Salmonellosis among Pediatric Population in Qatar: Prevalence, Antibiotic Resistance and Molecular Epidemiology

Faculty and Postdoc, Medical, Biomedical and Health Sciences

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BACKGROUND

Salmonellosis is a significant public health burden worldwide and being one of the most common bacterial diarrheal illnesses among infants and young children (Wang et al 2017). Qatar reports multiple incidences of salmonellosis outbreaks among the pediatric population every year, coupled with a significant increase of multidrug-resistant (MDR) among gram-negative bacteria including Salmonella in the last few years, resulting in a serious public health hazard. Salmonella ranks among the four commonly isolated Enterobacteriaceae from clinical samples at Hamad Medical Corporation (HMC). The identification of Salmonella isolates into specific serovars is essential for epidemiologic studies, and tracing the source of outbreaks (Hong et al., 2003) but it is laborious and time-consuming. Many genotyping techniques, including pulsed-field gel electrophoresis (PFGE), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), multiplex PCR and whole-genome sequencing have been applied as alternative methods for Salmonella subtyping. Data relevant to these typing methods are scarce in Qatar. The recurrent Salmonella outbreaks in Qatar and the increasing number of salmonellosis cases (MOPH, Salmonella Work Shop, 2017, Qatar) mandates more attention and efforts to hinder the spread of these bacteria. This partially depends on the understanding of the bacterial phenotypic and genotypic characteristics that influence its pathogenicity and transmission with the potential to cause an outbreak.

OBJECTIVES

This study aims to

- **Characterize the phenotypic resistance profile of *Salmonella* to relevant antibiotics among pediatrics population.**
- **Elucidate the molecular mechanisms underlying resistance to ceftriaxone, cefepime, amoxicillin- clavulanate, tetracycline, trimethoprim-sulfamethoxazole, chloramphenicol, colistin and azithromycin in *Salmonella* isolates identified from pediatric among 2 – 16 years old in Qatar.**
- **Characterize the 16S rRNA gene region by restriction fragment length polymorphism (RFLP) to investigate if this region constitutes an appropriate 'coincidental' marker to distinguish important pathogenic *Salmonella* species.**
- **Determine the lineages of *Salmonella* species and evolutionary relationships among bacteria classified within the same genus.**

Methods

Phenotypic profile of antibiotic

- **246 Salmonella isolates were collected from children under 16 years old during Jan. 2018 - Dec 2019, presented with gastroenteritis at Hamad Medical Corporation.**
- **Isolates were tested for antibiotic susceptibility against nineteen relevant antibiotic using E-test.**

Genotypic of AMR

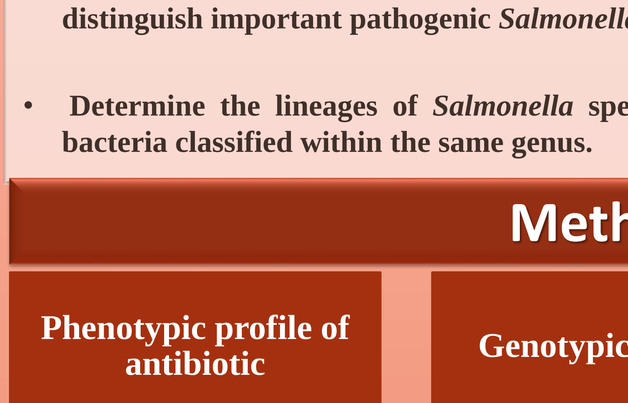
- **Isolates that harbor antibiotic resistance were confirmed using PCR specific primers for 38 genes.**
- **In addition, the variable region of class 1 and 2 integrons were studied by PCR among amoxicillin-clavulanate (AMC) resistance samples.**

Restriction fragment length (RFLP)

- **16S rRNA PCR amplicons were enzymatically digested with 7 restriction enzymes including *Alu*I, *Bgl*I, *Bgl*II, *Eco*RI, *Sma*I, *Hinf*I & *Hae*III according to instructions of the manufacturer.**

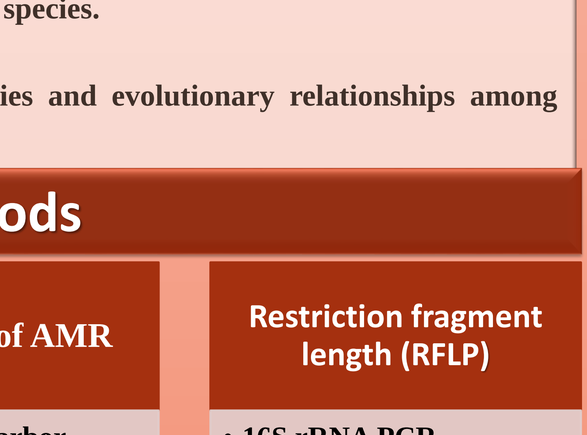
Results

Fig. 1 Relationship between Age of Children and no. of Isolated *Salmonella*.



