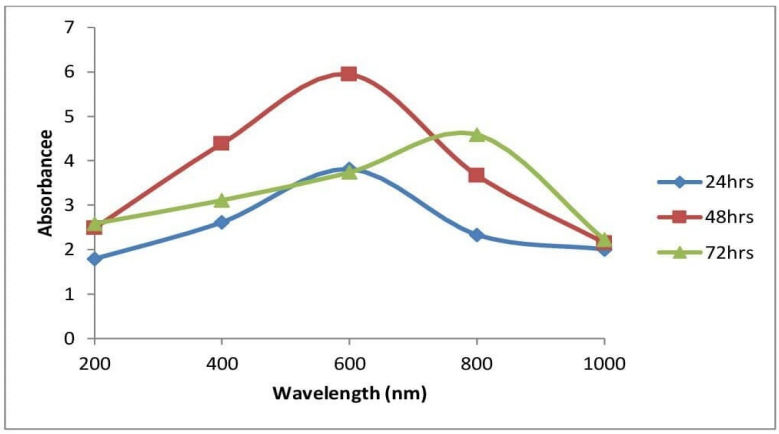


Figure-2a-c: UV-Visible spectra of (a) *Bacillus subtilis* Silver Nanoparticles (BsaSNPs), (b) *Bacillus licheniformis* Silver Nanoparticles (BlbSNPs) and (c) *Bacillus megaterium* Silver Nanoparticles (BmcSNPs) at different incubation time.

3.2.3. Fourier Transform Infra-Red (FTIR) determination of BsaSNPs, BlbSNPs, and BmcSNPs

BsaSNPs was characterized using FTIR and 7 bands were present at 3448.00, 2937.14, 1638.92, 1552.59, 1404.40, 1103.65, 607.24cm^{-1} as shown in Figure 3a. The peak indicated O-H stretch of alcohol, presence of C-H symmetrical stretch, presence of amide and NH bend respectively. The presence of C=O stretch was shown by the peak at 1435.09. The peak at 1103.65 indicated the presence of a C-O stretch of alcohol. The peak at 607.24 signified the presence of acetylenic CH of alkynes. The functional groups observed generally signified the presence of amino acids, alcohol, aldehydes and carboxylic acid in the sample may be responsible for the reduction of silver nitrate to SNPs (Figure 3a).

BlbSNPs were characterized by FTIR as shown in Figure 3b. The spectrum has 9 peaks ranging between 3423.00 cm^{-1} and 352.84 cm^{-1}. The peak at 3423.00 cm^{-1} could be attributed to the O-H stretch vibration of alcohol. The peak at 2948.57 cm^{-1} indicated the stretching vibration of symmetrical C-H. The peak at 1641.33 cm^{-1} and 1566.83 cm^{-1} corresponded to the C=O stretch of carboxylates and NH stretch of secondary amides. The absorption peak at 1412.23 cm^{-1} indicated the presence of O-H bends of esters, phenol, and tertiary alcohol or C-C bend of aldehyde. The absorption peaks at 1266.67 cm^{-1} and 1108.18 cm^{-1} signified C-O stretching vibration of carboxylic esters and C-O stretching vibration of a secondary alcohol.

The peak at 610.24 indicated the presence of acetylenic CH of alkynes. The peak at 352.84 depicts the presence of a weak peak of an aromatic benzene ring. Therefore from the FTIR spectrum observed, it was clear that the SNPs were surrounded by carboxylic acid, aldehyde, esters, protein, and amino acids which may be responsible for the biosynthesis and stability of the SNPs (Figure 3b).

