OXFORD UNIVERSITY PRESS | Journal of Antimicrobial Chemotherapy

Multiple antibiotic resistance index (MARI) of human-isolated Salmonella species: A practical bacterial antibiotic surveillance tool

Journal:	*Journal of Antimicrobial Chemotherapy*
Manuscript ID	JAC-2022-1254.R1
Manuscript Type:	Original research
Date Submitted by the Author:	13-Mar-2023
Complete List of Authors:	Woh, Pei Yee; The Hong Kong Polytechnic University Yeung, May Pui Shan; The Chinese University of Hong Kong, Jockey Club School of Public Health and Primary Care Goggins, William ; The Chinese University of Hong Kong, Jockey Club School of Public Health and Primary Care
Keywords:	Antibiotic resistance, Antimicrobial susceptibility, Multidrug resistance, Non-typhoidal Salmonella

Multiple antibiotic resistance index (MARI) of human-isolated *Salmonella* species: A practical bacterial antibiotic surveillance tool

Pei Yee WOH[1*], May Pui Shan YEUNG[2], William Bernard III GOGGINS[2†]

[1] Department of Food Science and Nutrition, Faculty of Science, The Hong Kong Polytechnic University, Hong Kong Special Administrative Region, China.

[2] Jockey Club School of Public Health and Primary Care, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong Special Administrative Region, China.

† Deceased.

*Corresponding authors:

Email: peggy.woh@polyu.edu.hk (PYW), phone: +852 3400 8880

Running title: Multiple antibiotic resistance of *Salmonella*

Abstract

Objective: Drug-resistant *Salmonella* plays a significant role in disease morbidity and mortality worldwide. The present study aimed to determine the multiple resistance index (MARI) of *Salmonella* isolated from children hospitalized for gastroenteritis in Hong Kong.

Methods: *Salmonella* isolates from stool samples of children aged from 30 days to below 5 years were confirmed by using MALDI-TOF MS and subjected to serotyping methods according to the White-Kauffmann-Le Minor scheme. Antimicrobial susceptibility was determined by agar disc diffusion.

Results: A total of 101 *Salmonella* isolates were serogrouped into Group B (n=46, 45.5%), Group C (n=9, 9.0%), Group D (n=46, 45.5%), and successfully classified into *S.* Enteritidis (n=15) and *S.* Typhimurium (n=7). Overall *Salmonella* susceptibilities demonstrated the highest level of resistance to ampicillin (76.2%), ciprofloxacin (54.0%), and tetracycline (61.2%) while MDR strains had high resistance toward ampicillin (100%), tetracycline (100%), cotrimoxazole (84.6%), chloramphenicol (83.3%), and ciprofloxacin (83.3%). MARI revealed that 80.2% of *Salmonella* including all MDR strains (n=13) had indexes greater than 0.2.

Conclusion: The MARI captures a snapshot of a high rate of antibiotic use and resistance in the isolated *Salmonella*, indicating the urgent need for continuous antimicrobial susceptibility surveillance and control of antibiotic prescription in selecting effective treatments for human diseases.

Introduction

Rising antibiotic resistance poses a significant threat to global health, and has been driven by unnecessary antibiotic use, which has grown by 66% since 2000 worldwide.[1] Drug-resistance infections cause nearly 750,000 mortalities worldwide each year, and this number could reach 10 million by 2050.[2]

Salmonella is one of the 12 drug-resistant superbugs that urgently require new treatments.[3] Nontyphoidal *Salmonella* (NTS) in particular is responsible for an annual estimation of 93.8 million gastroenteritis cases and 155,000 deaths globally.[4] In the United States, the yearly statistic for nontyphoidal salmonellosis reported 1.2 million illnesses, 23,000 hospitalizations, and 450 deaths[5]; while in China, NTS annually caused 9.87 million cases of gastroenteritis and 792 deaths from 200 to 2009.[6] Although NTS gastroenteritis is mostly self-limiting, children aged younger than 5 years are most vulnerable to this infection.[7-9]

