Salmonella enterica serovar Paratyphi A isolated from a hard-to-heal diabetic ulcer: a case report

Abstract: Chronically infected diabetic wounds have a polymicrobial aetiology. However, *Salmonella paratyphi A* is a very rare cause of wound infection. A 76-year-old female patient with type II diabetes, presented with a wound on the left leg of two months' duration. The wound was painful, erythematous and a thick, foul-smelling discharge was present. There was a history of delayed wound healing. *Salmonella paratyphi A* and *Pseudomonas aeruginosa* were isolated from the wound tissue. The patient was treated with cefuroxime and cloxacillin empirically and following the antibiotic susceptibility testing (ABST) report, ciprofloxacin was continued for 10 days [AQ1 - needs clarifying as per main text]. The wound was treated with multiple debridements and topical antiseptic. On follow-up, the patient remained afebrile with subsiding discharge from the ulcer. This is the first reported case of *Salmonella paratyphi A*, from an infected diabetic ulcer in Sri Lanka and it serves to further define the spectrum of illnesses caused by this uncommon pathogen.

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diabetes • hard-to-heal wound • infection • *Pseudomonas* • *Salmonella*

Infections caused by *Salmonella* spp. usually manifest as gastroenteritis, septicaemia or bacteraemia. Superficial wound infections due to *Salmonella* are very rare and not reported in Sri Lanka [AQ1: please clarify - does this mean no official reporting requirement? if so to who?]. This case report describes isolation of *Salmonella paratyphi A* from an infected diabetic ulcer in Sri Lanka.

Case report

A 76-year-old female with a hard-to-heal ulcer on the left leg of two months' duration was admitted to Colombo South Teaching Hospital in Sri Lanka. She had a past history of type II diabetes of 15 years' duration, hypertension, and hyperlipidaemia. She was on metformin 500mg and gliclazide 80mg twice a day. The ulcer was on the lateral aspect of the middle third of the left lower limb, 7cm up from the left lateral malleolus, and was about 5.5cm [AQ2: is this cm^2] in size with an irregular margin. The ulcer was painful, erythematous and a thick, foul-smelling discharge was present. The ulcer was infected and superficial, i.e. it did not involvie the tendon, capsule or bone and did not have ischaemia. According to the University of Texas wound classification system, the ulcer was a Grade 1B wound.

On admission, the patient was afebrile. However, she had elevated C-reactive protein (51mg/l), white blood cells (17.37x103/µl), with a neutrophil count of 9.12x103/µl showing evidence of systemic infection and decreased haemoglobin (9.7g/dl). Haemoglobin A1c level was 7%, and liver and renal functions were within normal limits.

Ethical approval

Ethical approval was obtained from the Ethics Review Committee, University of Sri Jayewardenepura (Ref. 12/16), Colombo South Teaching Hospital (Ref. 506) and Medical Research Institute (Ref. 67/2015). Written informed consent was obtained from the patient.

Tissue sampling and testing

Tissue specimens (two in total) were collected from deep within the wound bed after debridement; one in a sterile container for aerobic culture and another in a sterile Robertson Cooked Meat Medium for anaerobic culture. [AQ3: how was the wound cleaned? in discussion states

Direct Gram stain of the specimen [AQ4: from both specimens?] and aerobic and anaerobic cultures were performed according to the Standard Operating Procedures.[1] Direct Gram stain revealed presence of polymorphonuclear leukocytes (>25 cells/low power field) with Gram-negative *bacilli*. [AQ5: in bothspecimens?] However, Gram-positive *cocci* and fungal filaments were absent. A tissue specimen [AQ:

Ayomi Dilhari,[1] PhD Candidate; **Sujatha Pathirage**,[2] Consultant Microbiologist; **Chinthika Gunasekara**,[1] Senior Lecturer; **Neluka Fernando**,[1] Professor; **Deepaka Weerasekara**,[3] Professor; **Andrew MacBain**,[4] Professor; **Manjula Weerasekera**,[1] Senior Lecturer*
***Corresponding author email:** mmweera@sjp.ac.lk
1 Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka. **2** Department of Bacteriology, Medical Research Institute, Sri Lanka. **3** Department of Surgery, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka. **4** School of Health Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, UK.