BmcSNPs were characterized by FTIR and the spectrum observed is shown in Figure 3c. The spectrum showed 12 absorption peaks which ranged between 3760.00 cm^{-1} and 361.39 cm^{-1}. The peak at 3760.00 cm^{-1} signified the presence of an O-H stretch-free, strong alcohol. The absorption peak at 3417.00 cm^{-1} could be attributed to O-H stretch vibration of alcohol and 2938.61 cm^{-1} could be attributed to C-H symmetrical stretching. The peak at 2114.28 cm^{-1} indicated the carbonyl stretching of transition metals. The absorption peak at 1648.28 cm^{-1} and 1575.38 cm^{-1} corresponded to the NH bend of amide and NH stretch of secondary amides respectively.

The absorption peaks between 1266.70 cm^{-1} and 1110.12 cm^{-1} indicated a C-O stretch of esters and carboxylic acids. The absorption peak at 612.00 cm^{-1} indicated the presence of acetylenic CH of alkynes. The peak at 361.39 indicated weak aromatic benzene. The functional groups observed generally indicated the presence of amino acids, alcohol, aldehydes and carboxylic acid in the sample may be responsible for the reduction of silver nitrate to SNPs (Figure 3c).

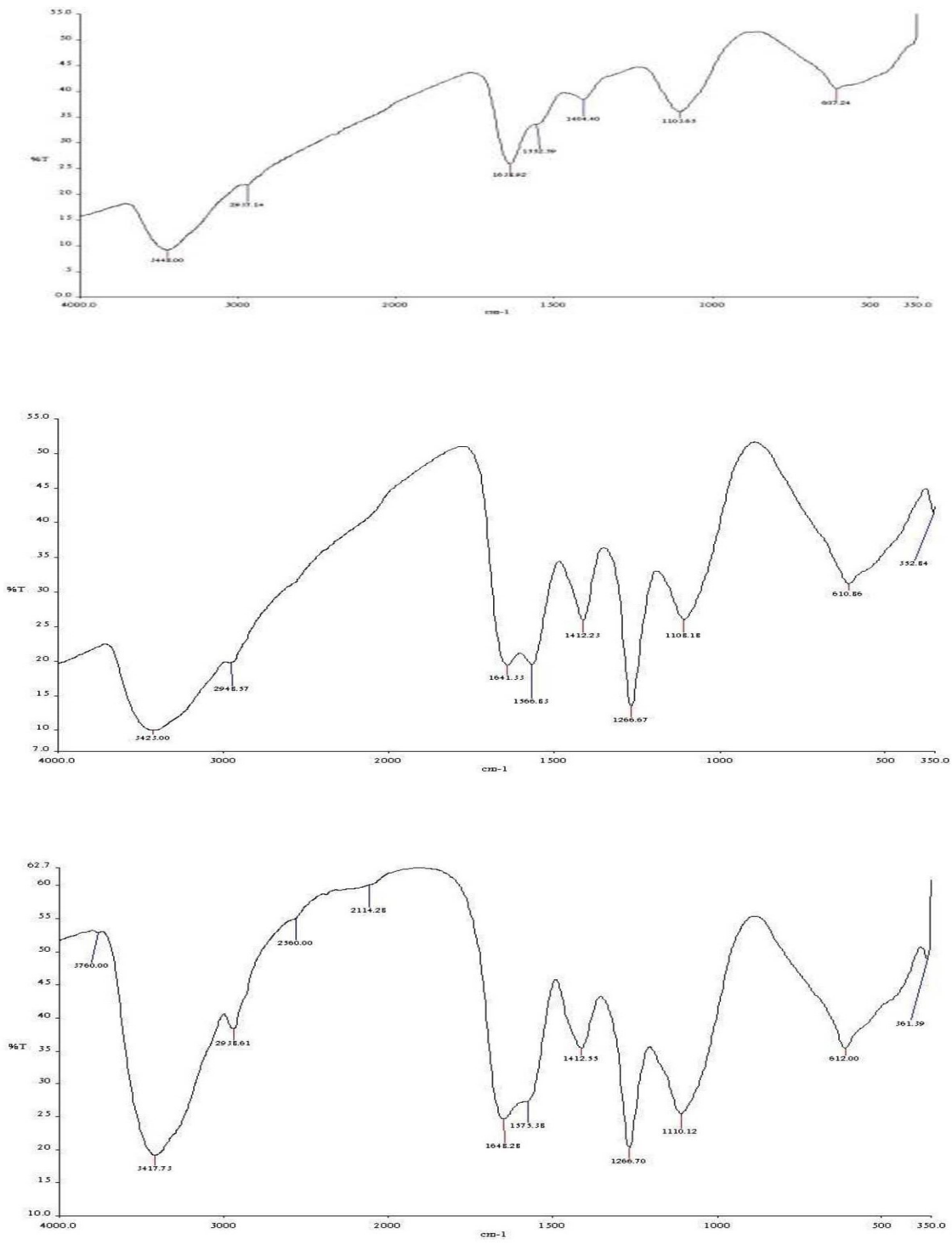


