A novel procedure on next generation sequencing data analysis using text mining algorithm

Weizhong Zhao\textsuperscript{1,2}, James J. Chen\textsuperscript{1}, Roger Perkins\textsuperscript{1}, Yuping Wang\textsuperscript{1}, Zhichao Liu\textsuperscript{1}, Huixiao Hong\textsuperscript{1}, Weida Tong\textsuperscript{1} and Wen Zou\textsuperscript{1*}

Abstract

Background: Next-generation sequencing (NGS) technologies have provided researchers with vast possibilities in various biological and biomedical research areas. Efficient data mining strategies are in high demand for large scale comparative and evolutionary studies to be performed on the large amounts of data derived from NGS projects. Topic modeling is an active research field in machine learning and has been mainly used as an analytical tool to structure large textual corpora for data mining.

Methods: We report a novel procedure to analyse NGS data using topic modeling. It consists of four major procedures: NGS data retrieval, preprocessing, topic modeling, and data mining using Latent Dirichlet Allocation (LDA) topic outputs. The NGS data set of the \textit{Salmonella enterica} strains were used as a case study to show the workflow of this procedure. The perplexity measurement of the topic numbers and the convergence efficiencies of Gibbs sampling were calculated and discussed for achieving the best result from the proposed procedure.

Results: The output topics by LDA algorithms could be treated as features of \textit{Salmonella} strains to accurately describe the genetic diversity of \textit{fliC} gene in various serotypes. The results of a two-way hierarchical clustering and data matrix analysis on LDA-derived matrices successfully classified \textit{Salmonella} serotypes based on the NGS data. The implementation of topic modeling in NGS data analysis procedure provides a new way to elucidate genetic information from NGS data, and identify the gene-phenotype relationships and biomarkers, especially in the era of biological and medical big data.

Conclusion: The implementation of topic modeling in NGS data analysis provides a new way to elucidate genetic information from NGS data, and identify the gene-phenotype relationships and biomarkers, especially in the era of biological and medical big data.

Keywords: Data mining, Topic modeling, Next-generation sequencing (NGS), Genetic diversity, Biomarker

Background

Next generation sequencing (NGS) [1] is a term that refers to post-Sanger sequencing methods. The primary advantage offered by NGS technologies over traditional sequencing methods is the production of large volumes of sequence data inexpensively and with a high degree of flexibility for the level of resolution required for given experiments. The production of large numbers of low-cost, high-quality sequences has enabled the scientific community to address an increasingly diverse range of biological and medical problems, including clinical diagnostics [2, 3], epidemiological investigation [4], species classification and gene discovery in metagenomics studies [5], virology [6] and genomic analysis [7].

Although NGS technologies are increasingly used in many areas, the large amounts of data produced by NGS technologies present a significant challenge for data analysis and interpretation. Advanced high-performance computing and intensive bioinformatics support is

\* Correspondence: wen.zou@fda.hhs.gov
\textsuperscript{1}Division of Bioinformatics and Biostatistics, National Center for Toxicological Research, U.S. Food and Drug Administration, 3900 NCTR Road, HFT-20, Jefferson, AR 72079, USA

Full list of author information is available at the end of the article

© 2016 Zhao et al. Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
The two matrices were then analyzed by hierarchical algorithm was run and two matrices were generated. The procedures for data preprocessing are shown in Fig. 1(b-d). The sequences in the constructed dataset were aligned by an algorithm of multiple sequence alignment (MSA), such as MUSCLE [27] or CLUSTAL [28], generating a dataset of aligned sequences. Better performance and accuracy was observed when comparing this method with Hamming distance method [21] by clustering analysis and classification. The results showed that the topic modeling provides a promising novel approach to analysis of NGS data for the purpose of understanding and decoding hidden genetic information in a biological system.

**Methods**

**Dataset construction**

In this study, the whole genome sequences of 119 strains (Additional file 1: Table S1) of *Salmonella* O antigen group B [22] were retrieved from the NCBI database, including 75 strains of *S. Agona*, 14 strains of *S. Heidelberg*, one strain of *S. Paratyphi B*, two strains of *S. Saintpaul*, two strains of *S. Schwarzengrund*, one strain of *S. Stanley*, 22 strains of *S. Typhimurium*, one strain of *S. Typhimurium* var.5- and one strain of *S. 4, 12:i:-* [23]. The dataset was constructed and preprocessed by our developed pipeline [24] as briefly described in the following: The retrieved sequence reads or contigs were collected to a data pool. For each strain, the sequence fragment best matching the reference *flIC* gene was selected by blasting with the serotype-specific reference *flIC*, using the Basic Local Alignment Search Tool (BLAST) [25]. In this study, the reference *flIC* genes used for the strains of *S. Agona*, *S. Heidelberg*, *S. Paratyphi B*, *S. Saintpaul*, *S. Schwarzengrund* and *S. Stanley* were those of *S. Agona* SL483, *S. Heidelberg* SL476, *S. Paratyphi B* SPB7, *S. Newport* SL254, *S. Schwarzengrund* CVM19633 and *S. Typhi* CT18, respectively. The strains of *S. Typhimurium*, *S. Typhimurium* var.5- and 1 *S. 4, 12:i:-* [26] used the *flIC* of *S. Typhimurium* LT2 as the reference gene. All the reference genes were retrieved from NCBI by the same pipeline. The metadata of the *flIC* gene-containing NGS data of 119 *Salmonella* strains are listed in Additional file 1: Table S1.

