

CASE REPORT

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Carbuncle due to *Salmonella* Enteritidis: a novel presentation

Wadha Alfouzan^{1,5}, Dieter Bulach², Hidemasa Izumiya³, Khaled AlBassam⁴, Simin Sheikh⁵, Nasser Alrubai'aan⁵ and M. John Albert^{1*}

Abstract

Background: *Salmonella* Enteritidis causes intestinal and extra-intestinal infections, but rarely cutaneous infections. It has never been reported to cause carbuncle (a collection of interconnected furuncles with multiple pustular openings). We report a case of carbuncle due to *S. Enteritidis*.

Case presentation: An adult Bangladeshi patient with type 2 diabetes presented with a carbuncle on the left-side of his neck. A pure culture of *S. Enteritidis* was grown from the pus of the carbuncle. The patient was successfully treated with ciprofloxacin to which the isolate was susceptible. Whole genome sequencing of the strain showed that it possessed three additional virulence genes—*pef* (for plasmid-encoded fimbriae), *spv* (for salmonella plasmid virulence), *rck* (for resistance to complement killing) -responsible for systemic infections that were absent in the genome of a reference *S. Enteritidis* strain. In phylogenetic analysis, the strain clustered with other *S. Enteritidis* strains from different parts of the world.

Conclusions: A weakened immune system of the patient due to diabetes mellitus and the additional virulence genes of the isolate may have contributed to the unusual presentation of carbuncle. The possibility of *S. Enteritidis* to cause carbuncle should be considered.

Keywords: *S. Enteritidis*, Carbuncle, Whole genome sequencing, Virulence genes

Background

Non-typhoid *Salmonella* infections lead to not only self-limited acute gastrointestinal infections, but also, bacteremia with or without extra-intestinal focal infections [1, 2]. Extra-intestinal focal infections include septic arthritis, osteomyelitis, cholangitis, aortitis, endocarditis, pneumonia, urinary tract infection, and meningitis [1, 2]. Non-typhoid salmonellae do not produce cutaneous infections in immunocompetent individuals, but a single case of leg abscesses has been reported in an immunocompromised patient [3]. Here we report carbuncle in a patient with type 2 diabetes mellitus. Carbuncle is an aggregate of connected furuncles (hair follicle infections) with multiple pustular openings. To our knowledge, this is the first case of carbuncle due to non-typhoid

salmonellosis. Clinical manifestation is the result of interaction between the host and the pathogen. In this report, we also investigated whether there are unique genetic properties of the *Salmonella* isolate that could have contributed to this unusual clinical manifestation. This was achieved by whole genome sequencing of the isolate.

Case presentation

A 55-year-old Bangladeshi male with a history of type 2 diabetes mellitus and hypertension presented to the Emergency Department of Farwaniya Hospital, Kuwait in December 2015 with a left-sided neck swelling (8 cm × 6 cm) discharging pus through multiple sinuses. He was suffering from this condition for the past 2 weeks. On examination on alert, voice, pain and unresponsive (AVPU) scale, he was conscious and oriented with stable signs and without fever (oral temperature of 36.7 °C). Examinations covering the respiratory, cardiovascular, gastrointestinal and respiratory systems were unremarkable.

*Correspondence: john@hsc.edu.kw

¹ Department of Microbiology, Faculty of Medicine, Kuwait University, Jabriya, Kuwait

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

His body mass index (BMI) was 27.4 (classified as overweight [4] and a risk factor for type 2 diabetes [5]). Blood examination by flow cytometry (Sysmex 9000, Bornbarch, Norderstedt, Germany) showed leukocytosis ($18 \times 10^9/L$, mainly neutrophils, normal range is $3.7 \times 10^9/L$) and by oxygen rate method (Beckman Coulter DxC 800, Brea, CA, USA) hyperglycemia (random blood sugar of 20 mmol/L; normal range is 3.9–6.1 mmol/L). A diagnosis of carbuncle with hyperglycemia was made. He was given parenteral insulin on a sliding scale (1–14 units with increments of 1–2 units during a 24 h period). Incision and drainage of the carbuncle were done and pus was sent for microbiological studies. An empirical therapy with clindamycin (300 mg intravenously every 8 h) was started suspecting infection with *Staphylococcus aureus* which is a common cause of carbuncle [6]. This therapy was continued for 3 days until culture and susceptibility report became available (see below).