Figure-3a-c: FTIR spectra of (a) *Bacillus subtilis* Silver Nanoparticles (BsaSNPs), (b) *Bacillus licheniformis* Silver Nanoparticles (BlbSNPs) and (c) *Bacillus megaterium* Silver Nanoparticles (BmcSNPs)

3.4. *Scanning Electron Micrograph of BsaSNPs, BlbSNPs, and BmcSNPs*

The Scanning Electron Micrograph of BsaSNPs, BlbSNPs, and BmcSNPs are shown in Figure 4 a-c. BsaSNPs, BlbSNPs, and BmcSNPs were aggregate, rod, and crystalline in shape respectively.

Figure-4 a-c: Scanning Electron Micrograph of (a) *Bacillus subtilis* Silver Nanoparticles (BsaSNPs), (b) *Bacillus licheniformis* Silver Nanoparticles (BlbSNPs) and (c) *Bacillus megaterium* Silver Nanoparticles (BmcSNPs)

3.5. *Energy Dispersion X-Ray Fluorescence Analysis of the SNPs*

Characterization of the biosynthesized silver nanoparticles using Energy dispersion X-Ray fluorescence (EDXRF) was done at a voltage of 40.0KV and a current of 350μA. EDX analysis of BsaSNPs, BlbSNPs, and BmcSNPs was shown in Figure 5a-c. The intensity ranged from 0.0001 – 0.2976, 0.0001 – 0.1318 and 0.0001 – 0.2500 respectively. Ag had the highest intensity. Elements such as Magnesium (Mg), Potassium (k), Chromium (Cr), Lead (Pb), Gold (Au), and Cadmium (Cd) had zero intensity and content. The intensity and content observed generally indicated the reduction of $AgNO_3$ and subsequent formation of SNPs.