**NGS data preprocessing**

The procedures for data preprocessing are shown in Fig. 1(b-d). The sequences in the constructed dataset (Fig. 1(a)) were aligned by an algorithm of multiple sequence alignment (MSA), such as MUSCLE [27] or CLUSTAL [28], generating a dataset of aligned sequences (including insertions and deletions, or indels) (Fig. 1(b)). The nucleotide differences at each site, called single nucleotide polymorphisms (SNPs), were collected and are shown in Fig. 1(c). Each of the 119 strains had its corresponding file of words consisting of both SNPs and the locations of the SNPs in the sequences (Fig. 1(d)).
Fig. 1 Flowchart of the proposed procedure

(a) Original sequences

(b) Preprocessing

(c) Topic modeling

(d) Data mining
**Topic modeling**

After preprocessing, a text corpus was generated in which each of the documents corresponded to one of the 119 strains, and all the documents had the same number of words. The LDA program implemented in Mallet [29] was utilized to model the corpus to get the latent topics and the topic mixture distribution for each strain.

LDA is a generative probabilistic model whose graphical model representation is shown in Fig. 2. LDA generates a given corpus according to the following process [10]:

1. For each topic $k$, where $k$ in $\{1...K\}$, pick a distribution over words $\varphi_k \sim \text{Dir}(\beta)$;
2. For each strain $D_s$ where $s$ in $\{1...S\}$,
   a. Pick a distribution over topics $\theta_s \sim \text{Dir}(\alpha)$;
   b. For each word $w_n$ with $n$ in $\{1...N\}$,
      (1) Pick a topic $z \sim \text{Multinomial}(\theta_s)$;
      (2) Pick word $w_n \sim \text{Multinomial}(\varphi_z)$;

In the generative process, $\text{Dir}$ represented a Dirichlet distribution and $\text{Multinomial}$ represented a Multinomial distribution. The distributions of words for topics and distributions of topics for documents were viewed as random variables obeying Dirichlet distributions with parameters $\beta$ and $\alpha$, respectively. Words in documents were treated as random variables obeying the Multinomial distribution of topics.

Given a corpus of $S$ strains $D = \{D_1, D_2, ..., D_S\}$, the probability of the corpus:

$$ p(D|\alpha, \beta) = \prod_{s=1}^{S} \int p(\theta_s|\alpha) \left( \prod_{n=1}^{N} \sum_{z=1}^{K} p(z_n|\theta_s) p(w_{sn}|\varphi_z) p(\varphi|\beta) \right) d\theta_s d\varphi_z $$

(1)

In this study, Gibbs sampling [11], a special case of Markov chain Monte Carlo (MCMC) [16] approach, was used to sample posterior distribution of $\theta, Z_{sn}$ and $\varphi_{sn}$. The LDA algorithm was run on the corpus several times with different number of topics. For all the runs, we assigned $\alpha = 0.1$ and $\beta = 0.01$ as initial values for the two hyper-parameters, and 2000 iterations in Gibbs sampling. The default values were applied for the other parameters in the LDA model in Mallet.
Perplexity measurement
Leave-one-out cross-validation was used to measure the perplexity of LDA algorithm. The LDA algorithm was first trained by 118 samples, and then the obtained LDA model was applied to calculate the perplexity of the left-out sample. This process was repeated 119 times until each of the samples had been left out once. The average perplexity of the 119 samples was taken as the final perplexity for the corresponding number of topics.

Data mining
Strain characterization The per-document topic distributions and the per-topic word distributions were obtained after LDA processing. Words with high probability for given topics were selected to characterize and differentiate bacterial strains.

Two-way hierarchical clustering Two-way clustering is a data mining technique which allows simultaneous clustering of the rows and columns of a matrix. In the two-way hierarchical clustering analysis in this study, the topic mixture distributions of strains were viewed as the strain representatives. The dissimilarities between strains and topics were calculated by Euclidean distance of topic mixture distributions of strains. The result describes simultaneously the subgroups of samples and the relationships among topics. Hierarchical cluster analysis using the complete linkage was applied on the dissimilarities to perform a two-way hierarchical clustering. Function “heatmap.2” in package “gplots” of R [30] was utilized to implement the two-way clustering. The colors from blue to red indicate the values of the topic probabilities of the strains ranging from 0 to 1.

Distance Matrix Analysis In the distance matrix analysis, Euclidean distance measure was used to measure the dissimilarity between strains based on the strain-topic mixtures derived from the flic SNPs dataset. The program “dist” in package “stats” of R (with default values of parameters) [31] was utilized to calculate the Euclidean distances between all the strains. The colors from blue to red represent the values of the Euclidean distances as they range from 0 to 1.

