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Resistance Evaluation, Gene Sequence and Plasmid Profile of *Salmonella typhi* Isolated from Human Anal Swab

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Abstract

Antibiotic resistance in Enterobacteriaceae are of great global concern. *Salmonella* Sp. is widespread in the environment, but the main reservoir is the intestinal tract of livestock animals and particularly pig, poultry, and cattle and can be transmitted to humans through the food chain. Ten rectal swab samples were collected from five (5) male and five (5) females. One organism of clinical importance amongst the isolates was identified as *Salmonella typhi* through colonial, morphological and biochemical tests carried out following standard procedures. The identified isolate was investigated for its antibiotic resistance profile, Multiple Antibiotic Resistance index (MARi), pathogenicity status and its resistance genes were determined through molecular means using plasmid amplification and primers. Primers used include. *ermB, BlaTem, qnrB* genes. Result obtained showed *Salmonella typhi* to have α heamolysis. It was sensitive to 25% of the tested antibiotics, especially to all classes of Cephalosporin used. The Multiple antibiotic resistance index (MARi) was 0.66. The plasmid profiling revealed *Salmonella* to have low molecular weight plasmid and the molecular investigation using gene primers *ermB, BlaTem*, and *qnrB* genes showed *Salmonella typhi* to have resistance genes for macrolides (*ermB* gene) and betalactam (*BlaTem*) but no resistance gene quinolones (qnrB gene). The high antibiotic resistance of *Salmonella typhi* to antibiotics is a cause for concern and alternative means of intervention into the treatment of Salmonella associated infection should be investigated as a matter of urgency. Identifying and monitoring resistance in Salmonella isolates from human-related environments are of clinical and epidemiological significance in battling antimicrobial resistance.

Keywords: *Salmonella* sp, rectal swab, antibiotic resistance, gene primers, Plasmids, heamolysis

1.0 Introduction

Salmonella spp is a food borne Gram-negative rod-shaped bacteria belonging to the Enterobacteriaceae and responsible for some gastrointestinal diseases. Infections by *Salmonella* Sp constitute a major cause of morbidity and mortality, especially in developing countries **(**Smith *et al*., 2016). The rate of Salmonellosis cases is as a result of increased antibiotic resistance by Salmonella Sp. Antibiotic resistant clones of *Salmonella* is associated with outbreaks leading to more treatment failures and more hospital cases. It is therefore imperative that the antibiotic resistance profile of isolated Salmonella Sp be monitored over time (VTN *et al.*, 2018).

Changes in the composition of the gut flora, due to constant exposure to antibiotics and horizontal transfer, can happen silently, leading to the selection of highly resistant bacteria (Carlet, 2012). It is on the basis that gut microbiome can acquire resistance to antibiotics from continuous

exposure to antibiotics and also from the environment that this research work was carried out.

2.0. Materials and Methods

2.1. Sample collection

Swab samples were collected from ten humans (5males and 5 females) and isolation procedure was carried out on the samples using laid down procedures.

2.2 Characterization and identification of isolates

The isolate was identified using colonial, morphological and biochemical means. Biochemical tests carried out including Gram staining, sugar fermentation, Citrate utilization Test, Oxidase Test, Indole Test, Urease Test, Methyl red Test and Voges Proskauer Test (Tankeshwa, 2022).

2.3 Pathogenicity Test

Blood agar was prepared by weighing blood agar powder and dispensed into a conical flask. 100ml of distilled water was added and shaken thoroughly. The medium was then sterilized in an autoclave. Afterwards, the medium was poured into plates and allowed to gel. The plates were then inoculated with the isolate by streaking method and incubated at 37°C for 24hrs. The pathogenicity test was confirmed by determining α or β hemolytic zone of the isolates on the plate. i.e., ability of the isolate to lyse red blood cells.

2.4 Antibiotic sensitivity tests

Identified isolates were tested against standard antibiotics using the method described by Kirby –Bauer (1966) pour plate method, after which the rings of standard antibiotics were placed on the surface of gelled agar and allowed to incubate at 37^0C for 24 hrs.

Antibiotics used include; Amoxicillin (30 µg) Cefotaxime (30µg), Ceftriaxone (45 µg), Cefexime (25 µg), Levofloxacin (5 µg), Ciprofloxacin (45 µg) Imipenem (10 µg), Cefuroxime (25 µg), Ofloxacin (5 µg), Erythromycin (15 µg) Gentamycin (10 µg), Azithromycin (15 µg). Augmentin (30 µg), Nitrofurantoin (300 µg).

2.5 MARi

The multiple antibiotics resistance index for the resistant bacteria isolates was determined according to the procedure described by Krumpman (1983). This is essentially to determine the degree of bacteria resistance to antibiotics. These indices will be determined by dividing the numbers of antibiotics to which the organisms were resistant to (a) number of antibiotics tested (b) resistance to two or more antibiotics is taken as multiple antibiotics resistance. MAR greater than 0.2 shows high antibiotic resistance index.