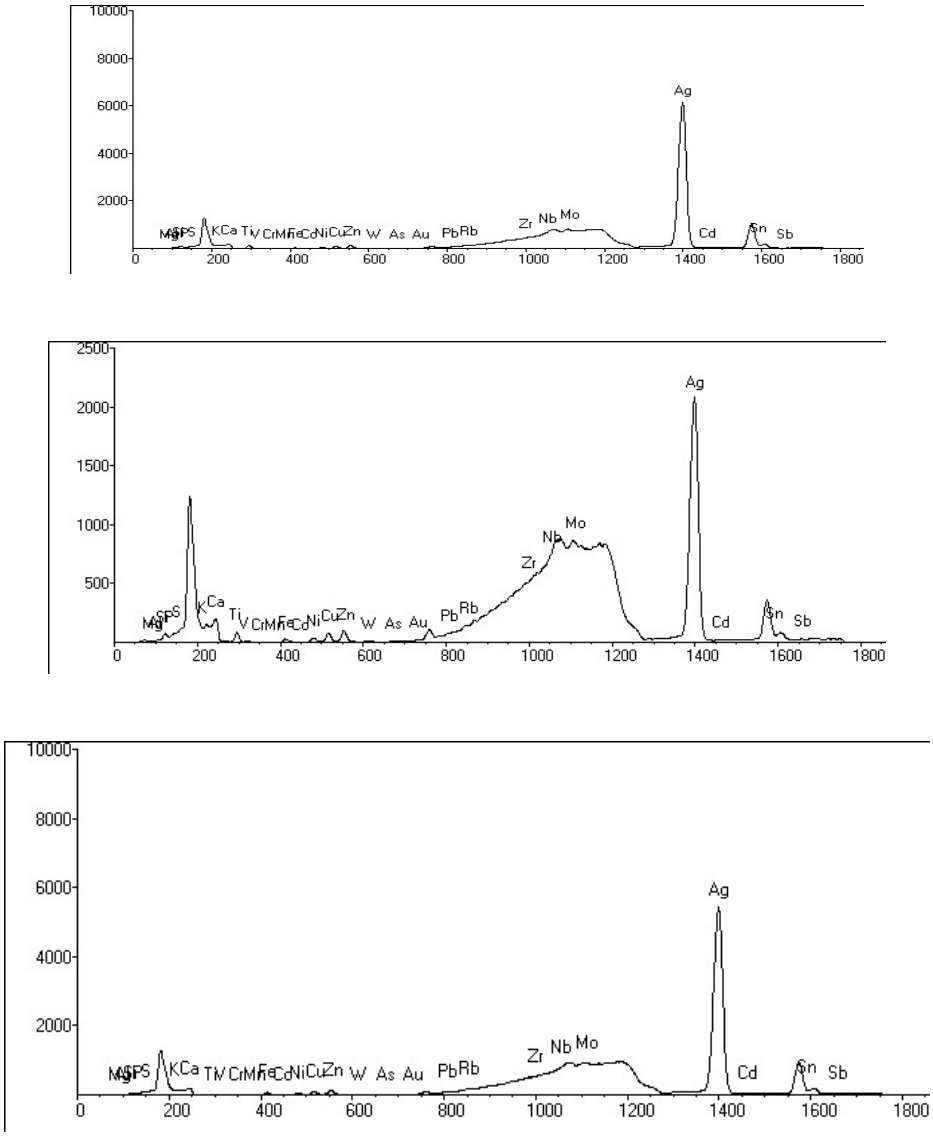


Figure-5a-c: EDX analysis of (a) *Bacillus subtilis* Silver Nanoparticles (BsaSNPs), (b) *Bacillus licheniformis* Silver Nanoparticles (BlbSNPs) and (c) *Bacillus megaterium* Silver Nanoparticles (BmcSNPs)

3.6. *Antagonistic activity of the nanoparticles*

Antibacterial activity of BsaSNPs, BlbSNPs, and BmcSNPs against the MDR *Listeria innocua* LA22A and *Listeria ivanovii* LA6 is shown in Table 2. The antagonistic activity ranged from 9.0 – 18.0 mm and 13.0-19.0 mm, *Listeria ivanovii* L6 and *Listeria innocua* L22A had the highest susceptibility to BlbSNPs.

Table 2: Antibacterial activity of the biosynthesized SNPs from the three (3) selected *Bacillus* species against the selected (MDR) *Listeria* spp.

Biosynthesized SNPs	Antagonistic activity (mm) of the nanoparticles against the MDR *Listeria* spp.	
	Listeria innocua LA22A	*Listeria ivanovii*LA6
BsaSNPs	9.0	17.0
BlbSNPs	18.0	19.0
BmcSNPs	11.0	17.0
Ciprofloxacin	10.0	12.24
$AgNO_3$	17.00	13.0

The MIC of BsaSNPs, BlbSNPs, and BmcSNPs against the MDR *Listeria innocua* LA22A and *Listeria ivanovii* LA6 is shown in Table 3. BsaSNPs, BlbSNPs and BmcSNPs had a MIC of 12.5%, 6.25% and 6.25% against *Listeria innocua*LA22A and MIC of 6.25%, 3.125% and 6.25%, respectively, against Listeria ivanoviiLA6. Generally, all the SNPs had the highest MIC while the least MIC of all the SNPs was on *Listeria innocua*LA22A.

