DETECTION OF 16S rRNA AND 23S rRNA GENE MUTATIONS IN MULTIDRUG RESISTANT SALMONELLA SEROVARS ISOLATED FROM DIFFERENT SOURCES USING RNA SEQUENCING METHOD

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SUMMARY

The rapid emergence of resistant bacteria is occurring worldwide. Antibiotic resistance is a serious problem for human beings because pathogenic microorganisms that acquire such resistance void antibiotic treatments. Bacterial antibiotic resistance mechanisms include efflux, reduced influx, modification and degradation of the drug, as well as mutation, modification or overexpression of the target. However, our knowledge as to how bacteria acquire antibiotic resistance is still fragmented, especially for ribosome-targeting drugs. Salmonella is a leading cause of foodborne salmonellosis in the world. The number of antibiotic resistant isolates identified in humans is steadily increasing, suggesting that the spread of antibiotic resistant strains is a major threat to public health. Salmonella is commonly identified in a wide range of animal hosts, food sources, and environments, but our knowledge as to how Salmonella resistance to antibiotics is still fragmented in this ecologically complex serovar. Therefore, the aim of this study was to support for finding novel mechanisms that render bacteria resistant to the ribosome targeting antibiotics, we screen for antibiotic resistant 16S and 23S ribosomal RNAs (rRNAs) in multidrug resistant Salmonella serovars isolated from raw retail meats isolated from Hanoi, Vietnam. Bioinformatic analysis identified 193 unknown novel mutations (64 mutations in 16S rRNA and 129 mutations in 23S rRNA genes). These mutations might play a role in streptomycin resistant in Salmonella serovars. These results suggest that uncharacterized antibiotic resistance mutations still exist, even for traditional antibiotics. This study is only a preliminary kind, further validation before they are applied in Salmonella or other closely related species are required.

Keywords: MDR Salmonella, mutation, 16S rRNA gene, 23S rRNA gene, RNAsequencing

INTRODUCTION

Aminoglycosides are used in treating a wide range of infections caused by gram-negative bacteria and has been classified by the World Health Organization as critically important antimicrobial drugs. They inhibit bacterial protein synthesis by binding to the 16S ribosomal subunit, leads to bacteria death. Resistance to these antimicrobial agents usually results from the production of aminoglycoside-modifying enzymes, reduced intracellular antibiotics accumulation, or mutation of ribosomal proteins or rRNA. An additional mechanism, methylation of the aminoacyl site of 16S rRNA, confers high level resistance to clinically crucial aminoglycosides such as streptomycin and gentamicin (Bonomo, Szabo, 2006; Fair, Tor, 2014; Katie et al., 2010; Kohanski et al., 2010). Exogenously acquired 16S rRNA methyltransferase (16S-RMTase) genes responsible for a really high level of resistance to various aminoglycosides have been widely distributed among Enterobacteriaceae including Salmonella serovars. This genetic apparatus may thus contribute to the rapid worldwide dissemination of the resistance mechanism among pathogenic bacteria. The worldwide dissemination of 16S-RMTases is becoming a global concern and this implies the necessity to continue investigations on the trend of 16S-RMTases to restrict their further worldwide dissemination (Wachino, Arakawa, 2012).
The ribosome is functionally critical sites exist mainly on RNAs, many antibiotic target sites exist on rRNAs, as several resistant point mutations (Moazed, Noller, 1987; Yassin et al., 2005). This is because ribosomes play a crucial role in protein biosynthesis, translating messenger RNA encoded genetic information into proteins, which consists of sequential multistep reactions such as initiation, elongation, termination, and recycling. Owing to these extremely elaborate reaction dynamics, there are different sorts of inhibitors targeting each step of the translation process (Wilson, 2013). Acquisition of mutations in target sites of the antimicrobial mechanism is often observed for ribosome targeting drugs such as aminoglycosides (e.g., streptomycin, gentamycin), tetracycline, chloramphenicol, macrolides, lincomycins, streptogramin A, and oxazolidinones; the former three are known to target the 30S subunit that contains the 16S rRNA as its main component, whereas the others are known to attack the 50S subunit that contains the 23S rRNA as its main component (Wilson, 2006).