Evaluation of topic modeling performance
To evaluate the performance of topic modeling, we applied clustering and classification algorithms on the sample-topic matrices (Fig. 1(f)) generated by LDA and similarity matrices generated by Hamming Distance [21], and the results were compared based on the serotypes (Additional file 1: Table S1) of the samples which were viewed as the sample’s true labels.

1. Hamming Distance measures
The documents (samples) in Fig. 1(d) were transformed into matrix of Vector Space Model (VSM) [32] which is a commonly used model for representing text documents. In the VSM matrix, the number of row is the number of samples and the number of column represents the size of the vocabulary. Using Hamming Distance measures, the traditional clustering and classification methods were conducted and the results were compared with those conducted on the sample-topic matrices from LDA (Fig. 1(f)).

2. Cluster analysis and result comparison

3. Classification analysis and comparison

Topic model-derived clustering method [33] was applied, in which LDA was utilized as a feature reduction approach for cluster analysis. The LDA-derived topics were considered as the new features of datasets. The sample-topic matrix (Fig. 1(f)) was treated as a new representation of the original dataset. Based on the sample-topic matrix (topic number was chosen as 5 and 30, respectively), conventional clustering algorithms, such as k-means, was used for the clustering analysis. The number of clusters was set as 7 in the k-means method due to 7 different serotypes in the dataset. While in comparison, k-means algorithm was also applied on VSM matrix using Hamming Distance similarities. For further comparison, due to the dimension reduction of topic modeling approach, the traditional tool of PCA was used to reduce features (Numbers of 2, 5, 10 and 30 were randomly selected as the reduced features, respectively) of VSM matrix followed by the k-means cluster analysis. Moreover, clustering by only LDA referred as “highest probable topic assignment” [33] (5 and 30 topics were used) was also used for comparison. In “highest probable topic assignment”, the LDA-derived topics were made as the clusters of the dataset. Then, each sample was assigned to the cluster (Topic) with the highest probability in the row of the sample-topic matrix. To interpret the clustering results obtained by the k-means algorithm, samples in each cluster were labeled as the dominant serotype of the samples in the cluster. The predicted labels of samples were compared with the true labels (serotypes) to evaluate the clustering quality. The clustering results were evaluated by Normalized mutual information (NMI) [34] and Adjusted Rand Index (ARI) [35]. NMI and ARI are two external validation metrics to evaluate the quality of clustering results with respect to the given true labels of datasets. The range of NMI and ARI values is 0–1. In general, the larger the value is, the better the clustering quality is.
Two commonly used classification algorithms, Support Vector Machine (SVM) [36] and Random Forest (RF) [37], were applied on the sample-topic matrix obtained by LDA with the topic number set to 5 and 30, respectively. SVM and RF were also applied to the VSM matrix for comparison. The accuracy rate was used to evaluate and compare the classification results. Since classification is a supervised learning task, training dataset is required to include all of the true labels in the testing dataset. Therefore, the new data set for classification consists of 117 samples with two serotypes (true labels) Paratyphi B and Stanley samples removed from the original dataset due to the insufficient samples. Leave-One-Out Cross-Validation (LOOCV) was conducted on the 117 samples and the predicted accuracy rate was calculated. In this study, the function “svm” (with “Polynomial” kernel and default values of other parameters) in R package “e1071” and function “randomForest” (number of trees setting as 500 and default values of other parameters) in R package “randomForest” were utilized to train the classifiers.

Results
In this study, we propose a novel procedure for applying the concept of topic modeling to the analysis and mining of NGS data. Assuming that the SNPs composition (four nucleotides and their locations) in NGS sequences can be considered and treated as “words”, the original sequence data are transformed to a file of “a bag of words”. A topic modeling procedure is then run on the document file to create two digital matrices, on which various data mining algorithms are applied to reveal the hidden genetic information in the sequences. A NGS data set of 119 Salmonella outbreak strains was used as a case study to show the workflow and its applications.

Procedure
Figure 1 shows a schematic representation of the proposed procedure to transform the original sequence data to two digital matrices by topic modeling. The data set, first constructed (Fig. 1(a)) by our developed pipeline [24], is described in detail in “Methods”. The best fliC gene-matching sequences from all of the 119 Salmonella strains were collected in the dataset (Fig. 1(a)). Nucleotides A, T, G, and C are shown by different colors. In the procedures for data preprocessing (Fig. 1(b-d)), the best fliC gene-matching sequences were multi-aligned and the variant nucleotides were found at 840 sites in 119 strains. All the nucleotides in the study at the 840 sites in the 119 sequences are designated as SNPs in this study (displayed in yellow in Fig. 1(b)). The collection of all the SNPs in the dataset (Fig. 1(c)) was then transformed into a text corpus, in which each of the 119 Salmonella strains had its unique file of words (Fig. 1(d)). The final vocabulary size is 2379 and the obtained corpus had a total of 99,960 words (occurrences) from the 119 strains.

LDA algorithms run on the corpus and generated two digital matrices: Fig. 1(e) shows per-topic word distributions; and Fig. 1(f) exhibits per-document topic distributions. The two matrices provided vast information pools available for data mining.