Table 3: Determination of MIC of biosynthesized SNPs on the selected (MDR) *Listeria* species

MIC Concentration (%)	Antagonistic activity (mm) of the nanoparticles					
	MDR *Listeria innocua* LA22A			MDR *Listeria ivanovii* LA6		
	BsaSNPs	BlbSNPs	BmcSNPs	BsaSNPs	BlbSNPs	BmcSNPs
50	14.0	10.0	12.0	15.0	18.0	15.0
25	11.0	10.0	10.0	12.0	14.0	16.0
12.5	10.0	6.0	12.0	9.0	11.0	12.0
6.25	00	6.0	5.0	8.0	7.0	9.0
3.25	00	00	00	00	5.0	00
Ciprofloxacin	12.0	12.0	9.0	11.0	13.0	10.0

3.6. *Anti-listeria potential of the nanoparticles, selected antibiotics, and functionalized antibiotics against the MDR Listeria species*

The antagonistic activity of the silver nanoparticles (BsaSNPs/ BlbSNPs/BmsSNPs), the antibiotics, and the functionalized nanoparticles (FSNPs) against *Listeria innocua*LA22A and *Listeria ivanovii*LA6 is shown in Table 4a. Antagonistic activity of BsaSNPs, the antibiotics, and the functionalized BsaSNPs against *Listeria innocua* LA22A and *Listeria ivanovii* LA6 ranged from 4.0-20.0 mm and 10.0 – 28.0 mm. LA22A had the highest susceptibility to Ciprofloxacin FBsaSNPs. *Listeria ivanovii*LA6 showed high susceptibility to Erythromycin, Amoxicillin, and GentamycinFBsaSNPs with antagonistic zones of 25.0 mm and 24.0 mm respectively. *Listeria innocua* LA22A had the lowest susceptibility to Chlorotetracycline FBsaSNPs while LA6 had the lowest susceptibility to Oxytetracycline.

*Listeria innocua*LA22A was not susceptible to oxytetracycline and Oxytetracycline FBsaSNPs. *Listeria ivanovii*LA6 were susceptible to the nanoparticles, the antibiotics, and the functionalized nanoparticles. All the functionalized BsaSNPs had better antagonistic activity against *Listeria ivanovii*LA6. Antagonistic activity of BlbSNPs, the antibiotics, and the functionalized BlbSNPs against *Listeria innocua*LA22A and *Listeria ivanovii*LA6 ranged from 6.0 – 24.0 mm and 10.0 – 26.0 mm. Ciprofloxacin FBlbSNPs had the highest antagonistic effect against *Listeria innocua* LA22A while Erythromycin FBlbSNPs had the highest antagonistic activity against *Listeria* ivanovii LA6. The anti-listeria activity of the BlbSNPs had better anti-listeria activity against *Listeria innocua* LA22A compared to ordinary antibiotics and the functionalized antibiotics except for Ciprofloxacin FBlbSNPs. 2 of the antibiotics and 4 of the functionalized BlbSNPs exhibited a better antibacterial activity against *Listeria ivanovii*LA6. Functionalization improves the antagonistic potentials of some of the antibiotics.

Table 4a: The antagonistic activity of the SNPs (BsaSNPs / BlbSNPs / BmsSNPs), the antibiotics, and the functionalized SNPs from *Bacillus* spp. metabolites against *Listeria innocua*LA22A and *Listeria ivanovii*LA6