Our knowledge as to how bacteria acquire antibiotic resistance is still fragmented, especially for the ribosome targeting drugs. Therefore, tremendous effort is being made to identify the mechanisms and mutations that lead to bacterial resistance to antibiotics. There are many unfound and uncharacterized antibiotic resistance point mutations in rRNA genes. Understanding this can help ensure we can effectively treat bacterial infections such as Salmonella serovars. Researchers have long tried to list as many resistant point mutations in rRNAs as possible (Miyazaki, Kitahara, 2018). There is limited information about point mutations in 16S rRNA and 23S rRNA genes in Salmonella isolated from retail meats in Vietnam. Thus, this study aims to detect point mutations in 16S rRNA and 23S rRNA genes, which is one of keys to prevent the spread of multidrug-resistant Salmonella serovars.

MATERIALS AND METHODS

Collection and preparation of samples

A total of 25 Salmonella serovars were serotyped and received from laboratory in Institute of Genome Research, Vietnam Academy of Science and Technology, including 2 S. warragul, 1 S. london, 4 S. derby, 2 S. indiana, 1 S. meleagridis, 1 S. give, 2 S. rissen, 11 S. typhimurium and 1 S. assine. The originated strains from pork, beef and chicken meat at retail markets in Hanoi, Vietnam.

Antibiotic susceptibility tests

The antimicrobial susceptibility test was performed according to the Clinical and Laboratory Standards Institute (CLSI-2015) and used the disk diffusion method as Kirby-Bauer’s description. Drug susceptibility was tested on the Muller Hinton agar plates. Cultures were grown at 37°C for 18-24 h in Brain Heart Broth Infusion (Biolife-Italia) and prepared on Mueller-Hinton agar. The antibiotic disks were placed aseptically on it and plates were incubated at 37°C for 16-18 h.

The eight tested antimicrobials were often used in husbandry and treatment of animal farms as well as human diseases in Vietnam such as ampicillin (AMP) 10 µg, ceftazidime (CAZ) 30 µg, gentamicin (GEN) 10 µg, streptomycin (STR) 10 µg, ciprofloxacin (CIP) 5 µg, chloramphenicol (CHL) 30 µg, tetracycline (TET) 30 µg, and trimethoprim/sulfamethoxazole (SXT) 1.25/23.75 µg (BD Diagnostics).

RNA sequencing and virulence gene detection

Total RNA was extracted from Salmonella spp. according to the manufacturer’s instructions (TRizol Reagent, Life Technologies Inc.). RNA was concentrated and purified with an RNA MinElute kit (Qiagen). mRNA-seq libraries were produced from 1 µg of genomic RNA libraries, following the TruSeq Nano DNA Sample Preparation Guide, Part # 15041110 Rev. Library preparations were sequenced on a HiSeq4000 (Illumina) platform (Next Generation Sequencing Div. MACROGEN, Inc., Daejeon, Korea) using TruSeq Nano DNA Kit. The trimmingomatic program was used to remove adapter sequences. The trimmingomatic program was used to remove adapter sequences. All subsequent analyses were based on high quality, clean data. Transcriptome de novo assembly using automated parameters in Geneious R11 software (Kearse et al., 2012). The 16S rRNA gene mutations were analyzed using ResFinder (Center for Genomic Epidemiology) (Zankari et al., 2012).

RESULTS

Antibiotic resistance of Salmonella isolates

Twenty-five Salmonella spp. were tested for
antibiotic resistance against 8 antibiotics. All strains were susceptible to CAZ, and 52% of the isolates were resistant to at least one antibiotic (data not shown). Total 9 *Salmonella* isolates were shown the multi-antimicrobial resistance, including one *S. meleagridis*, one *S. derby*, one *S. give*, three *S. typhimurium*, one *S. warragul*, one *S. indiana*, and one *S. rissen*). In addition, *S. indiana* isolate from chicken showed resistance to 8 antibiotics (Table 1).