The antibiotic resistance of *Salmonella* varies between serotypes. A study in Guangzhou China depicted that annual resistance rates of NTS to ceftazidime in 2015 (31.43%), had doubled compared to the previous year (16%), and the rates of antibiotic resistance to ampicillin between isolates *S.* Typhimurium and *S.* Enteritidis were significantly higher than other serotypes.[10] Another study in Hong Kong has reported a high prevalence of ESBL-producing Enterobacteriaceae of 52.8% and of carbapenem resistance from gut carriage among healthy subjects.[11] However, ESBL and carbapenem phenotypic susceptibilities are not routinely tested for extraintestinal NTS species in microbiology diagnostic laboratories, especially if first-line antimicrobial agents (ampicillin, fluoroquinolone, and trimethoprim-sulfamethoxazole) are susceptible. Furthermore, a report by Klein *et al* (2018) on antibiotic consumption from 2000 to 2015 in 76 countries, reported that the increase was associated with the gross domestic product per capita growth, and the antibiotic consumption rates in low- and middle-income countries (LMICs) have been rapidly converging towards rates similar to high-income

countries.[1] However, many LMICs continue to be burdened with a high rate of infectious disease, consequently inequities in drug access and the supply of newer diagnostic applications.[12] Thus, the need to track the trend of antibiotic therapy in a simple and effective manner is crucial.

Multiple antibiotic resistance indexes (MARI) is a practical tool employing a combination of measurements of antibiotic consumption and resistance of the pathogen to create a single metric that represents the level of drug resistance, thereby formulating the ratio of the number of resistant antibiotics to the total number of antibiotics being exposed to the microorganism.[13] High-risk sources of contamination can be identified through the MAR indexing values of environmental bacteria, for example, *Escherichia coli*,[13, 14] *Klebsiella* spp.[15] and *Pseudomonas* spp.[15, 16] Here we applied this approach to generate MARI for clinical *Salmonella* isolates from gastroenteritis patients.

Material and Methods

Collection of *Salmonella* isolates

Between April and November 2019, the *Salmonella* isolates that were isolated from faecal samples of hospitalized children aged from 30 days to below 5 years in three Hong Kong public hospitals, was confirmed by MALDI-TOF MS, and subjected to serotyping methods according to the White-Kauffmann-Le Minor scheme.[17]

Antimicrobial susceptibility test (AST)

The AST was performed by means of the Mueller-Hilton agar disc diffusion method. The susceptibilities to nine antimicrobials (BD BBL): ampicillin (10 µg), cefotaxime (30 µg), ceftriaxone (30 µg), meropenem (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), trimethoprim-sulfamethoxazole (23.75µg/1.25µg), ciprofloxacin (5 µg), and azithromycin (15 µg), were assessed according to CLSI 2022 resistance breakpoint. Zones of diameter were

measured and interpreted as percent resistant-susceptible. MDR strains were referred to as those with phenotype resistance to >3 antimicrobials tested.[18]

Determination of MARI

Multiple antibiotic resistance index for each isolate was calculated by the method described by Krumperman 1983 using the following equation $MAR^{index} = a/b$, where 'a' represents the number of antibiotics to which the isolate was resistant, and 'b' represents the total number of antibiotics to which isolate was tested. MARI >0.2 indicates the existence of isolate from high-risk contaminated sources with frequent use of antibiotics while values ≤0.2 shows that bacteria from sources have been exposed to less antibiotic usage.[15]

Ethics Statement

Ethics approvals were granted by the Institutional Review Boards of the Joint Chinese University of Hong Kong—New Territories East Cluster Clinical Research Ethics Committee (Ref: CRE-2018.416) and Kowloon Central/Kowloon East Research Ethics Committee (Ref: KC/KE-19-0116/ER-2). Written consents were informed and obtained from parents or guardians.

Results

Among 101 *Salmonella* isolates, 46 (45.5%) of them were serogroup B, while 9 (9.0%) were serogroup C and 46 (45.5%) were serogroup D (Table 1). The laboratory serology further classified 15 isolates and 7 isolates into *S*. Enteritidis and *S*. Typhimurium, respectively.

The overall antimicrobial susceptibilities revealed that ceftriaxone (100%), meropenem (100%), azithromycin (98.1%), cefotaxime (92.9%), cotrimoxazole (76.2%), and chloramphenicol (73.5%) were efficacious against *Salmonella* infection while high resistance was observed for other antimicrobial tested. Among the MDR isolates (n=13), the majority had high resistance toward ampicillin (100%), tetracycline (100%), cotrimoxazole (84.6%),

chloramphenicol (83.3%), and ciprofloxacin (83.3%). The most promising drug against MDR *Salmonella* was cefotaxime (n= 10/13, susceptibility 76.9%).

MARI revealed 80.2% of isolates with MARI greater than 0.2 and 19.8% of isolates with MARI less than 0.2 (Table 2). Thirteen *Salmonella* isolates were susceptible to all antimicrobials tested, of which 7 were Group B while 4 were Group C/other and 4 were Group D. All the MDR isolates were Group B *Salmonella* and one of them had shown a MARI of 1.0 (i.e., resistant to all the antimicrobials tested). The resistance phenotypes of *Salmonella* species varied among different serogroups. For Group B *Salmonella*, the predominant phenotypes were AM-SXT (n=6), AM-T (n=8), and AM-CHL-MCIP-SXT-T (n=7); while for Group D *Salmonella*, resistance to AM (n=27) and AM-MCIP (n=10) was observed. No obvious patterns were observed in Group C *Salmonella*. For MDR isolates, the most frequently observed pattern was AM-CHL-MCIP-SXT-T (n=7) with a MAR index of 0.83.