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IN PROOF

from which sample as two collected?] was sliced into small pieces [AQ6: what size?], weighed and homogenised in 1ml of sterile phosphate buffered saline.[2] Homogenised tissue was then subjected to serial dilution for quantitative culture. Following this, 100µl of each dilution was inoculated on to blood agar (Oxoid, UK). A further 50µl from each of the initial homogenate was inoculated on to chocolate agar, MacConkey agar and Sabouraud's Dextrose agar. All plates were incubated at 37°C for 18–24 hours and the chocolate agar was incubated in 5–10% CO_2 for 18–24 hours at 37°C. Anaerobic cultures were performed as per Standard Operation Procedures.[1]

Colony count from the blood agar plates were obtained and recorded as colony forming units/gram of tissue (CFU/g). The culture isolates were presumptively identified based on the colony morphology, Gram stain and appropriate biochemical tests as per the Standard Operating Procedures.[1] *Salmonella paratyphi A* was isolated from the specimen together with *Pseudomonas aeruginosa* with observed colony counts of 1.2x107CFU/g and 11.6x107CFU/g, respectively. Identification of *Salmonella paratyphi A* was confirmed at Enteric Reference Laboratory, Medical Research Institute, Colombo, Sri Lanka by biochemical tests, and serotyping using *Salmonella antisera* (Denka Seiken Co. Ltd, Japan). Anaerobic organisms were not isolated from the anaerobic culture.

The *Salmonella paratyphi A* isolate was subjected to DNA extraction and nearly full length 16S rRNA genes were amplified using the 27F primer (5'AGAGTTTGATCMTGGCTCAG) and 1492R primer (5'TACGGYTACCTTGTTACGACTT),[3] followed by sequencing for molecular identification. Following PCR (polymerase chain reaction) product purification, bidirectional sequencing was done using inner primers of 785F (5'GGATTAGATACCCTGGTA) and 905R (5'CCGTCAATTCMTTTRAGTTT).[AQ7: does this need a reference as previous is ref'd] The bacterial species were identified by nucleotide sequence analysis of the PCR product followed by comparison of this sequence with known sequences stored in the Basic Local Alignment Search Tool (BLAST) database (in the National Center for Biotechnology Information, NCBI) to identify the species with a similarity cut-off of 100%. Sequencing results identified the organism as *Salmonella enterica* sub sp. *enterica serovar paratyphi A* strain and was submitted to the GenBank database at the NCBI (Accession No: MH001399).

Antimicrobial susceptibility testing by the Clinical and Laboratory Standards Institute (CLSI) method for *Salmonella paratyphi A* revealed susceptibility to ciprofloxacin, gentamicin, cefotaxime, ceftriaxone, amoxicillin-clavulanic acid, cefepime, imipenem, aztreonam, meropenem, and piperacillin-tazobactam and resistance to ampicillin. *Pseudomonas aeruginosa* was susceptible to ciprofloxacin, ceftazidime, gentamicin, piperacillin-tazobactam, cefepime, aztreonam, amikacin, imipenem and was resistant to meropenem.

The patient was treated with cefuroxime 750mg (eight-hourly) and cloxacillin 500mg six-hourly empirically and following the ABST report, ciprofloxacin 500mg (12-hourly) was continued for 10 days [AQ8: please clarify duration of a/b use - was it only ciprofloxacin that was continued for 10 days?]. The systemic antibiotics were indicated due to the presence of systemic symptoms [AQ9: which were?]. The patient was followed up with ulcer debridement three times a week [AQ10: for how many weeks?], at Colombo South Teaching Hospital. After each debridement, a topical medication, betadine 7.5% (w/v), was used. On follow-up, the patient remained afebrile with subsiding discharge from the ulcer. [AQ11: do we know ultimate outcome? did the ulcer heal?]