### Table 1. Susceptibility results of multidrug-resistant *Salmonella* isolates.

| Salmonella serovar | Antibiotics |
|--------------------|-------------|
| Indiana            | AMP | CAZ | GEN | STR | CIP | CHL | TET | SXT |
| Rissen             | S   | S   | R   | R   | R   | R   | R   | R   |
| Warragul           | S   | S   | S   | S   | S   | R   | R   | R   |
| Typhimurium S384   | R   | S   | R   | R   | S   | R   | R   | R   |
| Typhimurium S181   | R   | S   | S   | S   | R   | S   | R   | S   |
| Typhimurium S360   | R   | S   | S   | R   | S   | R   | R   | R   |

Abbreviations: R (resistant); S (sensitive)

### Table 2. Mutations in 16S rRNA and 23S rRNA genes among isolates.

| Sal 4 | Sal 6 | Sal 7 | Sal 8 | Sal 11 | Sal 12 |
|-------|-------|-------|-------|--------|--------|
| 16S_rsD.r.45A>G | 16S_rsD.r.54A>G | 16S_rsD.r.54A>G | 16S_rsD.r.45A>G | 16S_rsD.r.45A>G |
| 16S_rsD.r.54A>G | 16S_rsD.r.54A>G | 16S_rsD.r.54A>G | 16S_rsD.r.54A>G |
| 16S_rsD.r.248C>T | 16S_rsD.r.642G>C | 16S_rsD.r.248C>T | 16S_rsD.r.726T>C |
| 16S_rsD.r.260A>G | 16S_rsD.r.756G>C | 16S_rsD.r.260A>G | 16S_rsD.r.756G>C |
| 16S_rsD.r.891C>T | 16S_rsD.r.756G>C | 16S_rsD.r.891C>T | 16S_rsD.r.1047C>T |
| 16S_rsD.r.933G>A | 16S_rsD.r.1164T>C | 16S_rsD.r.900G>T | 16S_rsD.r.1047C>T |
| 16S_rsD.r.1017C>T | 16S_rsD.r.1017C>T | 16S_rsD.r.1017C>T |
| 16S_rsD.r.1050C>T | 16S_rsD.r.1050C>T | 16S_rsD.r.1050C>T |
| 16S_rsD.r.1057C>T | 16S_rsD.r.1057C>T | 16S_rsD.r.1057C>T |
| 16S_rsD.r.1057C>T | 16S_rsD.r.1057C>T | 16S_rsD.r.1057C>T |
| 16S_rsD.r.1095T>G | 16S_rsD.r.1095T>G | 16S_rsD.r.1095T>G |
| 16S_rsD.r.1154T>C | 16S_rsD.r.1154T>C | 16S_rsD.r.1154T>C |
| 16S_rsD.r.1154T>C | 16S_rsD.r.1154T>C | 16S_rsD.r.1154T>C |
| 16S_rsD.r.1154T>C | 16S_rsD.r.1154T>C | 16S_rsD.r.1154T>C |
| 16S_rsD.r.1154T>C | 16S_rsD.r.1154T>C | 16S_rsD.r.1154T>C |

In silico 16S rRNA and 23S rRNA gene mutation analysis

Six out of nine multidrug resistance samples were selected for mRNA sequencing, including *S. indiana* (Sal 4), *S. derby* (Sal 6), *S. give* (Sal 7), *S. typhimurium* S360 (Sal 8), *S. typhimurium* S384 (Sal 11), and *S. typhimurium* S181 (Sal 12). A total of 193 point mutations were identified (64 point mutations in 16S rRNA and 129 point mutations in 23S rRNA). A listing over the mutations among isolates was presented in Table 2.
DISCUSSION

The rRNA is the most commonly exploited RNA target for antibiotics. The bacterial ribosome comprises 30S and 50S ribonucleoprotein subunits, contains a number of binding sites for antibiotics and is an target for novel antibacterial agents (Howard et al., 1996). Bacterial ribosomes have two ribonucleoprotein subunits. The bacterial rRNA includes 5S, 16S and 23S rRNA, the smallest (5S rRNA) being an approximately 120 nt RNA. The smaller 30S subunit contains a single approximately 1500 nt RNA (16S rRNA) and about 20 different proteins while the larger 50S subunit contains an approximately 2900 nt RNA (23S rRNA) and about 30 different proteins (Moore, 2001). Aminoglycosides are a group of well-known antibiotics that have been used successfully for more than half a century. Streptomycin and gentamycin are typical antibiotics which function by binding to specific sites on bacterial rRNA and affecting the fidelity of protein synthesis. The rRNA aminoacyl-tRNA site (rRNA A-site) is a major target for aminoglycosides which selectively kills bacterial cells. Binding of drug to the 16S subunit near the A-site of the 30S subunit leads to a decrease in translational accuracy and inhibition of the translocation of the ribosome (Thomas, Hergenrother, 2008).