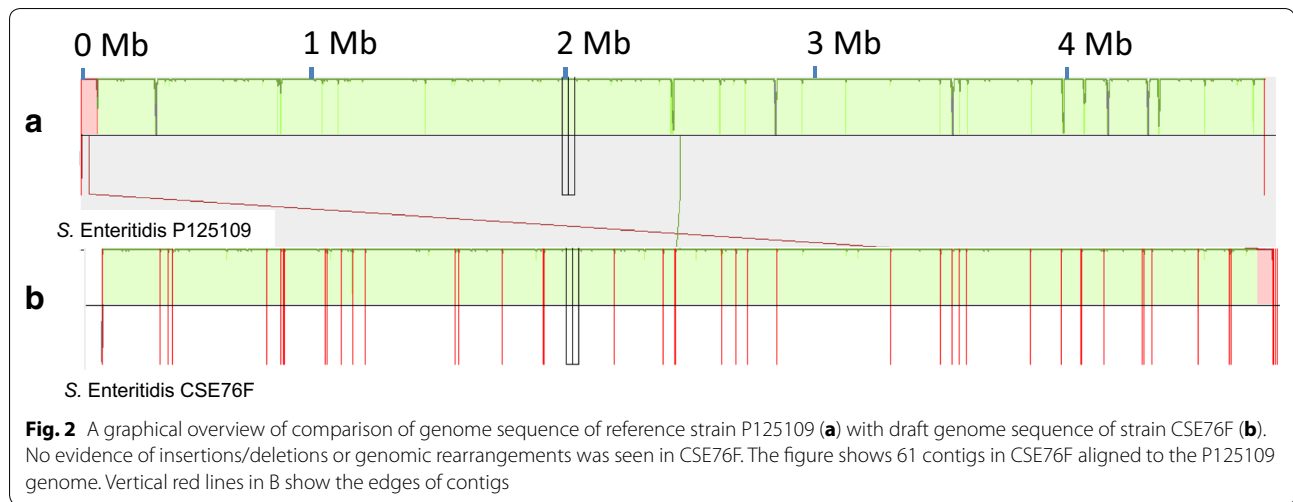
Pus was cultured on blood agar, MacConkey agar, chocolate agar and gentamicin blood agar. Blood agar and MacConkey agar were incubated aerobically, chocolate agar microaerobically and gentamicin blood agar anaerobically. Incubation was done at 37 °C for 24–48 h. All plates except the gentamicin plate grew a pure culture (organism was susceptible to gentamicin, see below) which was identified as a *Salmonella* species using Phoenix method (Becton–Dickinson, Franklin Lakes, NJ, USA) and Vitek 2 and Vitek-MS methods (Biomerieux, Marcy l’Etoile, France). Blood, urine and stool were sent for culture. Blood culture was done using BD BACTEC system (Becton–Dickinson) and there was no growth of any organism. Urine culture was done on blood agar and cysteine-, lactose-, and electrolyte-deficient (CLED) agar. There was no bacteriuria and *Salmonella* species was not isolated. Stool was cultured on MacConkey agar, Campy agar and *Salmonella–Shigella* agar (SSA) and enriched in selenite F broth with subsequent subculture on SSA. No bacterial diarrheal pathogen including *Salmonella* species was isolated. In vitro susceptibility of the *Salmonella* isolated from carbuncle (designated as CSE76F) to antibiotics was done by Vitek II system (bioMerieux) and E test (AB Biodisk, Solna, Sweden) and interpreted by Clinical and Laboratory Standards Institute (CLSI) guidelines [7]. It was susceptible to amikacin, amoxicillin–clavulanic acid, chloramphenicol, tetracycline, ceftazidime, ceftriaxone, cefuroxime, cephalothin, imipenem, meropenem, ciprofloxacin, gentamicin, piperacillin–tazobactam, tigecycline, and trimethoprim–sulfamethoxazole, but resistant to ampicillin and clindamycin. The antibiotic therapy was changed to ciprofloxacin (400 mg intravenously every 12 h) for 14 days at which time he recovered. This prolonged intravenous therapy was necessitated to avoid relapse because the patient was immunocompromised

(due to diabetes mellitus) and the wound was large. After debridement of the wound (Fig. 1), the patient was referred to plastic surgery for skin grafting.