Topic analysis
LDA-derived topics classify a group of words which share the similar characteristics. Table 1 lists the top 10 most probable words in each of the five topics when the number of topics was set to 5 (T0, T1, T2, T3, and T4). In each topic, the words were shown in the order of the word probability, from high to low. Each topic has its unique word composition, and the top 10 words among the five topics were different. Each strain had a unique file consisting of various topics and corresponding probabilities (Additional file 2: Table S2). Strains of the same serotype exhibited similar topic mixture coefficients (Additional file 2: Table S2). We have grouped the strains by serotypes and calculated the average probabilities of the topics for each serotype (Fig. 3). The topic distributions varied with serotypes. Three serotypes, Typhimurium, Typhimurium var. 5-, and 4,[5],12:i:-, harbored only one topic T0 (shown in red bar); while the absences of topics T0 and T3 differentiated the serotype Agona from the other eight serotypes. Serotype Heidelberg was unique, lacking topics T1, T2, and T4. Serotype Saintpaul distinguished itself from serotypes Schwarzengrund and Stanley by missing topic T4. The various topic distributions could be used to differentiate serotypes.

Two-way hierarchical clustering
The two mixtures derived from LDA (Fig. 1(e) and (f)) provided vast probabilities for data mining. Hierarchical clustering analysis was first conducted on the strain-topic mixtures (Fig. 1(f)) of all 119 strains in the data set to identify the relationships between the strains, serotypes, and the obtained five topics (Fig. 4). The heat map shows that the strains with the same serotype were

| Table 1 | Topic-10 most probable words obtained when topic number is set to five |
|---------|---------------------------------------------------------------|
| Topic ID | Topic-10 most probable words |
| Topic 0  | 1724 T; 1662 G; 1654 A; 456 C; 434 C; 415 G; 383 G; 382 G; 372 C; 361 A |
| Topic 1  | 1443 T; 1020 G; 842 C; 827 C; 706 A; 616 C; 1594 T; 1532; 1433 C; 1416 A |
| Topic 2  | 1137 G; 1010 T; 1676 A; 1520 T; 1378 G; 1354 A; 1314 T; 1297 G; 1294 G; 1249 A |
| Topic 3  | 1115 C; 656 T; 913 G; 1100 G; 1059 C; 1027; 1026; 746 T; 705 T; 660 G |
| Topic 4  | 1835 T; 1748 T; 1627 A; 1626 A; 1549 T; 1511 C; 1469 T; 1348 A; 1317 T; 1147 A |
grouped together and all 119 strains were clustered into four groups (I to IV): 75 strains of Agona expressing f, g, and s factors of the *fliC* gene were clustered in group I; 24 strains of serotypes Typhimurium, Typhimurium var.5- and 4,[5],12:i:- with i factor of the *fliC* gene were clustered together in group III; 14 strains of Heidelberg with factor “r” of the *fliC* gene were grouped into group IV; and the six strains from serotypes Schwarzengrund, Stanley, Paratyphi B, and Saintpaul were in group II. The results are consistent with those shown in Fig. 3.

![Fig. 3](image1.png)

*Fig. 3* The topic distribution among different serotypes when the topic number is set to five. The five topics and the average probabilities for each serotype were calculated and the colored bars represent topics T0 to T4, respectively.

![Fig. 4](image2.png)

*Fig. 4* Two-way hierarchical clustering analysis of the LDA-derived strain-topic matrix. The complete-link hierarchical clustering algorithm was applied on the Euclidean distance measures of the topics in any two of the strains in the dataset. The heat map shows that the 119 strains are clustered into four groups (I to IV groups): I: Agona; II: Saintpaul, Paratyphi B, Schwarzengrund and Stanley; III: Typhimurium, Typhimurium var.5- and 4,[5],12:i:- ; IV: Heidelberg. The color histogram from blue to red shows the value of the topic weights of the strains ranged from 0 to 1.
Serotypes Agona and Heidelberg, as well as the group of three serotypes Typhimurium, Typhimurium var.5- and 4,[5],12:i:-, have unique and distinguishable topic compositions; while the serotypes Schwarzengrund, Stanley, Paratyphi B, and Saintpaul share more or less similar topic compositions. The clustering patterns on the data matrix derived from the topic modeling reflected the genetic truth of the serotype determinant \( fliC \) gene. According to the CDC’s annual report [38], I 4,[5],12:i:- is the monophasic variant of Typhimurium (formula I 4,[5],12:i:1,2) and lacks the second phase H antigen 1,2. In surveillance reports, Typhimurium var. 5- has been considered an O:5-negative variant of Typhimurium or reported as Typhimurium [38]. Strains of all three serotypes express factor \( i \) of the \( fliC \) gene and were classified in one group. Figure 4 clearly showed not only the topic distributions among serotypes (as shown in Fig. 3), but also the relationships between the topics. The topic distributions and SNPs compositions in topics were confirmed to be important features that could be used to characterize bacterial strains and distinguish serotypes.

