Distribution and factors associated with *Salmonella enterica* genotypes in a diverse population of humans and animals in Qatar using multi-locus sequence typing (MLST)

Yu C. Chang[a], Joy Scaria[a], Mariamma Ibraham[b],
Sanjay Doiphode[b], Yung-Fu Chang[a], Ali Sultan[c],
Hussni O. Mohammed[a,*]

[a] *Department of Population Medicine and Diagnostic Sciences, College of Veterinary
Medicine, Cornell University, Schurman Hall, Ithaca, NY 14853, USA*
[b] *Hamad Medical Corporation, Bin Omran Area, Doha 3050, Qatar*
[c] *Weill Cornell Medical College-Qatar, Education City, Al Luqta Street, Ar-Rayyan, P.O.
Box 24144, Doha, Qatar*

Summary *Salmonella enterica* is one of the most commonly reported causes of bacterial foodborne illness around the world. Understanding the sources of this pathogen and the associated factors that exacerbate its risk to humans will help in developing risk mitigation strategies. The genetic relatedness among *Salmonella* isolates recovered from human gastroenteritis cases and food animals in Qatar were investigated in the hope of shedding light on these sources, their possible transmission routes, and any associated factors. A repeat cross-sectional study was conducted in which the samples and associated data were collected from both populations (gastroenteritis cases and animals). *Salmonella* isolates were initially analyzed using multi-locus sequence typing (MLST) to investigate the genetic diversity and clonality. The relatedness among the isolates was assessed using the minimum spanning tree (MST). Twenty-seven different sequence types (STs) were identified in this study; among them, seven were novel, including ST1695, ST1696, ST1697, ST1698, ST1699,

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* Corresponding author. Tel.: +1 6072533566.
 E-mail addresses: yc483@cornell.edu (Y.C. Chang), js567@cornell.edu (J. Scaria), mabraham2@hmc.org.qa
(M. Ibraham), sdoiphode@hmc.org.qa (S. Doiphode), yc42@cornell.edu (Y.-F. Chang), als2026@qatar-med.cornell.edu (A. Sultan),
hom1@cornell.edu (H.O. Mohammed).

ST1702, and ST1703. The pattern of overall ST distribution was diverse; in particular, it was revealed that ST11 and ST19 were the most common sequence types, presenting 29.5% and 11.5% within the whole population. In addition, 20 eBurst Groups (eBGs) were identified in our data, which indicates that ST11 and ST19 belonged to eBG4 and eBG1, respectively. In addition, the potential association between the putative risk factors and eBGs were evaluated. There was no significant clustering of these eBGs by season; however, a significant association was identified in terms of nationality in that Qataris were six times more likely to present with eBG1 compared to non-Qataris. In the MST analysis, four major clusters were presented, namely, ST11, ST19, ST16, and ST31. The linkages between the clusters alluded to a possible transmission route. The results of the study have provided insight into the ST distributions of *S. enterica* and their possible zoonotic associations in Qatar.
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Introduction

Salmonella enterica is a major cause of enteric illness in both humans and animals [1,2]. Sequelae that arise from salmonellosis vary from mild gastroenteritis to systemic infections [3]. Globally, human salmonellosis is one of the common food-borne diseases [4]. It has been estimated that over 90 million cases of gastroenteritis due to *Salmonella* infections occur worldwide each year, which results in more than 150,000 deaths [5]. Furthermore, *Salmonella* infection in food animals is a great challenge because food animals are a major source of outbreaks in humans [6]. Therefore, defining the relatedness and association among *S. enterica* isolates in a population is critical for human health and food security. In addition, several epidemiological investigations are available from areas such as North America and Europe; however, fewer were reported in Africa and the Middle East region. The combination of molecular and epidemiologic approaches is more valid and has been used in part or total to address similar objectives around the world, with varying results [7—10].

One of the new and valuable diagnostic techniques in molecular epidemiologic investigations is multilocus sequence typing (MLST) for the typing of multiple loci [2]. In this technique, housekeeping genes along with other conserved genes are analyzed according to the nucleotide variation for the characterization of bacterial pathogens. The traditional *Salmonella* MLST technique employs seven housekeeping genes; however, other approaches were used in the literature. It is commonly believed that in comparison to other strain typing techniques, the MLST has many advantages; for example, it is consistent and reproducible, and it combines sequencing and bioinformatics with a population genetics approach to produce accurate and reliable results.

The long-term objective of the study was to investigate the occurrence of *S. enterica* in human and non-human populations and to shed light on the factors that contribute to their perpetuation. This knowledge would help in designing cost-effective strategies to mitigate its associated risk. An integrated approach of MLST was conducted to investigate the genotype distribution, MST was conducted to clarify the relationship among the STs and possible pathways of transmission, and epidemiological studies were conducted to identify the factors that contribute to the perpetuation of the pathogen.

Materials and methods

Target and study population

Two populations (human and non-human) were targeted in this repeat cross-sectional study to address the stated objectives. All of the isolates were sampled either in the hot months (April—October) or cold months (November—March) throughout the year of 2011 to capture potential seasonal variations in the shedding of individual pathogens. The human subjects consist of individuals who were admitted to any of the Hamad Medical Corporation (HMC) hospitals in Qatar and had fecal samples collected from them, and the samples were tested bacteriologically for *Salmonella* spp. This population consisted of individuals of varying ethnicity, nationality, age, gender, and received tentative diagnosis. The samples were collected as routine patient care, and we have obtained ethical approval from the Institutional Review Board to use the samples and the data.