SNPs/Antibiotics/functionalized antibiotics	Antagonistic activity (mm)					
	BsaSNPs		BlbSNPs		BmsSNPs	
	Listeria innocua LA22A	*Listeria ivanovii*LA6	*Listeria innocua* LA22A	*Listeria ivanovii*LA6	*Listeria innocua* LA22A	*Listeria ivanovii*LA6
SNPs(BsaSNPs/ BlbSNPs/BmsSNPs)	9.0	17	18	19	11	17
Chlorotetracycline	11	20	11	20	11	20
Amoxicillin	14	19	14	19	14	19
Colistin	8.0	15	8.0	15	0	15
Oxytetracycline	0	10	0	10	0	10
Gentamycin	12	21	12	21	12	21
Erythromycin	10	17	10	17	10	17
Ciprofloxacin	15	13	15	13	15	13
Chlorotetracycline FSNPs	4.0	22	6.0	19	10	25
Amoxicillin FSNPs	20	24	13	12	11	20
Colistin FSNPs	12	21	10	18	0	11
Oxytetracycline FSNPs	0	14	14	22	0	22
Gentamycin FSNPs	15	24	16	23	10	20
Erythromycin FSNPs	9.0	25	7.0	26	6.0	27
Ciprofloxacin FSNPs	16	28	24	24	10	30
$AgNO_3$	17	13	17	13	12	21

Antagonistic activity of BmcSNPs, the antibiotics, and FBmcSNPs against *Listeria innocua* LA22A and *Listeria ivanovii* LA6 ranged from 6.0 -15.0 mm and 10.0 – 30.0 mm. The highest antagonistic activity against *Listeria innocua*LA22A was recorded when Ciprofloxacin was used while Ciprofloxacin FBmcSNPs had the highest activity against *Listeria ivanovii* LA6.

ErythromycinFBmcSNPs and Oxytetracycline had the lowest antagonistic activity against *Listeria innocua*LA22A and *Listeria ivanovii*LA6 respectively. *Listeria innocua*LA22A was not susceptible to Colistin, Oxytetracycline, Colistin FBmcSNPs, and Oxytetracycline FBmcSNPs. *Listeria ivanovii* LA6 were 100 % susceptible to BmcSNPs, the antibiotics, and the FBmcSNPs respectively. 18.75 % (3) and 43.75% (7) of the antibiotics and the FBmcSNPs had higher activity against *Listeria ivanovii*LA6 compared to BmcSNPs.

5. DISCUSSION

The metabolites from *Bacillus* species biologically reduce $AgNO_3$ for silver nanoparticles production. Vithiya et al. (2014) reported *Bacillus* spp. produced silver nanoparticles extracellularly. El-Batal et al. (2013) reported the antimicrobial activity of the SNPs produced by *B. stearothermophilus.* Kamani and Lim, (2013) reported the biosynthesis and structural evaluation of silver nanoparticles using bacterial exopolysaccharides and their antimicrobial effect against food and Multi-Drug Resistant pathogens. Colour changes from darkish brown is an indication of the silver nanoparticles formation from *Bacillus* species. This is in agreement with the work of Nanda and Raghavan, (2014) on colour change of EPS supernatants to yellowish-brown. Furthermore, the green production of silver nanoparticles using *Bacillus subtilis* and its antimicrobial potential has been reported by Natarajan and Selvaraj (2010).

Characterization of silver solution exposed to the supernatant of *Bacillus* species by UV-Visible spectrophotometer confirmed the biological reduction of silver ions to silver nanoparticles. The Surface Plasmon Resonance peak recorded for the nanoparticles produced from the samples was within the range of 500-600nm. This observation is also in contrast to the work of Vithiya et al. (2014) that extracellular *Bacillus* mediated SNPs showed a strong SPR peak at 400-430nm with broadband, indicating varied shape and size SNPs formation. The peak absorbance of SNP produced by *Bacillus subtilis* investigated by Natarajan and Selvaraj (2011) was 440nm, this is contrary to the absorbance peak of 500-600nm which was evaluated in this present study. Shahverdi et al. (2011) proposed that as the particle size increments caused narrower peaks and reduction in bandwidth and increased band intensity. This work is in contrast the report the of Balashanmugam et al. (2013) who reported 386 nm SPR.
The FTIR spectra measurement was carried out to inspect the available functional groups of the *Bacillus* species metabolites responsible for the biological reduction and stabilization of the SNPs. FTIR is very useful for SNPs characterization as reported by many authors (Kanmanni and Lim 2013; Magdi et al., 2014). The formation and stabilization of SNPs may be due to the release of extracellular protein molecules from *Bacillus* spp. (El-Batal et al., 2013). Carboxylic acid, amino acid, alcohol, esters, and aldehydes were the functional group present in the production of SNPs. This is similar to the work of Chitra and Annadurai (2013) who reported these functional groups as the reducing agents responsible for silver nanoparticles production. The stabilization of the nanoparticles may be as a result of the presence of proteins and amino acids in the metabolites.