**Distance matrix analysis**

Distance matrix analysis was performed on the LDA-derived (topic number set to 5) strain-topic matrix of the 119 strains (Fig. 5). Colors ranging from blue to red indicate various degrees of similarity of topic mixtures between every pair of strains. The blue squares in the diagonal, which are distinguishable from the other squares, represent concordance among the strains within the same serotypes. All of the 119 strains were classified into four subgroups.
Strains of Typhimurium and its variants were grouped into subgroup A, while strains of Heidelberg were grouped into subgroup B. The distances of the strains between subgroups A and B are much smaller (light blue), compared to the distances to the strains in other subgroups (light red to red). The results show that serotypes Heidelberg, Typhimurium and its variants share the same \textit{flc} factor i and are genetically close to each other. Strains of serotype Agona were clustered into subgroup D, containing similar topics. The distances of Agona strains to other serotype strains are much further. The results shown in Fig. 3, 4 and 5 are concordant with each other.

We also ran the LDA algorithms on the SNPs corpus with the topic numbers (\(k\)) set to 2, 3, 5, 10, 20, 40, 60, and 80, respectively, to clarify the effect of topic number variation on the resulted biological importance. Data mining was then performed on the obtained strain-topic mixtures representing the SNPs in the \textit{flc} gene of 119 \textit{Salmonella} strains. Figure 6 shows the heat map of the distance matrices performed at the LDA outputs with various topic numbers. A, B, C, and D represent the same serotype groups as shown in Fig. 5. When \(k\) is set to two, the groups A and B cannot be separated, and the distance between the groups A/B and C is relatively close (Fig. 6(a)), indicating that the strain-topic matrices are not able to provide sufficient information to distinguish the serotypes included in groups of A, B and C. When \(k\) is increased, the blue squares in the diagonal increase and become more differentiated from each other (Fig. 6(b-g)) until they are stable (Fig. 6(h-i)). The groups A and B were separated from each other when the topic number was set to three (Fig. 6(b-g)). When \(k\) was increased to 20, the strains in subgroup C were classified into three groups (Fig. 6(e)) representing three groups of serotypes. The strains in group A/B were classified into two small closely related subgroups when \(k\) was set to 30 (Fig. 6(f)) or more, indicating the existing two patterns of serotype Typhimurium var.5- and 4,[5],12:i:-. The pattern differentiation and subgroup classification remain the same when the topic number is set to 60 or more (Fig. 6(h-i)). Therefore, we consider that the strain-topic matrices derived from the LDA algorithm best distinguished the serotype-determinant \textit{flc} gene when the topic number was set to 30 for this NGS dataset.

**Model evaluation**

We compared the performance and accuracy of our proposed topic modeling method with the Hamming method, which is one of the existing methods on sequence similarity analysis, by clustering analysis and classification. Table 2 shows the NMI and ARI values of different methods which were applied to evaluate the clustering results. It is obvious that both NMI and ARI measurements were the highest when \(k\)-means clustering analysis was applied on LDA-derived sample-topic matrix with topic number set to 30, indicating the better accuracy of topic modeling on similarity detection. However, when topic number was set to 5, the clustering quality was not satisfactory due to the insufficient distinction among serotypes. The results are consistent with those in Fig. 6(c). The distinction between different serotypes becomes more significant when the topic number increases from 2 to 30. Five topics were not enough to distinguish the samples with serotypes Paratyphi B, Saintpaul, Schwarzengrund and Stanley. Therefore, the clustering quality was worse than that when 30 topics were applied. In addition, it was noticed that when Hamming distance was used to calculate the sequence similarities, PCA feature extraction had no obvious effect on the qualities of \(k\)-means clustering, comparing the similar ARI and NMI values of \(k\)-means, PCA(2) + \(k\)-means, and PCA(5) + \(k\)-means, inferring that topic modeling was more efficient than PCA on this dataset even as a dimension reduction tool. Comparing the results of various methods, the method of “highest probable topic assignment” performed the worst, indicating that the highest probable topic only is not sufficient to describe the distinctions among samples.

Table 3 shows the classification results using both SVM and RF algorithms to compare the proposed topic modeling method and Hamming method. Overall, the LDA derived sample-topic matrix with 30 topics had higher classification accuracies than the matrix from Hamming method. Especially, RF algorithm reached 100 % accurate prediction on the sample-topic matrix with 30 topics.

**Biomarker identification and visualization**

The LDA-derived strain-topic and topic-word matrices could also be combined to analyze the SNPs in the \textit{flc} gene. The LDA algorithm was run on the text corpus of the \textit{flc} SNPs from 119 strains with the topic number set to five, resulting in two matrices: strain-topic matrix (119 × 5), and topic-SNP matrix (5 × 2379). We multiplied the two matrices and generated a new matrix of strain-SNPs, from which the top 10 SNPs were then collected for each strain. A sketch was plotted to show the dissemination of these highly emerged SNPs in 119 strains (Fig. 7). The colored dots represent SNPs of A, T, C, G, and deletions as well as their locations in the \textit{flc} gene. The dissemination of the top ten SNPs for the 119 strains was shown to be serotype-dependent. Especially, the top 10 SNPs are identical for the strains of the same serotypes of Heidelberg, Typhimurium group (including Typhimurium, Typhimurium var.5- and 4,[5],12:i:-,), Schwarzengrund and Saintpaul. The strains of serotype Agona exhibit more diversity on SNPs dissemination, with a majority of the SNPs located between 1000–1500 bp. The accuracies of these SNPs in the 119 strains have been confirmed by the sequences from NCBI (Additional file 1: Table S1). The information about the commonalities and diversities in and between serotypes
shown in Fig. 7 provides vast possibilities for potential biomarker discrimination, strain evolution, source tracking, and genomic knowledge interpretation (more details in Discussion).