The *Salmonella* organism was typed as Enteritidis species by multilocus sequence typing (MLST) [8] and a *sefA* (*Salmonella* Enteritidis *fimbria*)A gene-specific PCR [9]. A draft genome sequence for isolate CSE76F was determined using the whole genome shotgun method. The sequencing library was prepared using the Nextera XT DNA sample preparation kit (Illumina, San Diego, CA) and the sequence read data were produced on the Illumina NextSeq instrument (paired end, 150 base reads). A total of 3,213,288 reads yielded 477,626,349 bases of usable sequence data after filtering to remove low quality sequence data and adapter sequences (approximately 100-fold read coverage of the genome) (Sequence Read Archive [SRA] Accession Number, SRR5198927). De novo assembly of the read data with MegaHit [10] yielded a draft genome sequence comprising a total of 4,743,613 bases in 61 contigs (minimum contig size 500 bases). The MLST type was confirmed from the draft genome sequence (SenTera scheme, ST 11) from the genome sequence [11] and the antimicrobial resistance gene profile of the isolate was determined using Abricate [12] and the ResFinder database [13]. This showed that the isolate carried a *bla*_{TEM-1b} gene (encoding a class A beta-lactamase). This fits with the observed resistance of the isolate to ampicillin [14]. The most related closed genome sequence was that from *Salmonella enterica* subsp. *enterica* serovar Enteritidis strain P125109 isolated from an outbreak of human food-poisoning (RefSeq: NC_011294) with 55 SNPs identified across 98.82% of the strain P125109 genome sequence. Aligning the CSE76F contigs to the P125109 genome sequence revealed no apparent major genomic deletions, inversions or rearrangements (Fig. 2). Analysis of the virulence gene profile was performed using Abricate with the VFDB database of virulence genes [15]. CSE76F strain



Fig. 1 Photo of debrided carbuncle on the left-side of patient's neck after successful antibiotic therapy



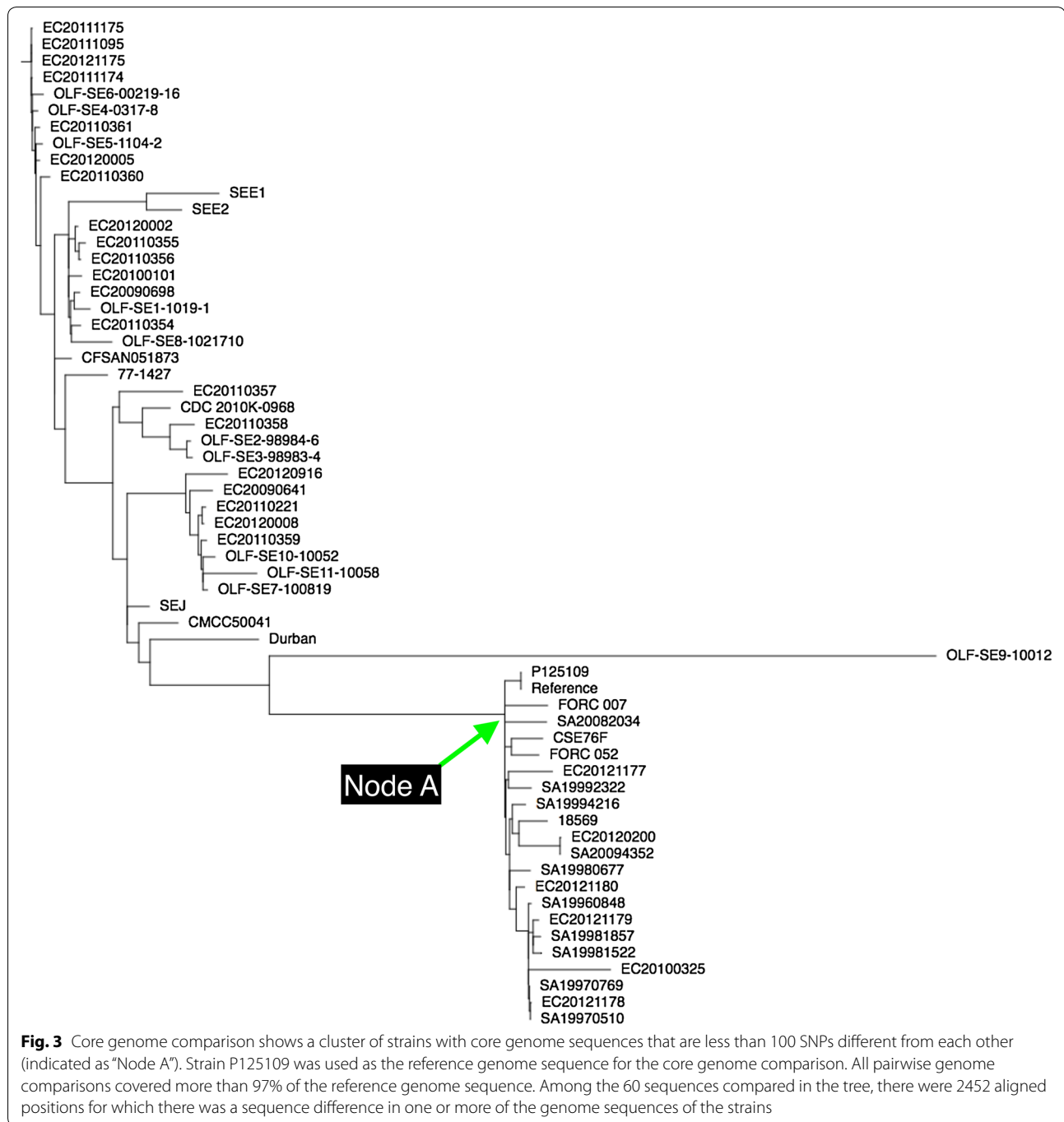
possessed three additional virulence genes-*pef* (*plasmid-encoded fimbriae* with all four subunits-*pefA*, *pefB*, *pefC*, *pefD*), *spv* (*salmonella plasmid virulence* with all three subunits- *spvA*, *spvB*, *spvC*) and *rck* (*resistance to complement killing*)-that were absent in strain P125109. Phylogenetic relationship of CSE76F strain based on single nucleotide polymorphism (SNP) with that of 60 closed genomes of *S. Enteritidis* strains available in GenBank (shown in Additional file 1: Table S1) was constructed using FastTree [16]. Core SNP differences were called using Nullarbor [17]. Strain CSE76F did not occupy a unique position, but clustered with several other strains from the United Kingdom, South Korea, Canada and the United States of America (Fig. 3).