There are three main mechanisms for microorganisms to acquire antibiotic resistance such as (i) enzymatic inactivation or modification of antibiotics (e.g. β-lactamases inactivate penicillin) (Li, Nikaido, 2009); (ii) acquisition of mutations in target sites of the antibiotics; and (iii) decreasing the

| **Sal 4** | **Sal 6** | **Sal 7** | **Sal 8** | **Sal 11** | **Sal 12** |
|----------|----------|----------|----------|-----------|-----------|
| 23S r.1400T>C | 23S r.1737C>T | 23S r.2688G>C | 23S r.1523T>C | 23S r.1731G>A | 23S r.1865T>C |
| 23S r.1523T>C | 23S r.1740G>T | 23S r.1732G>A | 23S r.1749G>T | 23S r.2210T>G | 23S r.1882T>C |
| 23S r.1712T>C | 23S r.1741G>C | 23S r.1720C>T | 23S r.2212G>C | 23S r.2803G>C | 23S r.2203T>G |
| 23S r.1723G>A | 23S r.1753A>G | 23S r.2800C>G | 23S r.2793C>G | 23S r.2794C>A | 23S r.2798T>A |
| 23S r.1727C>T | 23S r.1872T>C | 23S r.2802G>T | 23S r.2802G>T | 23S r.2803G>C | 23S r.2803G>C |
| 23S r.23S r.1885T>C | 23S r.2809G>T | 23S r.2810G>C | 23S r.2810G>C | 23S r.2810G>C | 23S r.2810G>C |
net drug concentration in the cell by reducing drug permeability via cell wall or by increasing the activity of efflux pumps (e.g. tetracycline resistance) (Bassetti et al., 2017). Among these, acquisition of mutations in target sites of the antibiotics is often observed for ribosome targeting drugs such as streptomycin, gentamycin; the former three are known to target the 30 S subunit that contains the 16 S rRNA as its main component, whereas the others are known to attack the 50 S subunit that contains the 23 S rRNA as its main component (Wilson, 2006). There are a large number of antibiotics that target the ribosome. This is because ribosomes play a crucial role in protein biosynthesis, translating messenger RNA-encoded genetic information into proteins, which consists of sequential multistep reactions such as initiation, elongation, termination, and recycling. There are different kinds of inhibitors targeting each step of the translation process (Lambert, 2012; Wilson, 2006; Wilson, 2014). As the ribosome is RNA-rich, and functionally critical sites exist mainly on RNAs (the decoding center in 16S rRNA and peptidyl transferase center in 23S rRNA), many antibiotic target sites exist on RNAs, as do several resistant point mutations (Hong et al., 2014; Noller, Yassin et al., 2005).

Many antibiotics inhibit the growth of bacteria by targeting protein biosynthesis. Streptomycin has been shown to interact directly with the small ribosomal subunit. The ribosome accuracy center is a highly conserved component of the translational apparatus, comprising an rRNA domain and several polypeptides of the small subunit. Mutations within rRNA genes have been found to confer drug resistance; for some of these mutations experimental proof for a cause-effect relationship has been provided (Andersson, Hughes, 2011; Cocozaki et al., 2016; Smith et al., 2013; Springer et al., 2001).

We have provided a comprehensive summary of the point mutations in 16S rRNA and 23S rRNA genes expression across the multidrug-resistant Salmonella. The mRNA-seq of Salmonella isolates showed the collective expression of 193 point mutations genes conferring resistance to gentamycin and streptomycin. The presence of these genes could contribute to the pathogenicity of these Salmonella isolates and also indicates the potential for these isolates to resist various antibiotics. In this study, point mutations detected in Sal 4 and Sal 11 exhibited 100% concordance, with all isolates displaying phenotypic resistant to gentamycin and streptomycin and all containing point mutations typically associated with resistance to these antimicrobials (Table 1 and Table 2). Likewise, all of six streptomycin resistant strains carried point mutations. Despite the concordance between genotypic and phenotypic in Sal 4 and Sal 11, there were some examples of disagreement. Most notably, there were four isolates (Sal 6, Sal 7, Sal 8, and Sal 12) that possessed point mutation genes (Table 2) but were not resistant to gentamycin (Table 1). A blast search released that these novel point mutation has not been reported previously in any organism. This result suggested that these point mutations are associated with resistance to streptomycin, and mutations expression in Sal 4 and Sal 11 are involved with gentamycin and streptomycin resistance in our isolates. Further studies are necessary in order to conclude association between these point mutations and gentamycin and streptomycin resistant in six isolates.