2.6 Plasmid amplification and Molecular fingerprints using primers

The antibiotic resistant isolates were subjected to plasmid profiling to determine if the resistance factors are plasmid mediated using laid down procedure as described by (Barghouthi, 2011).

3.0 RESULTS

Morphological, colonial and biochemical tests carried out on the isolate showed a Gram-negative indole negative, citrate negative, oxidase negative, methyl red positive and urease negative rod. The organism was able to ferment glucose, galactose and fructose but was not able to utilize sucrose and lactose. The suspected organism was identified as *Salmonella* Spp.

3.1 Pathogenicity

The pathogenicity test showed the isolate to be alpha (α) heamolytic i.e it can partially break down red blood cells (Fig 1).

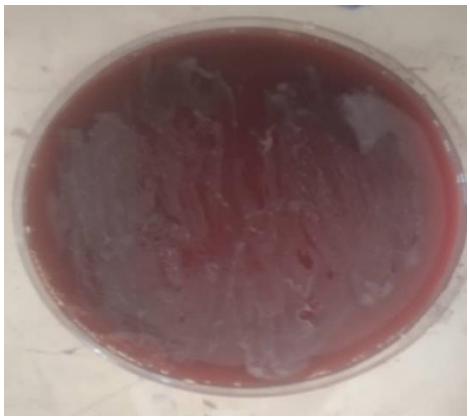

Fig 1. Alpha heamolysis of Salmonella spp.

3.2 Antibiotic resistance status

Salmonella Spp was sensitive to 33% of the antibiotics used. These include; Ofloxacin (12mm), Gentamycin (12mm), Nalidixic Acid (5mm) and levofloxacin (13mm) while it was resistant to Amoxicillin C, Cefotaxime, Imipenem, Nitrofurantoin, Cefuroxime, Ceftriaxone S, Ampliclox and Cefexime (Fig 2).

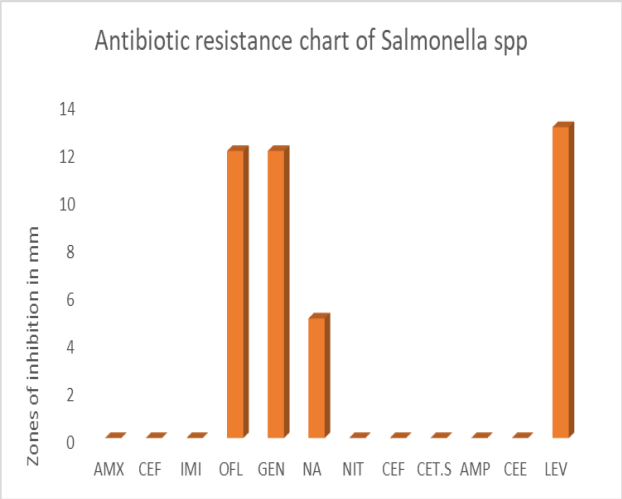

Fig 2. Antibiotic resistance chart of Salmonella spp

3.3 Multiple Antibiotic Resistance Index of Salmonella spp

Salmonella sp had MARI of 0.66. Resistance to two or more antibiotics is taken as multiple antibiotics resistance and MAR greater than 0.2 shows high antibiotic resistance index.

3.4 Plasmid profile of isolates

The isolate subjected to plasmid amplification showed Salmonella spp (well 2) to have plasmid borne resistant factor (Fig 3).

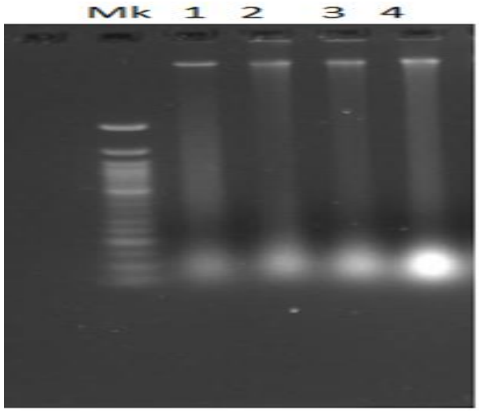

Fig 3: The plasmid profile of *Salmonella* spp (well 2)

3.5 Molecular fingerprints using gene primer

Figure 4 shows the molecular fingerprints of the isolate using primers. Salmonella spp (well 2) was positive for resistance to *ermB* (macrolides) with a basepair of 450 kbp but negative for *BlaTem* and qnrB genes.