Discussion and conclusions

pef gene mediates adhesion and biofilm formation [18], *spv* gene cytotoxicity [19] and *rck* gene serum-resistance and survival inside the macrophage [20]. All the three

genes are located in a 60 MDa virulence plasmid that enhances the growth rate during the systemic phase of the disease [21]. It is not surprising that the strain CSE76F possessed the additional virulence genes which would have contributed to its ability to produce the unusual manifestation of carbuncle in the immunologically weakened diabetes mellitus patient. However, the strain clustered with several other *S. Enteritidis* strains from different parts of the world.

Since there was no history of diarrhea in this patient or in his close family contacts, he would have had an asymptomatic intestinal infection with *S. Enteritidis*. The organism would have entered the blood stream from the intestinal tract and then the skin. Another possibility that the patient might have introduced the organism into his skin by scratching it by fecally contaminated fingers. This case shows that the possibility of non-typhoid *Salmonella* (*S. Enteritidis*) causing carbuncle should be taken into consideration.



Additional file

Additional file 1: Table S1. Complete genome sequences to which strain CSE76F was compared.

Abbreviations

AVPU: alert voice pain unresponsive scale; CLED: cysteine lysine electrolyte deficient agar; SSA: *Salmonella-Shigella* agar; CLSI: Clinical and laboratory Standards Institute; *sef*: *Salmonella* Enteritidis fimbriaA; *pef*: plasmid-encoded fimbriae; *spv*: *Salmonella* plasmid virulence; *rck*: resistance to complement killing; SRA: sequenced read archive; SNPs: single nucleotide polymorphisms; BMI: body mass index.

Authors' contributions

WA, SS and NA did clinical microbiology work, DB did whole genome analysis, HI typed the isolate, KA did surgical debridement of the wound, and MJA supervised collection of data and wrote the manuscript. All authors read and approved the final manuscript.

Author details

¹ Department of Microbiology, Faculty of Medicine, Kuwait University, Jabriya, Kuwait. ² Microbiological Diagnostic Unit Public Health Laboratory, Peter Doherty Institute for Infection and Immunity and Melbourne Bioinformatics, The University of Melbourne, Victoria, Australia. ³ National Institute of Infectious Diseases, Tokyo, Japan. ⁴ Department of Surgery, Farwaniya Hospital, Al Farwaniyah, Kuwait. ⁵ Microbiology Unit, Farwaniya Hospital, Al Farwaniyah, Kuwait.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

Available.

Consent for publication

Available from the patient.

Ethics approval and consent to participate

Not needed as this case is a routine case admitted in the hospital for treatment. Therefore, ethics approval was waived by Farwaniya Hospital Ethics Committee. Local, national and international guidelines were followed in the conduct of this study.

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Alfouzan, W; Bulach, D; Izumiya, H; AlBassam, K; Sheikh, S; Alrubai'aan, N; Albert, MJ

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