CONCLUSION

Antibiotic resistance is a serious problem, more and more pathogenic bacterial are developing immunity to widely used antibiotics, rendering them useless. Tremendous effort is being made to identify the mechanisms and mutations that lead to bacterial resistance to antibiotics. Understanding this can help ensure we can effectively treat multidrug Salmonella resistant infections. Our results suggest that there are many unfound and uncharacterized antibiotic resistance point mutations in rRNA genes. These mutations might contribute to streptomycin resistant in Salmonella serovars. This result is only a prediction, further validation is required.

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REFERENCES

Andersson DI, Hughes D (2011) Persistence of antibiotic resistance in bacterial populations. FEMS Microbiol Rev 35: 901-911.

Bassetti M, Poulakou G, Ruppe E, Bouza E, Van Hal SJ, Brink A (2017) Antimicrobial resistance in the next 30 years, humankind, bugs and drugs: a visionary approach. Intensive Care Med 43: 1464-1475.
Bonomo RA, Szabo D (2006) Mechanisms of multidrug resistance in Acinetobacter species and Pseudomonas aeruginosa. Clin Infect Dis 43 Suppl 2: S49-56.

Cocozaki AI, Altman RB, Huang J, Buurman ET, Kazmirski SL, Doig P, Prince DB, Blanchard SC, Cate JHD, Ferguson AD (2016) Resistance mutations generate divergent antibiotic susceptibility profiles against translation inhibitors. Proc Natl Acad Sci U S A 113: 8188-8193.

Fair RJ, Tor Y (2014) Antibiotics and bacterial resistance in the 21st century. Perspect Medicin Chem 6: 25-64.

Hong W, Zeng J, Xie J (2014) Antibiotic drugs targeting bacterial RNAs. Acta Pharm Sin B 4: 258-265.

Howard M, Frizzell RA, Bedwell DM (1996) Aminoglycoside antibiotics restore CFTR function by overcoming premature stop mutations. Nat Med 2: 467.

Katie LH, Jose AE, Laura H, Bruno GZ (2010) 16S rRNA Methyltransferase RmtC in Salmonella enterica Serovar Virchow. Emerg Infect Dis 16: 712.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647-9.

Kohanski MA, Dwyer DJ, Collins JJ (2010) How antibiotics kill bacteria: from targets to networks. Nat Rev Microbiol 8: 423-35.

Lambert T (2012) Antibiotics that affect the ribosome. Rev Sci Tech 31: 57-64.

Li ZX, Nikaido H (2009) Efflux-mediated drug resistance in bacteria: an update. Drugs 69: 1555-1623.

Miyazaki K, Kitahara K (2018) Functional metagenomic approach to identify overlooked antibiotic resistance mutations in bacterial RNA. Sci Rep 8: 5179.

Moazed D, Noller HF (1987) Interaction of antibiotics with functional sites in 16S ribosomal RNA. Nature 327: 389.

Moore PB (2001) The ribosome at atomic resolution. Biochemistry 40: 3243-3250.

Noller HF Evolution of protein synthesis from an RNA world. Cold Spring Harb Perspect Biol 4: a003681-a003681.

Smith T, Wolff KA, Nguyen L (2013) Molecular biology of drug resistance in Mycobacterium tuberculosis. Curr Top Microbiol Immunol 374: 53-80.

Springer B, Kidan YG, Prammananan T, Ellrott K, Böttger EC, Sander P (2001) Mechanisms of streptomycin resistance: Selection of mutations in the 16S rRNA gene conferring resistance. Antimicrob Agents Chemother 45: 2877-2884.