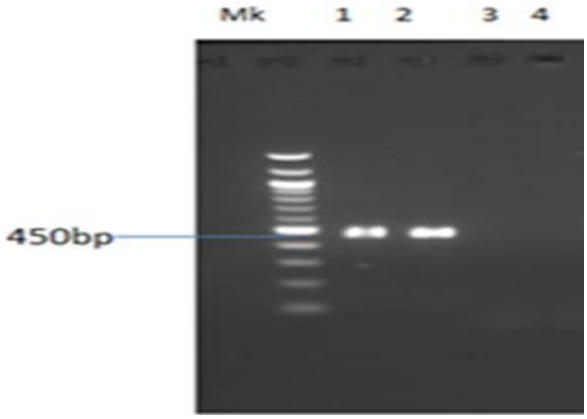

Fig 4: Gel indicates positive ermB amplification in Salmonella spp (well 2)

4.0 Discussion

Indiscriminate use of β-lactam antibiotics in both community and hospitals has transformed the human healthy intestinal gut flora into a reservoir of antibiotic-resistant organisms. The present study was planned to ascertain antimicrobial resistance among members of Enterobacteriaceae from the gut flora of healthy individuals at the community level.

Bacteria in the gut not only acquire Antimicrobial Resistance Genes (ARGs) but also contribute to the transfer of ARGs to other bacteria in the gut ((Ravi *et al*., 2014). These bacteria possess great danger, have become a global issue, and it is now essential to develop strategies for treatment options against infections caused by them. Therefore, understanding the resistance pattern is essential in fighting the battle against bacterial drug resistance.

Salmonella Spp are Gram-negative rod-shaped bacteria belonging to the Enterobacteriaceae. Bacteria in the genus Salmonella are implicated in the widespread foodborne diseases and have a significant global economic impact (Abebe *et al*., 2020). Resistance of Salmonella Sp to antibiotics have been severally reported, including ampicillin, amoxicillin, tetracycline, and chloramphenicol, to varying degrees (Alam *et al*., 2020; Mridha *et al*., 2020).

In this study, S. Sp isolated was sensitive to 25% of the standard antibiotics it was tested against. Many authors have recorded multidrug resistance of Salmonella Spp to antibiotics (Ray *et al*,. 2007; Genovese *et al*., 2004; Hleba *et al*. 2011). The increase of Salmonella strains resistant to antibiotics may be an indicator of the ecological impact of use of antibiotics in animals. A large amount of antibiotics is been used in veterinary medicine as therapy and as feed additives, which producing resistant organism (Cummings *et al*., 2013). These resistant bacteria and/or their resistant genes can be transferred from animals to humans through the food chain (Van den Bogaard *et al*., 2001). The MARi of Salmonella spp isolated 0.66. The higher MARI value that was observed in the present study might be attributed to the widespread use of antibiotics in the locality and the indiscriminate use of antibiotics either at recommended doses or at sub-therapeutic doses. The work of Elkenany, and his counterparts recorded similar observation of high MARI in Salmonella Spp (Elkenany *et al*., 2019) Mthembu *et al.,(*2019) also recorded high MARI for Salmonella spp.

In this study, Salmonella Spp was found to bear plasmid for antibiotic resistance (Fig 5). The presence of plasmids for antibiotics in Salmonella spp was reported by Rakov and Kuznetsova (2021). It was also observed that Salmonella Sp isolated in this work was positive for ermB (macrolide) and BlaTem (Betalactam) genes but negative for qnrB gene (quinolones) (Fig 3 & 4). Several authors have also reported the presence of resistance of Salmonella to these antibiotics (Chen *et al*., 2004; Uddin, *et al.*, 2021).

5.0 Conclusion

The result of this research emphasizes the fact that intestinal bacteria are prone to developing antibiotic resistance through horizontal transfer of plasmids, genetic mutation or constant exposure to antibiotics. The human gut microbiota harbours both commensals and opportunistic pathogens which can acquire resistance to antibiotics through mutation and horizontal gene transfer.

The development of antibiotic resistance in bacteria has been traced to uncontrolled use of antibiotics both in human therapy and agricultural practices. There is therefore a need to promulgate laws on the consumption and recommendation of antibiotics in hospital setting.

The need to understand how bacteria adapt to the antibiotic environment will lead to new therapeutic strategies for antibiotic-resistant infections. Interventions measures to minimize the abundance of antibiotic-resistant commensals and opportunistic pathogens may include faecal microbiota transplantation and the use of live biotherapeutics. Also, with the pandemic nature of bacteria resistance to cephalosporins and

deaths reportedly to have been caused by plasmid transfer and the fast spread of this resistant pathogens, a restriction in the use of these antibiotics (especially the third and fourth generations), would be recommended, and then the ceftolozane – tazobactam combination encouraged since it has demonstrated an excellent activity against Enterobacteriaceae.

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