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RESEARCH ARTICLE

GENOMIC ANALYSIS OF AN OUTBREAK ISOLATE OF *SALMONELLA ENTERICA*SEROVARENTERITIDIS ST11 FROMCAYAR, SENEGAL

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Abstract

Salmonella enterica is a bacterial species that groups +2,600 serotypes, many of which being capable of infecting humans and animals. *Salmonella* infects its hosts by the oral route, and causes two types of diseases, a gastroenteritis and an invasive infection. *Salmonella* gastroenteritis is a self-limited infection that is restricted to the gastrointestinal tract, and is frequent worldwide. In contrast, invasive salmonellosis is a systemic, life-threatening disease that is mostly found in low-and-middle income countries (LMIC) where *Salmonella* transmission is favored by poor hygiene conditions. In Senegal, like in other sub-Saharan African countries, *Salmonella* gastroenteritis outbreaks are frequent, and, in many cases, investigations, when conducted, are typically limited to isolating, identifying and performing antimicrobial susceptibility testing. In this study, we used a whole genome sequencing approach to conduct a genomic characterization of a *Salmonella enterica* outbreak in Cayar, a coastal town located 40 km from Dakar, capital of Senegal. Our results showed that the responsible clone belonged to a global epidemic group of serovarEnteritidis isolates that are frequently associated with gastroenteritis in various countries worldwide.

Introduction:-

Salmonella enterica(*S. enterica*) is a bacterial species that groups +2,600 serotypes defined by the variability of the lipopolysaccharide (O-antigen) and flagellar subunit (H-antigen)(1). *S. enterica* infects various human and animal hosts by the oral route, and causes two types of diseases, a gastroenteritis and an invasive infection. *Salmonella* gastroenteritis manifests as diarrhea, abdominal pain and vomiting, and is characterized by bacterial colonizationrestricted to the gastrointestinal tract(2). In contrast,invasive salmonellosis, including typhoid fever, paratyphoid fever(3) and invasive nontyphoidal salmonellosis (iNTS)(4), is a life-threateningdisease, characterized by the translocation of the bacteria to the systemic compartment of the infected host. *Salmonella*gastroenteritis is

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frequent worldwide while invasive salmonellosis is mostly found in low-and-middle income countries (LMIC) where *Salmonella*transmission is favored by poor hygiene conditions(5). In humans, *Salmonella* gastroenteritis can be caused by a variety of serovars that are known to infect different hosts, the most frequently reported being Typhimurium and Enteritidis(6). In contrast, only a few serovars are responsible of invasive salmonellosis,the most prevalent being human-restricted Typhi andParatyphi, the causative agents of human Typhoid and Paratyphoid fever respectively, and a few iNTSserovars. Interestingly, iNTSserovars are mainly associated with gastroenteritis and, as for this disease,Typhimurium and Enteritidis are the two serovars mostly causingiNTS. The prevalence of iNTS is high in LMIC (7), especially in sub-Saharan Africa (sSA) where several studies reported higher bloodstream infection cases due to iNTS than to Typhi(8,9). (7,8,9)

In Senegal, *Salmonella* gastroenteritis outbreaks are frequent (10–13), mostly being at low scale and, in many cases, without detailed characterization of the responsible strains. Investigations, when conducted, are typically limited to isolating, identifying and antimicrobial susceptibility testing. The development of genomics and its introduction to African countries give the opportunity to conduct more in-depth investigation of outbreaks to support public health management. Recently, we built capacity in bacterial genomics(13) and added this approach to our outbreak response toolbox. In this study, we report the genomic characterization of a *Salmonella enterica* outbreak in Cayar, a coastal town located 40 km from Dakar, capital of Senegal.

Materials and Methods:-

Outbreak cases description

In June 2021, the Senegalese Ministry of Health and Social Action requested from our institute a microbiological investigation following several cases of acute gastroenteritis in Cayar, a coastal town located 40 km from Dakar, capital of Senegal. We received seven (07) stool samples collected from five males and two females aged 22 – 50 years, all presenting symptoms of diarrhea.Among them, three (03) individuals also reported fever, while two (02) experienced vomiting. All the patients with fever returned a negative malaria rapid test.