Discussion

According to the available literature, this is the first reported case of isolation of *Salmonella paratyphi A* from an infected, superficial diabetic ulcer in Sri Lanka. Traditional culture-dependent microbial studies of wound microbiota have focused on the role of aerobic and facultative anaerobic pathogens.[AQ12: ref?] Studies report polymicrobial aetiology of wounds based on culture methods which suggest the presence of *Staphylococcus aureus*, β haemolytic *Streptococci* and in certain circumstance, *Pseudomonas aeruginosa*.[4]

The medical and research communities are now beginning to understand that the diversity of bacterial populations in hard-to-heal wounds may be a key contributor to the chronicity of the wounds, especially in open, diabetic hard-to-heal ulcers.[4] In this case study, both *Salmonella paratyphi A* and *Pseudomonas aeruginosa* were isolated from an infected chronic diabetic ulcer. *Pseudomonas aeruginosa* is a known pathogen associated with diabetic ulcers.

Salmonella spp. are known to cause enteric fever and have also rarely been reported to be associated with skin, soft tissue infections[5] and osteomyelitis.[6] Skin and soft tissue infections related to *Salmonella* spp. were reported, especially in immunocompetent patients.[7] *Salmonella osteomyelitis* has been reported in patients with gastroenteritis and with risk factors: haemoglobinopathies (such as sickle cell disease and thalassemia), and immunosuppressed status including human immunodeficiency virus (HIV), diabetes, chronic steroid use, and peripheral vascular disease (PVD).[8–10]

Approximately 50% of patients with diabetes are known to have a peripheral neuropathy that predisposes them to the development of unrecognised [AQ13: what does this mean?] soft tissue and bone infections.[10] Pak and Pham[6] in 2017, presented the case of a 67-year-old woman with diabetes who developed *Salmonella osteomyelitis* and subsequently underwent surgical excision of a tibial lesion followed by two months of intravenous antibiotic therapy. In another study, Dowd et al.[4] in 2008, reported the presence of three *Salmonella*

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spp. (7.5%) as well as other different bacterial species, in a group of 40 individuals with diabetic foot ulcers (DFUs) using Bacterial Tag Encoded FLX Amplicon Pyrosequencing. However, culture isolation of bacterial species was not carried out in that study.

In this case study, *Salmonella paratyphi A* isolated from microbiological investigations was further confirmed by gene sequencing. Similarly, in a case study published by Chander et al.[11] in 2011, both *Salmonella paratyphi A* and *Pseudomonas aeruginosa* were isolated from a 47-year-old female with a history of diabetes for 20 years, with a hard-to-heal wound of three months' duration having distal osteomyelitis [AQ14: was the wound caused by the d/m?].[11]

However, in the present case study, the ulcer was categorised as grade 1B and osteomyelitis was not present. In this patient, both *Salmonella paratyphi A* and *Pseudomonas aeruginosa* were isolated from the patient's infected, hard-to-heal diabetic ulcer. The fact that the specimen collected was a wound tissue specimen deep from within the wound bed and also that the patient had an infected ulcer strongly suggests the role of *Salmonella paratyphi A* as a pathogen rather than a coloniser. However, the lack of blood culture results is a limitation of the study which could have helped to confirm the pathogenic role of *Salmonella paratyphi A*.