Scanning Electron Microscope was further used to characterize the SNPs. SEM is an important tool for SNPs characterization (Begum et al., 2009; Balashanmugam et al., 2013). The greenly synthesized SNPs were partially aggregated, rod-shaped, and crystalline. The aggregation recorded may be as a result of the drying process. Sadowski et al. (2008) reported the effect of drying on the shape and size of SNPs from *Penicillium* strains. BIbSNPs were observed to be rod-shaped; this is agrees with the work of Gardea-Torresday et al. (2007) on gold nanoparticles from *Triticum aestivum* leaves.

BmcSNPs was a crystalline shape, Nanda and Raghavan (2014) reported that the shape of the biosynthesized SNPs from exopolysaccharides (EPS) produced by *Bacillus subtilis* was dispersed. Energy dispersion X-ray fluorescence was further used to characterize the biosynthesized SNPs. EDXRF is an essential tool for SNPs characterization that the EDXRF pattern peak is metallic silver (Vithiya et al., 2014). Kamani and Lim, (2013) has reported that EPS–reduced SNPs exhibited intense peak of silver ions. EDXRF optical absorption peak observed at 3keV is typical for metallic $AgNO_3$ (Magdi et al., 2014). The BsaSNPs, BlbSNPs, and BmcSNPs had antibacterial activity against MDR *Listeria* spp. The antagonistic activity may be due to functional groups present in the stabilized silver nanoparticles by the bioactive metabolites. This work also agrees with the report of Kanmanni and Lim (2013) who investigated the antibacterial activity of EPS-mediated SNPs against the pathogen *Escherichia coli*, the food-borne pathogen *Listeria monocytogenes*, and the MDR *Klebsiella pneumonia* and *Pseudomonas aeruginosa*.

MIC was checked with two MDR *Listeria* spp. The SNPs that were used to check the MIC was 10mM, different MICs were recorded for the two (MDR) *Listeria* spp. Balashanmugam et al. (2013) reported the highest MIC of their SNPs on some pathogens and *Escherichia coli* and *Bacillus subtilis*. The functionalization effect of the biosynthesized SNPs and some antibiotics was examined and the combined effect was more efficient than the SNPs alone and even the antibiotics alone. This result is in line with the findings of Krishna et al. (2015) using the biogenic synthesis of silver nanoparticles who reported an increase in who report an increase in antimicrobial activity of combination of antibiotics and silver nanoparticles. Yua et al. (2021) reported the enhanced antibacterial properties of silver nanoparticles from secondary metabolites of *Bacillus subtilis* Silver nanoparticles biosynthesis using *Bacillus licheniformis* isolated from Quail manure from Vietnam has been reported (Tan et al., 2021).

In conclusion, the *Bacillus* strains metabolites exhibit anti-listeria activity against the MDR *Listeria* species. The metabolites bio-reduce $AgNO_3$ for SNPs biosynthesis. The biosynthesized silver nanoparticles and combination of silver nanoparticles with some antibiotics had an antimicrobial effect on MDR *Listeria* strains. Hence, SNPs in combination with some antibiotics could be used as an alternative to commercial antibiotics to combat MDR *Listeria* species.

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Statement of Human and Animal rights
This article does not contain any studies with human and animal subjects performed by any of the authors and complied with all ethical standards.

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