Specimen collection, molecular testing, bacterial isolation and identification

Fecal samples were collected in dedicated boxes and transported to the Virology and Bacteriology departmentsfor analysis. Molecular testing was carried out using a multiplex one-step real-time RT-PCR assay that detects sixmajor gastrointestinal virus types (Astrovirus, Sapovirus, Rotavirus, Norovirus G1, Norovirus G2, Adenovirus-F40/41) simultaneously (Allplex™ GI-virus assay, Seegene). For bacteriological analyses,five (05) stoolsampleswere directly inoculated onto Bromocresol Purple (BCP), Hektoen and *Salmonella-Shigella*(SS) agar plates that were incubated at 37°C (two samples that did not have sufficient materials for bacteriological analyses were discarded). After overnight culture, typical *Salmonella spp* colonies grew on Hektoen (bluish colonies with black centers) and SS (colorless, transparent colonies with a central black spot).These colonies were directly identified on a Matrix-Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF) biotyper platform (Bruker, Germany) and re-isolated on the same plates for further characterization.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) was performedusing the disc diffusion method on Mueller-Hinton agar plates. The antibiotic discs (Bio-Rad Antibiotic Disks, Marnes la Coquette, France) used included, ampicillin (10 µg), ticarcillin (75 µg),cefalotin (30 µg), cefoxitin (30 µg),cefotaxime (30 µg), ceftazidime (30 µg), cefepime (30 µg), imipenem (10 µg), gentamicin (10 µg), nalidixic acid (30 µg), norfloxacin (10 µg) and chloramphenicol (30 µg). The results were interpreted following the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2021 and were recorded as susceptible, intermediate or resistantaccordingly.

Extraction of bacterial genomic DNA, whole genome sequencing, and assembly

Bacterial genomic DNA was extracted using aQiagenDNA Mini Blood & tissues Kit (Qiagen, USA) according to the manufacturer's recommendations. The quality and concentration of the extracted DNA were determined using a NanoDropTM 2000/2000c Spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts) and a Qubit 3.0 Fluorometer (Thermo Fisher Scientific). DNA libraries were prepared using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA) following the manufacturer's instructions. The resulting DNA libraries were purified using AMPure XP beads (Beckman Coulter, Sharon Hill, PA) and re-quantified using the Qubit 3.0 Fluorometer (Thermo Fisher Scientific) in order to obtain a normalized library pool. Sequencing was performed on the MiSeq System using v2 sequencing reagent kits (Illumina).The quality of the sequencing reads wasexamined using FastQCv0.11.9 before trimming with Trimmomatic v0.39(14). Reads that passed quality control were

submitted to mash v1.1 to determine the similarity of our isolates to genomes at the NCBI Refseq database to select for the genetically closest strain that was used for a reference-guided assembly. The cleaned reads were mapped to *Salmonellaenterica*serovarEnetritidis NC_011294.1. Reads that did not map to the NC_011294.1 chromosome were considered as forming a putative plasmid and were assembled using SPAdes v3.15.3 tool(15). Comparison of the assembled chromosomes from the two isolates with each other revealed two SNPs demonstrating that they corresponded to a single clone.

Serotype prediction, detection of antimicrobial resistance and virulence genes, and phylogenetic analysis
*Salmonellaenterica*serotype was predicted by submitting the contigs from genome assembly to the SeqSero 2 (http://denglab.info/SeqSero2) platform(16). Sequence types were determined using the MLST 2.0 software(17). Antimicrobial resistance genes and alleles with a minimum of 90% coverage and identity were detected from assembled chromosomal and plasmidiccontigs usingAbricate 1.0.1 (https://github.com/tseemann/abricate). Virulence genes and *Salmonella* Pathogenicity Islands (SPIs) were detected by using blast on a locally downladed database from the Center for Genomic Epidemiology (https://www.genomicepidemiology.org/). For phylogenetic analysis, concatenated core genome SNPs were analyzed using the maximum likelihood method with a bootstrap of 1,000. Generated phylogenetic tree was visualized using Microreact (http://www.microreact.org/).