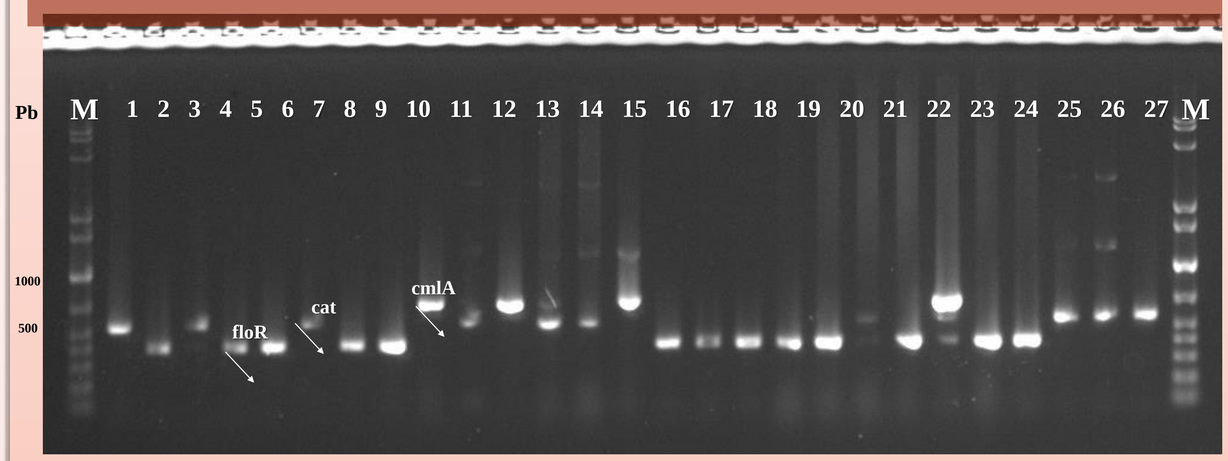
In total 246 *Salmonella* were obtained, the highest number was isolated from 2 years old (34.2%) followed by (37%) from 3 & 4 years while the lowest number of isolates (1.6%) from 15 & 16 years old

Fig. 2 Phenotypic Resistance Profile of *Salmonella* (246) isolated from Children suffering from Gastroenteritis