**Discussion**

In this study, we proposed a novel procedure to analyze NGS data and discussed its computational implementation as an integrated tool to analyze a target gene’s diversity from the whole genome sequence reads. The key element of the procedure is the application of topic modeling and its integration with current sequence analysis tools and data mining methods. Topic modeling has been widely used in large dataset analysis [10, 14, 15]. Its algorithms analyse the words of documents to discover the themes that pervade a large collection of documents [39]. The rational for
incorporating topic modeling on NGS data analysis was based on the fact that the four nucleotides as well as their orders in NGS sequences could be treated as “words”, therefore, the genetic information in sequences was translated and exhibited as “a bag of words”. Two matrices generated by the algorithms of topic modeling provide huge potential applications by combining with various data mining methods for different purposes. The main motivation of this study was to develop the text mining and data mining tools can be applied for deep analysis of bacterial pathogen populations with high rates of nucleotide substitutions.

The *Salmonella* *fliC* gene was used as a case study to show one of the applications of the proposed procedure on genic diversity and biomarker clarification for bacterial pathogen populations. Epidemiological investigations of *Salmonella* infections in humans and animals have depended on serotyping of the cultivated isolates for more than 70 years [40, 41]. Currently there are more than 2,500 serotypes within *S. enterica* and *S. bongori* due to the various combinations of 46 O antigens and 85 H antigens [41]. The limitations of traditional serotyping have stimulated the request for developing new DNA-based serotyping methods. Multiple methods have been investigated for their abilities as a replacement for *Salmonella* serotyping, such as PFGE (Pulsed-Field Gel Electrophoresis) [42–45], ribotyping [46], repetitive extragenic palindromic sequence-based PCR (rep-PCR) [46], microsphere-based liquid arrays, and Multilocus Sequence Typing (MLST) [47]. These methods are feasible for some serotypes, but lack widespread adoption and might misidentify a newly emergent serotype [48]. The nature of unbiased NGS approaches allows numerous applications in comprehensive pathogen detection, infectious disease diagnosis, outbreak investigation and surveillance at a global level [49]. However, the latest applications have mostly relied on phylogenetic clustering and comparisons based on whole genome reads [26, 49, 50]. It is challenging to globally estimate a targeted functional encoding gene’s diversity in bacterial pathogen populations from whole genome reads. Before the cost-effective NGS sequencing technologies became available, this was approached by sequencing PCR-amplified fragments of cultivated isolates, which was indirect, usually biased and inaccurate due to genetic diversity, oligonucleotide primer design, and experimental errors.

The proposed procedure is designed to work on NGS reads or contigs from the NCBI bio-project NGS sequence database (Additional file 1: Table S1) or any other data source, and the specific target gene-related reads or contigs (e.g. *Salmonella* *fliC*-related gene fragments) were retrieved for the following alignment. The SNPs we identified in this study were different from the original designation, in which all the variations in the same location were included (Fig. 1(c)). Therefore, the “bag of SNPs” (Fig. 1(d)) covered all possible variations in the *fliC*-coding region. These variations, as well as their locations, could be used as characteristics to distinguish strain similarities or identify new mutants (Fig. 7). Moreover, the original data of ATGC sequence reads were transferred into a file of texts on which the text mining/data mining tools can be applied for deep analyses. In the example used in this study, by using the strain-topic matrix derived from topic modeling, we investigated the relationships and similarities between 119 strains and nine serotypes by two-way hierarchical cluster analysis and distance matrix analysis (Fig. 4, 5, and 6). Since the *Salmonella* *fliC* is the coding gene for *Salmonella* phase 1 antigen, and is considered one of the *Salmonella* serotype determinant genes [20], the diversity of *Salmonella* *fliC* and the relationship with the corresponding serotypes (Fig. 3, 4, 5, 6, and 7) more accurately reflected gene-phenotype relationships than the whole genome sequence phylogenetic trees. The nucleotide-level *Salmonella* *fliC* gene diversity can be potentially used as the biomarker for serotype screening (Fig. 7). The more NGS sequences are added from more strains and serotypes, the higher the accuracy of the biomarker will be.

### Table 2 Comparison the results of clustering

| Method                        | ARI  | NMI  |
|-------------------------------|------|------|
| k-means                       | 0.9232 | 0.8861 |
| PCA(2) + k-means              | 0.9202 | 0.8547 |
| PCA(5) + k-means              | 0.9322 | 0.8547 |
| LDA(5)                        | 0.5325 | 0.4209 |
| LDA(30)                       | 0.8634 | 0.7946 |
| LDA(30) + k-means             | 0.4301 | 0.713  |
| LDA(30) + k-means             | **0.9543** | **0.912** |

* k-means: traditional k-means applying on VSM format of dataset, using Hamming distance.