Results and Discussion:-

Salmonella spp was isolated from the five stool samplesanalyzed as shown by MALDI-TOF and API gallery testing. No other pathogenic bacterium was isolated from the samples analyzed. Additionally, none of the tested viruses was found except for one sample in which a Norovirus GII was detected in coinfection with *Salmonella spp*. These results suggest an outbreak of *Salmonella* gastroenteritis. Antimicrobial susceptibility testing revealed a resistance to the quinolones nalidixic acid and norfloxacin, while the bacteria were susceptible to all the other antibiotics tested including β-lactams (ampicillin, ticarcillin, cephalothin, cefoxitin, cefotaxime, ceftazidime, cefepime and imipenem), gentamicin, and chloramphenicol. These results showed that the clone responsible for this outbreak was susceptible to historical *Salmonella*treatment (ampicillin and chloramphenicol) as well as to late resort antibiotics (cephalosporins and carbapenems). Additionally, they are in line with the increased frequency of resistance to quinolones in Senegal that our laboratory reported earlier(12,13).

Genomic characterization of outbreak *Salmonella*
Since outbreaks of gastroenteritis are frequent in Senegal, and since there are usually no advanced molecular investigations of the etiological agents of these infections, we wanted to characterize the recovered isolates further. For this purpose, we performed a whole genome sequencing of two isolates and conducted bioinformatics analysis of the obtained data.

Genome assembly yield a chromosome4,685,708bp long, with a 52.2% GC content. Additionally, a plasmid belonging to the IncX family was identified. MLST analysis showed that the clone belonged to ST11 of *S. enterica*serovarEnteritidis.As expected, we found a mutation of the*gyrA* gene (GyrA D87Y) that explains the isolates' resistance to quinolones (**Table 1**). Beside this, the bacteriaharbored an *aac(6')-Iaa* gene that encodes an aminoglycoside acetyl transferase (**Table 1**). However, we previously reported that the presence of this gene did not confer a resistance to aminoglycosides to *Salmonella*isolates from humans and poultry in Senegal(13). No AMR gene was detected in the plasmid harbored by the isolate. Analysis of the virulence determinants revealed the presence of several *Salmonella* Pathogenicity Islands (SPIs) including SPI1, SPI2, SPI3, SPI4, SPI5, SPI9, SPI10, SPI12, SPI13, SPI14, the pathogenicity island at centisome 63 (C63PI) and centisome 54 Island (CS54I) (**Table 1**).

Table 1:- Relevant genomic features of outbreak clone of Salmonella entericaserovarEnteritidis ST11.

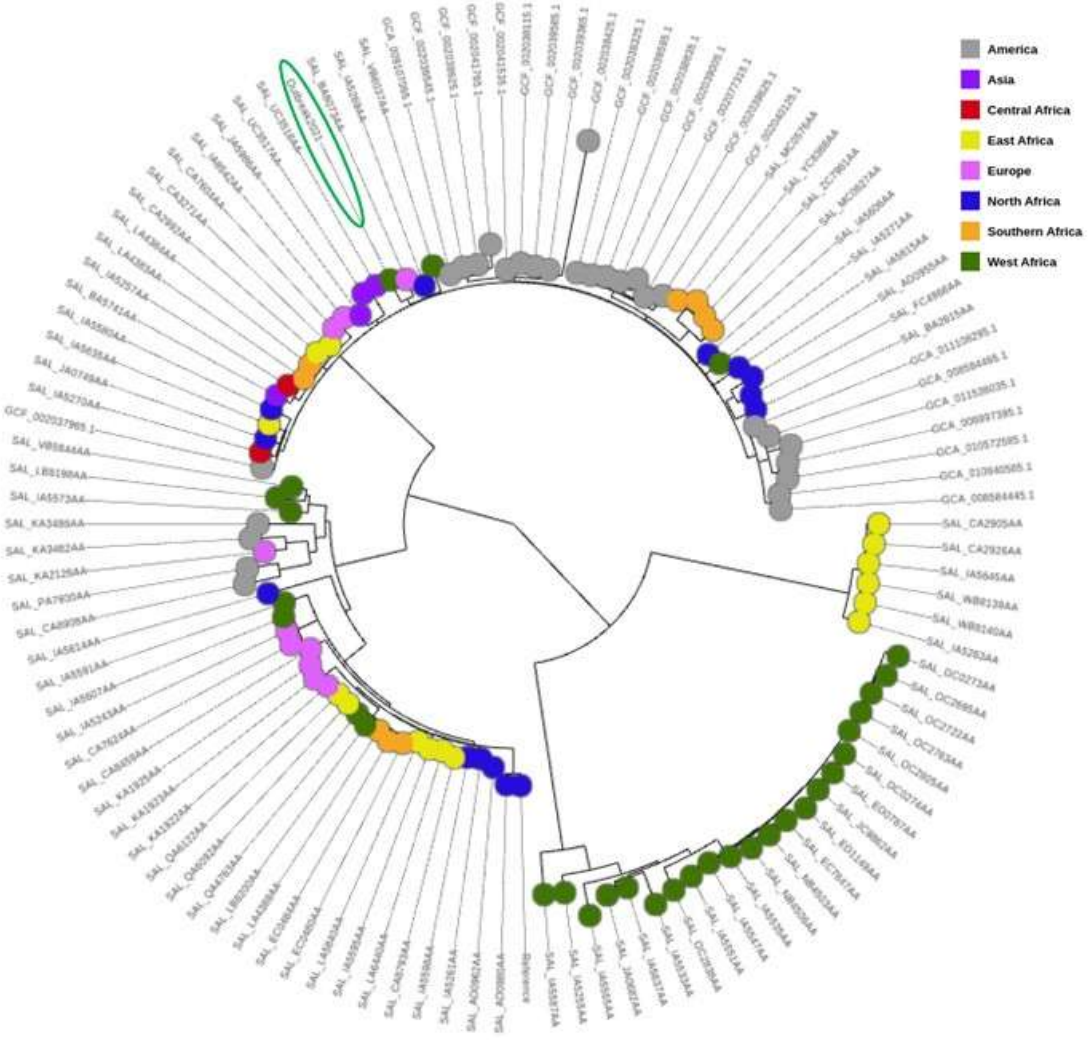
Features	Description
Chromosome	4,685,708 bp; 52.2% GC
Plasmid	IncXfamily
Pathogenicityislands	SPI-1, SPI1, SPI2, SPI3, SPI4, SPI5, SPI9, SPI10, SPI12, SPI13, SPI14, C63PI and CS54
Antimicrobialresistancedeterminants	*gyrA* (p.D87Y) conferring resistance to quinolones *aac(6')-Iaa*, not associated with resistance