Discussion

Antibiotic resistance in *Salmonella* over the past decades has significantly reduced the efficacy of first-generation antimicrobial agents.[5] Rapid urbanization and increased antibiotic use can have serious consequences for the effectiveness of local antibiotic resistance control programs in human and veterinary medicine. Extensive research to overcome the rising rates of bacterial resistance has recommended fluoroquinolones and third-generation cephalosporins for invasive *Salmonella* infections. However, the emergence of fluoroquinolone-resistance salmonellae highlights the need for strategic intervention to control resistance dissemination.[19] In our analyses, we found a large spread in the MARI with observed differences in resistance rates between *Salmonella* serotypes. The study revealed that the increase in antibiotic consumption was due to increased use of cephalosporins to replace penicillin and quinolones for infection management, which was likely due to changing prescribing practices for respiratory tract infections, skin and soft tissue infections, gonococcal infections, and enteric

fever.[20] Increasing occurrence of decreased susceptibilities to third-generation cephalosporins in human non-typhoidal *Salmonella* strains of *S.* Enteritidis and *S.* Typhimurium were observed in Hong Kong.[10, 11] This compromises the clinical efficacy of a very important class of antibiotic, consequently, ceftriaxone is now the drugs of choice for infection caused by *Salmonella* strains with resistance to ciprofloxacin.

MARI is a quick and easy-to-use tool without the need for training professional staff nor the complexity of setting up a new laboratory as its effectiveness is comparable across time and space. MARI can be helpful in determining the effectiveness of antibiotic therapy and communicating the issue of resistance to lay audiences as an early warning of future issues. The combination of antibiotic use and resistance into a MARI single measure informs clinicians whether to remain with current drug prescription practices or move to newer, more expensive antibiotics that reflect the affordability constraints of a region. Incorporating MARI into surveillance programs would improve patient therapy and allow for more detailed surveillance as a result of broader communication of antimicrobial resistance and consumption. Most importantly, long-term government funding for ongoing surveillance and commitments, as well as the involvement of clinicians to provide sample isolation from patient care are necessary to sustain this capacity.

The main limitation of our study was the non-uniform choice of antimicrobial agents for AST across the three hospitals. Although we have no way of assessing data differences, we did confirm the antimicrobial resistance profiles of the isolates were determined following the zone of the inhibition diameter breakpoints and interpretation for Enterobacteriaceae as recommended by CLSI. We were unable to add the MIC values which might improve the MAR index results. Without data on consistent antibiotic use-consumption of isolates from the three hospitals, the MARI presented here may not be fully representative of the effectiveness of

antibiotic therapy; however, the MARI can be calculated in different ways, because when additional data becomes available new indexes can be calculated.

All these findings contribute to a better understanding of the problems of resistance and provide insight into underlying trends in antibiotic use to aid the practical management of infections, continuous surveillance of antimicrobial susceptibility, and effective infection controls. Future research could include a detailed study of the factors driving MARI differences to provide insight into potential solutions to the resistance problem at hospital-level comparisons, as well as at regional and national levels, that could be more readily utilized as an indicator of hospital quality.

Acknowledgment

We are grateful to medical doctors, nurses and administrative staff at the Prince of Wales Hospital, the Alice Ho Miu Ling Nethersole Hospital, and the United Christian Hospital for subject recruitment and logistic arrangement. We also thank laboratory technicians from the Departments of Microbiology of these study hospitals for stool sample processing.

Funding

This work was supported by Chung Chi College Student Helper Award Scheme 2019 (project no. AF00008) and part of unrestricted grants from Division of Biostatistics (project no. 7103510 and 7051838), The Chinese University of Hong Kong.

Transparency declarations

We declare no conflicts of interest. Author William Bernard III GOGGINS was unable to confirm their authorship contributions. On their behalf, the corresponding author has reported their contributions to the best of their knowledge.