PCA(2) + k-means: traditional k-means applying on 2 feature matrix obtained by PCA.

PCA(5) + k-means: traditional k-means applying on 5 feature matrix obtained by PCA.

LDA(5): “highest probable topic assignment” by LDA with 5 topics.

LDA(30): “highest probable topic assignment” by LDA with 30 topics.

LDA(30) + k-means: traditional k-means applying on sample-topic matrix obtained by PCA.

LDA(30) + k-means: traditional k-means applying on sample-topic matrix by LDA with 5 topics.

Note that PCA(2) and PCA(5) exhibited better clustering qualities than PCA(10) and PCA(30), and are shown in the table.

Bold numbers indicate the best results among various methods.

### Table 3 Comparison the results of classification

| Data Format   | SVM   | RF    |
|---------------|-------|-------|
| VSM           | 0.8462| 0.9573|
| Sample-topic matrix(5) | 0.9573 | 0.9829 |
| Sample-topic matrix(30) | **0.9658** | 1|

Bold numbers indicate the best results among various methods.
Fig. 7 SNPs differentiation and locations in 119 strains. The identified SNPs and their relative locations in the *Salmonella* fliC gene were marked. The red, green, purple, black and grey dots represent A, T, G, C and deletions, respectively.
In this study, Gibbs sampling was used to maximize the probabilities of the obtained text corpus. An important property of the Gibbs sampling approach is its convergent efficiency. If it takes too many iterations to converge, Gibbs sampling approach will not be a feasible tool for real applications. To test if Gibbs sampling converged fast in the LDA model, we computed the likelihood of the model consisting of both a Dirichlet-multinomial for the SNPs in each topic and a Dirichlet-multinomial for the topics in each strain. The formula used for model likelihood is shown in Eq. 2. The larger the value of the model likelihood is, the better the model that is obtained. Gibbs sampling in LDA converges once the value is stable.

$$LL_{model} = \prod_{s=1}^{S} \left\{ \frac{\Gamma \left( \sum_{i=1}^{V} a_i \right)}{\Gamma \left( \sum_{i=1}^{V} (a_i + n_{si}) \right)} \right\} \prod_{k=1}^{K} \left\{ \frac{\Gamma (a_i + n_{si})}{\Gamma (a_i)} \right\} \prod_{k=1}^{K} \left\{ \frac{\Gamma \left( \sum_{j=1}^{V} \beta_j \right)}{\Gamma \left( \sum_{j=1}^{V} (\beta_j + n_{kj}) \right)} \right\} \prod_{j=1}^{V} \left\{ \frac{\Gamma (\beta_j + n_{kj})}{\Gamma (\beta_j)} \right\}$$

Here in Eq.2, $V$ represents the size of the vocabulary (number of different words in the corpus). The first part in right-hand side of Eq.2 is the Dirichlet-multinomial for the topics in $S$ documents and $n_{si}$ represents the number of topics $i$ picked in document $s$; The second part in right-hand side of Eq.2 is the Dirichlet-multinomial for the words in $K$ topics and $n_{kj}$ represents the number of words $j$ picked in topic $k$. The log of Eq. 2 for every 100 iterations was calculated (every 10 iterations for the first 100). Fig. 8 shows the results of the convergence test of Gibbs sampling in the LDA algorithm with the number of topics set at different numbers. The log likelihood of the model increases fast in the first 100 iterations, then becomes stable after about 300 iterations (500 iterations when the topic number is three). Gibbs sampling reaches the convergence around 300, indicating that the running of the LDA algorithm is fast to reach the best result. Therefore, the proposed procedure and the implemented tool will be a fast, efficient method and workflow for NGS data analysis and data mining.

Our procedure could be applied on NGS datasets for genetic diversity identification and biomarker development on various functional genes and gene clusters in various biological and biomedical areas. The proposed procedure has implemented the topic modeling algorithms and data mining algorithms in the NGS data analysis, and the resulting sample-topics and topic-words matrices provide huge possibilities for data mining and interpretation. Furthermore, the algorithms in the procedure are designed to accommodate billions of NGS
reads if the users’ computer capacity allows. Therefore, the tool we
developed in this study is suitable for over-
coming difficulties in big NGS data analysis in biological
and medical fields. We expect this procedure to be an
efficient tool to cope with high complexity and huge vol-
umes of sequence data for elucidating genetic informa-
tion, gene-phenotype relationships and biomarker
identification. The tool has the potential to give a more
complete view of the evolutionary dynamics of the bac-
terial population.

Conclusions
We have reported a novel procedure to analyze next-
generation sequencing data by introducing topic mod-
ing, which is an active research field in machine learning
and has been mainly used as an analytical tool to struc-
ture large text corpora for data mining. Four major steps
are included in this procedure: NGS data retrieval, pre-
processing, topic modeling, and data mining using Latent
Dirichlet Allocation (LDA) topic outputs. The perform-
ance was evaluated by a case study of the NGS data set of
the Salmonella enterica strains. The results illustrate that
the implementation of topic modeling in NGS data anal-
ysis provides a new way to elucidate genetic information
from NGS data, and identify the gene-phenotype relation-
ships and biomarkers, especially in the era of biological
and medical big data.