Salmonella paratyphi A is a facultative anaerobic, Gram-negative bacillus, belonging to the *Enterobacteriaceae* family and usually causes enteric fever. Most cases of salmonellosis are mild. However, sometimes it can be life-threatening. The development of extremity ulcers is a serious complication for approximately 15% [AQ15: is this worldwide?] of patients with diabetes.[4,12,13] Numerous factors such as wound infection, wound hypoxia, nutritional deficiencies, ischaemia and the disease itself are the most common predisposing factors, which can impair wound healing and lead to diabetes-related lower-limb amputations.[14–16] Wound infection and impaired healing dramatically increases the risk of mortality in patients with diabetes.[17] It is reported that inconsistent blood sugar levels and hypoxia may impair the ability of white blood cells to destroy pathogenic bacteria and fungi that increases the risk of infection.[18]

The patient in this study was treated with antibiotics due to the presence of systemic involvement [AQ16: what does it mean?] while subjected to local wound management with antiseptics and debridement every three days [AQ: previously state three times a week]. The patient was seen to improve on follow-up. However, the role of antibiotics in hard-to-heal wounds is a subject of debate. Systematic reviews have also found little evidence for the benefit of antibiotic therapy on wound healing.[19] [AQ17: statement says reviews though only one ref - please add more or amend] Another study claimed that although antibiotics are used for hard-to-heal wounds, their optimal use and benefit remain unclear.[20] Gardner et al.[21] in 2009, also reported that no statistical significance was observed between the presence of low and high microbial load in both patients on and patients not on systemic antibiotics at the time of data collection.[21]

Thus, antiseptics which have a broad spectrum of activity are commonly being used as a treatment strategy for prevention and control of infections in hard-to-heal wounds.[22] The use of antiseptics at the optimal time and concentration could promote healing of the wound. Furthermore, the optimal timing and frequency of debridement is also an important component of wound care.[23]

Wound debridement is carried out to remove non-viable tissue and to support the development of healthy, well perfused tissue to enable cell proliferation to populate the wound bed.[AQ18: ref] In this study, wound debridement was carried out every three days, as routinely practiced in the management of hard-to-heal wounds at the institution where the study was conducted. The patient underwent eight rounds of wound debridement, both during and after antibiotic treatment. Since the use of use of systemic antibiotics in treatment of hard-to-heal wounds is not beneficial [AQ19: is it definitely not beneficial or is it equivocal?], after each debridement, povidone-iodine (betadine) was used as a topical antiseptic for wound healing. Povidone-iodine application can enhance wound healing by reducing microbial bio-burden in hard-to-heal wounds. [AQ20: ref]

According to best practice guidelines for ASEAN Plus: management of diabetic foot wounds [AQ21: is this a publication - please provide ref], management of hard-to-heal diabetic wounds generally involves keeping the affected area clean and dry, periodic surgical debridement of any necrotic tissue, revascularisation procedures when indicated, washing of the area with antibacterial solution and antimicrobial dressings for greater effect.[24] It is believed that, in this study, the management of the patient's wound, with multiple debridement and topical antiseptic treatment, could have contributed to wound healing.

This case study reports the first finding of a gastrointestinal pathogen *Salmonella paratyphi A* in a chronically infected diabetic wound in Sri Lanka. Thus, it is an indication that rare pathogens can be present in this type of wound and, consequently, can lead to complications such as osteomylitis. There are few other similar published case reports of *Salmonella paratyphi A* in chronically infected wounds. Diabetes is a key risk factor for *Salmonella* infection in skin, soft tissue and osteomyelitis.[25] As the prevalence of diabetes continues to increase, the incidence of rare pathogens may play a role in the aetiology of hard-to-heal diabetic wounds due to the patient's immunocompromised state. Therefore, it would be advisable to maintain a high index of suspicion for rare pathogens such as *Salmonella paratyphi A* in this group of patients.

Limitations

The lack of blood culture results is a limitation of the

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study which could have helped to confirm the pathogenic role of *Salmonella paratyphi A*. [AQ22: please advise of any other limitations?]

Conclusion

This is the first reported case of isolation of *Salmonella paratyphi A* as an unusual pathogen from an infected diabetic wound in Sri Lanka and it serves to expand the spectrum of rare aetiological agents that could lead to chronic wound infections and complications. JWC

Acknowledgments

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Reflective questions [AQ23: please provide 3-4 questions]

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