SPI, *Salmonella* Pathogenicity Island; C63PI, Centisome 63 Pathogenicity island; CS54I, Centisome 54 Island

Within the Enteritidisserovar, ST11 is largely predominant accounting for 94.8% of the 26,670 isolates included in a large genomic analysis(18). Previous phylogenetic analyses based on core-genome SNPs showed that epidemic Enteritidisisolates can be grouped in three clusters corresponding to a global epidemic clade composed of isolates from various geographic origins, and additional clades predominantly constituted by clones from Western and Eastern/Central Africa respectively(19). In order to identify the closest genetic relative of our outbreak isolate, we performed a core-genome SNP-based phylogenetic analysis in which we included 114genomes of different geographical origins including 72 from Africa, available at the Refseq database of the NCBI (https://www.ncbi.nlm.nih.gov/). Our analysis revealed four main clusters, two of them being composed of isolates from Western and Eastern Africa respectively, and two comprising strains from different countries and continents (Figure 1). Interestingly, our outbreak isolate appeared in one of the international diverse cluster suggesting that it belongs to the global epidemic group of Enteritidisisolatespreviously described(19), whichare mostly associated with gastroenteritis in industrialized countries and are characterized by rare resistance to antimicrobials (Figure 1). It is important to note that the isolates composing the Western and Eastern Africa phylogenetic clades mentioned above are frequently multidrug resistant and associated with iNTS. These isolates deserve special attention in the sSA context where there are many people highly susceptible to *Salmonella* infections including children, immunocompromised individuals and patients of sickle-cell disease.

Legend Figure 1

Figure 1:- Phylogenetic analysis of *Salmonella enterica*serovarEnteritidis ST11. SNPs from core genome were compiled and used to build a maximum likelihood phylogenetic tree with a bootstrap of 1,000. The generated tree was visualized using Microreact. The geographical origins of the isolates are color-coded. The 2021 outbreakisolateiscircled in green.



Conclusion:-

We conducted a whole genome sequence and genomic analysis of a clone of *Salmonella enterica* that caused a gastroenteritis outbreak in Cayar, Senegal. Phylogenetic analysis suggested that this clone belongs to a global epidemic cluster composed of strains that caused enterocolitis in various countries, which are characterized by rare resistance to antimicrobials. Given the frequent occurrence of *Salmonella* outbreak in Senegal, our results call for the implementation of a genomic surveillance of *Salmonella* that will help manage future epidemics and provide means for evidence-based treatment in case of outbreak due to clones prone to invasive infections.

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