Thomas JR, Hergenrother PJ (2008) Targeting RNA with small molecules. Chem Rev 108: 1171-1224.

Wachino J, Arakawa Y (2012) Exogenously acquired 16S RNA methyltransferases found in aminoglycoside-resistant pathogenic Gram-negative bacteria: An update. Drug Resist Updat 15: 133-148.

Wilson DN (2006) Antibiotics and the inhibition of ribosome function (eds K. H. Nierhaus & D. N. Wilson) Ch. 12, 449–527. https://doi.org/10.1002/3527603433.ch12

Wilson DN (2013) Ribosome-targeting antibiotics and mechanisms of bacterial resistance. Nat Rev Microbiol 12: 35.

Wilson DN (2014) Ribosome-targeting antibiotics and mechanisms of bacterial resistance. at Rev Microbiol 12: 35-48.

Yassin A, Fredrick K, Mankin AS (2005) Deleterious mutations in small subunit ribosomal RNA identify functional sites and potential targets for antibiotics. Proc Natl Acad Sci U S A 102: 16620-16625.

Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV (2012) Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67: 2640-4.

PHÁT HIỆN ĐỘT BIỆN GEN 16S rRNA VÀ 23S rRNA TRONG CÁC CHỨNG SALMONELLA DA KHẢNG THUỘC DUỘC PHÂN LÁP TỪ CÁC NGƯỜI KHÁC NHAU BẰNG PHƯƠNG PHÁP GIẢI TRÌNH TỤ RNA-SEQ

Nguyễn Thanh Việt1,3, Võ Thị Bình Thủy1,3
1Viện Nghiên cứu ấu gen, Viện Hán lâm Khoa học và Công nghệ Việt Nam
2Viện Y Dược học Quản trị, Học viện Quản trị
3Học viện Khoa học và Công nghệ, Viện Hán lâm Khoa học và Công nghệ Việt Nam

TÔM TẮT

Sự gia tăng của vi khuẩn kháng thuốc đang xảy ra trên toàn thế giới. Kháng kháng sinh là một vấn đề...
Nguyễn Thanh Viết & Vo Thị Bích Thúy

nghiệm trong đổi với con người, vì các vi sinh vật gây bệnh có khả năng kháng thuốc sẽ làm mất tác dụng của kháng sinh. Cơ chế kháng thuốc ở vi khuẩn bao gồm các kênh bơm thuốc, cải biến và làm thay đổi protein, đặc biệt là đổi với các thuốc có dịch tác động là ribosome. Salmonella là một nguyên nhân hàng đầu gây ô nhiễm thực phẩm trên thế giới. Số lượng vi khuẩn kháng kháng sinh này phân lập được ở người lớn tăng lên, cho thấy sự lây lan của các loại vi khuẩn kháng kháng sinh là mối đe doa lớn đối với sức khỏe cộng đồng. Salmonella thường có mặt trong một lượng lớn các loại động vật, trong thức ăn, và môi trường, nhưng kiến thức của chúng ta về cách Salmonella kháng thuốc vẫn còn chưa rõ ràng. Do đó, mục đích của nghiên cứu này là hỗ trợ trong việc nghiên cứu các cơ chế mới giúp vi khuẩn các kháng kháng sinh có dịch tác động là ribosome. Chúng tôi sử dụng lọt biếu hiện đột biến của các gen 16S rRNA và 23S rRNA ở các loài Salmonella ta kháng kháng sinh phân lập được từ thị bản lề ở khu vực Hà Nội, Việt Nam. Kết quả đã xác định được 193 đột biến đếm (64 đột biến ở gen 16S rRNA và 129 đột biến ở gen 23S rRNA). Những đột biến này có thể có vai trò trong đề kháng kháng sinh streptomycin. Kết quả này cho thấy rằng có nhiều đột biến kháng kháng sinh vẫn còn chưa được biết đến, ngày càng đối với các kháng sinh có diện. Nghiên cứu này chỉ là kết quả sơ bộ, việc đánh giá thực nghiệm cần được tiến hành trước khi được áp dụng ở Salmonella và các loại vi khuẩn khác.

Tiểu khảo: Salmonella da kháng thuốc, đột biến đếm, 16S rRNA, 23S rRNA, giải trình tự mRNA