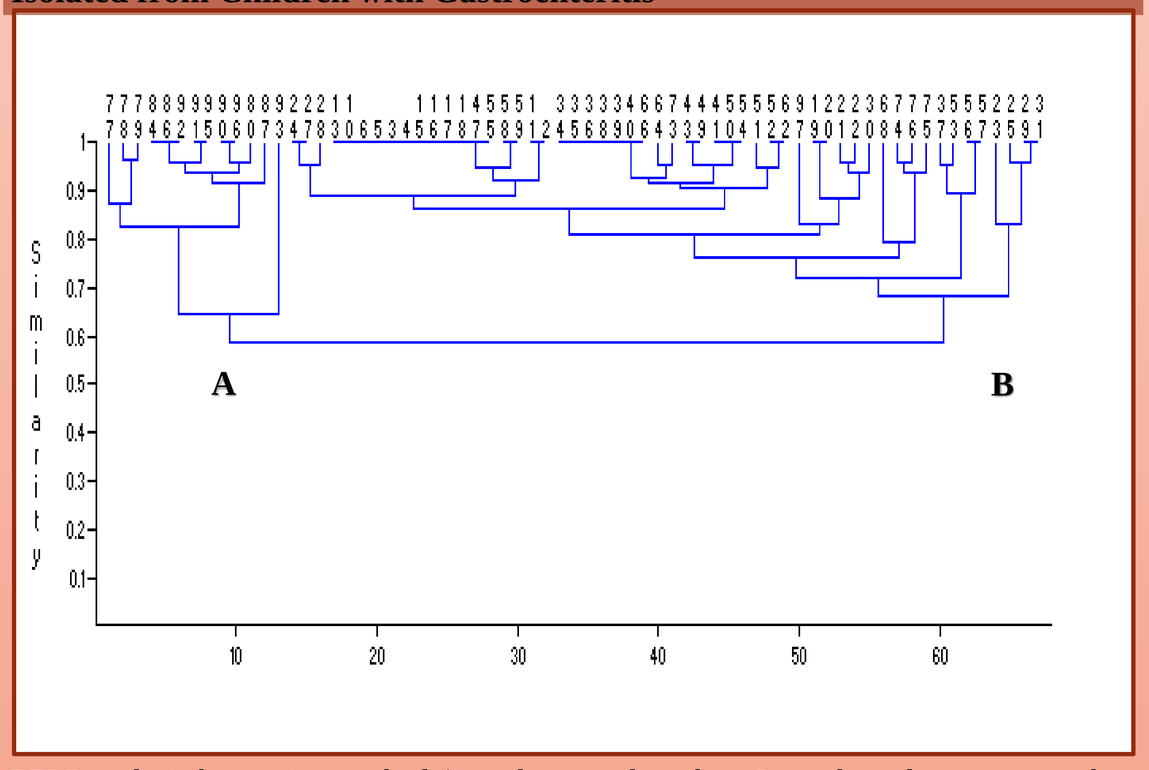
Resistance was detected against 15 antibiotics. 38.2% of isolates were resistant to at least one antibiotic. Overall, high resistance was reported to tetracycline (23.9%), ampicillin (21.1%), AMC (18.7%) and sulfamethoxazole-trimethoprim (13%). Further, 22.4% of the isolates were multidrug-resistant (MDR), with 4.1% being ESBL producers.

Fig.2: Identification of Genes Encoding chloramphenicol Resistance (*cat, cmlA* & *floR*) Isolated from Children Suffering from Gastroenteritis



Lane M; 1 kb ladder marker, lane1: (*cat*), lane 2: (*flo* R), lane 3: (*cat*), lane 4: sample 75 (*flo* R), lane 5: (*flo* R), lane 6: (*cat*) , lane 7: (*cat*, *flo*R), lane 8: (*flo*R) lane 9: (*cml* A), lane 10: (*cat*), lane 11: (*cml*A), lane 12: (*cat*), lane 13: (*cat*), lane 14: (*cmlA*), lane 15: (*floR*), lane 16: (*flor*), lane 17: (*floR*), lane 18: (*floR*), lane 19: (*floR*) , lane 20: (*cat*), lane 21: (*floR*), lane 22: (*cml*A, *flo*R), lane 23: (*flo*R), lane 24: (*flo*R) lane 25: (*cat*), lane 26: (*cat*), lane 27: sample 426 (*cat*)

Fig.3: Dendrogram for RFLP-(16s rRNA region) Pattern among *Salmonella* Isolated from Children with Gastroenteritis



RFLP analysis by scoring method (paired group algorithm- Jaccard similarity measure by using Past software)
This dendrogram divided *Salmonella* isolates to 2 main group. The similarity between these groups was 55%.

Conclusions

- **Our results indicate a high antimicrobial resistance pattern of Salmonella, which necessities the development of regulatory programs to combats antimicrobial resistance.**
- **In particular, our results showed high resistance to Class (1) AMC cassette that involves the transmission and expression of the resistance. This might lead to a concern of increased multidrug resistance in the future.**
- **This study provides evidence guidance to activate and implement the pillars of an antimicrobial stewardship program in animal and human health to reduce MDR salmonellosis**

References

- **Hong, Y., Liu, T., Hofacre, C., Maier,M., White, D.G., Ayers, S., et al.(2003). A restriction fragment length polymorphism-based polymerase chain reaction as an alternative to serotyping for identifying *Salmonella* serotypes. *Avian Dis.* 47, 387–395.doi:10.1637/0005-2086(2003)047[0387:ARFLPP]2.0.CO;2**
- **Wong, M. H., Yan, M., Chan, E. W., Biao, K., and Chen, S. (2014). Emergence of clinical *Salmonella enterica* serovar *Typhimurium* isolates with concurrent resistance to ciprofloxacin, ceftriaxone, and azithromycin. *Antimicrob. Agents Chemother.* 58, 3752–3756. doi: 10.1128/AAC.02770-13**

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