Availability of data and materials
The original whole genome sequences of 119 strains of
Salmonella O antigen group B were retrieved from the
NCBI database, including 75 strains of S. Agona, 14
strains of S. Heidelberg, one strain of S. Paratyphi B, two
strains of S. Saintpaul, two strains of S. Schwarzengrund,
one strain of S. Stanley, 22 strains of S. Typhimurium,
one strain of S. Typhimurium var.5–, and one strain of S.
4, 12:i–. The WGS Accession Numbers, Sequence Bio-
project Numbers, and strain names in NCBI database
are listed in Additional file 1: Table S1.

Additional files

Additional file 1: Table S1. Metadata of 119 samples used in this
study. (DOCX 25 kb)

Additional file 2: Table S2. Topic mixtures of 119 samples with 5
topics. (DOCX 29 kb)

Abbreviations
ARI: adjusted rand index; BLAST: basic local alignment search tool;
LDA: Latent Dirichlet Allocation; LOOCV: leave one out cross validation;
MCMC: Markov chain Monte Carlo; MLST: multilocus sequence typing;
MSA: multiple sequence alignment; NCBI: national center for biotechnology
information; NGS: next generation sequencing; NMI: normalized mutual
information; PFGE: pulsed-field gel electrophoresis; RF: random forest;
SNPs: single nucleotide polymorphisms; SVM: support vector machine;
VSM: vector space model; WGS: whole genome sequence.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
WZ (Zhao) performed all the calculations and data analysis, and wrote the
first draft of the manuscript. WZ developed the methods, had the original
idea, and guided the data analysis and presentation of results. WZ, WZ
(Zhao), JC, RP, YW, ZL, HH, and WT participated the dataset construction and
result presentation. WZ managed the research and guided the scientific
discussing and editing. All authors contributed to data verification, approach
evaluation, and assisted with writing the manuscript. All authors read and
approved the final manuscript.

Acknowledgements
This work and the publication were funded by FDA. Dr. Weizhong Zhao
acknowledges the support of a fellowship from the Oak Ridge Institute for
Science and Education, administered through an interagency agreement
between the U.S. Department of Energy and the U.S. Food and Drug
Administration. We are grateful to Ms. Beth Juliar and Dr. John Sutherland for
critical reading of this manuscript.

Disclaimer
The views presented in this paper are those of the authors and do not
necessarily represent those of the U.S. Food and Drug Administration.

Author details
1Division of Bioinformatics and Biostatistics, National Center for Toxicological
Research, U.S. Food and Drug Administration, 3900 NCTR Road, HFT-20,
Jefferson, AR 72079, USA. 2College of Information Engineering, Xiangtan
University, Xiangtan, Hunan Province, China.

Received: 20 October 2015 Accepted: 7 May 2016
Published online: 13 May 2016

References
1. Metzker ML. Sequencing technologies - the next generation. Nature reviews
Genetics. 2010;11(1):31–46.
2. Didelot X, Bowden R, Wilson DJ, Peto TE, Crook DW. Transforming clinical
microbiology with bacterial genome sequencing. Nature reviews Genetics.
2011;12(9):601 –12.
3. Kosar CU, Holden MT, Ellington MJ, Cartwright LJ, Brown NM, Ogilvy-Stuart
AL, Hsu LY, Chewapreecha C, Croucher NJ, Harris SR, et al. Rapid whole-
genome sequencing for investigation of a neonatal MRSA outbreak. The
New England journal of medicine. 2012;366(24):2267–75.
4. Lenau EK, Strain E, Wang C, Zheng J, Ottesen AR, Keys CE, Hammad TS,
Musser SM, Brown EW, Allard MW, et al. Identification of a salmonellosis
outbreak by means of molecular sequencing. The New England journal of
medicine. 2011;364(10):981–2.
5. Petrovsky NJ, Hightower S, Luna RA, Gibbs RA, Versalovic J. Metagenomic
pyrosequencing and microbial identification. Clinical chemistry.
2009;55(5):856 –66.
6. Radford AD, Chapman D, Dixon L, Chantrey J, Darby AC, Hall N. Application
of next-generation sequencing technologies in virology. The Journal of
general virology. 2012;93(Pt 9):1853–68.
7. Koboldt DC, Steinberg KM, Larson DE, Wilson RK, Mardis ER. The next-
generation sequencing revolution and its impact on genomics. Cell. 2013;
155(1):27–38.
8. Zhang J, Chiodini R, Badr A, Zhang G. The impact of next-generation
sequencing on genomics. Journal of genetics and genomics = Yi chuan xue
bao. 2011;38(3):105–9.
9. Hofmann T. Unsupervised learning by probabilistic latent semantic analysis.
Machine Learning. 2001;42:177 –96.
10. Blei DM, Ng AY, Jordan MI. Latent Dirichlet Allocation. Journal of Machine
Learning Research. 2003;3:993–1022.