References

181 1. Klein EY, Boeckel TPV, Martinez EM *et al*. Global increase and geographic
182 convergence in antibiotic consumption between 2000 and 2015. *Proceedings of the National*
183 *Academy of Sciences* 2018; **115**: E3463-E70.
184 2. World Health Organization. *New report calls for urgent action to avert antimicrobial*
185 *resistance crisis*. https://www.who.int/news/item/29-04-2019-new-report-calls-for-urgent-
186 action-to-avert-antimicrobial-resistance-crisis
187 3. World Health Organization. *WHO publishes list of bacteria for which new antibiotics*
188 *are urgently needed*. https://www.who.int/news/item/27-02-2017-who-publishes-list-of-
189 bacteria-for-which-new-antibiotics-are-urgently-needed
190 4. Majowicz SE, Musto J, Scallan E *et al*. The global burden of nontyphoidal *Salmonella*
191 gastroenteritis. *Clin Infect Dis* 2010; **50**: 882-9.
192 5. Medalla F, Gu W, Mahon BE *et al*. Estimated incidence of antimicrobial drug-resistant
193 nontyphoidal *Salmonella* infections, United States, 2004-2012. *Emerg Infect Dis* 2016; **23**: 29-
194 37.
195 6. Mao X, Hu J, Liu X. Estimation on disease burden of foodborne non-typhoid
196 salmonellosis in China using literature review method. *Chin J Dis Control Prev* 2011; **15**: 622-
197 5.
198 7. Wang P, Goggins WB, Chan EYY. Associations of *Salmonella* hospitalizations with
199 ambient temperature, humidity and rainfall in Hong Kong. *Environ Int* 2018; **120**: 223-30.
200 8. Katiyo S, Muller-Pebody B, Minaji M *et al*. Epidemiology and outcomes of
201 nontyphoidal *Salmonella* bacteremias from England, 2004 to 2015. *J Clin Microbiol* 2019;
202 **57**:e01189-18.
203 9. Woh PY, Yeung MPS, Nelson EAS *et al*. Risk factors of non-typhoidal *Salmonella*
204 gastroenteritis in hospitalised young children: a case-control study. *BMJ Paediatr Open* 2021;
205 **5**: e000898.

206 10. Liang B, Xie Y, He S *et al*. Prevalence, serotypes, and drug resistance of nontyphoidal
207 *Salmonella* among paediatric patients in a tertiary hospital in Guangzhou, China, 2014-2016. *J*
208 *Infect Public Health* 2019; **12**: 252-7.
209 11. Kwok K-O, Chan E, Chung P-H *et al*. Prevalence and associated factors for carriage of
210 Enterobacteriaceae producing ESBLs or carbapenemase and methicillin-resistant
211 *Staphylococcus aureus* in Hong Kong community. *Journal of Infection* 2020; **81**: 242-7.
212 12. Okeke IN, Laxminarayan R, Bhutta ZA *et al*. Antimicrobial resistance in developing
213 countries. Part I: recent trends and current status. *Lancet Infect Dis* 2005; **5**: 481-93.
214 13. Krumperman PH. Multiple antibiotic resistance indexing of *Escherichia coli* to identify
215 high-risk sources of fecal contamination of foods. *Appl Environ Microbiol* 1983; **46**: 165-70.
216 14. Parveen S, Murphree RL, Edmiston L *et al*. Association of multiple-antibiotic-
217 resistance profiles with point and nonpoint sources of *Escherichia coli* in Apalachicola Bay.
218 *Appl Environ Microbiol* 1997; **63**: 2607-12.
219 15. Osundiya OO, Oladele RO, Oduyebo OO. Multiple antibiotic resistance (MAR) indices
220 of *Pseudomonas* and *Klebsiella* species isolates in Lagos University Teaching Hospital.
221 *African Journal of Clinical and Experimental Microbiology* 2013; **14**: 164-8.
222 16. Paul S, Bezbaruah RL, Roy MK *et al*. Multiple antibiotic resistance (MAR) index and
223 its reversion in *Pseudomonas aeruginosa*. *Lett Appl Microbiol* 1997; **24**: 169-71.
224 17. Guibourdenche M, Roggentin P, Mikoleit M *et al*. Supplement 2003–2007 (No. 47) to
225 the White-Kauffmann-Le Minor scheme. *Research in Microbiology* 2010; **161**: 26-9.
226 18. Magiorakos AP, Srinivasan A, Carey RB *et al*. Multidrug-resistant, extensively drug-
227 resistant and pandrug-resistant bacteria: an international expert proposal for interim standard
228 definitions for acquired resistance. *Clinical Microbiology and Infection* 2012; **18**: 268-81.

19. Ling JM, Chan EW, Lam AW *et al*. Mutations in topoisomerase genes of fluoroquinolone-resistant salmonellae in Hong Kong. *Antimicrob Agents Chemother* 2003; **47**: 3567-73.

20. Kotwani A, Holloway K. Antibiotic prescribing practice for acute, uncomplicated respiratory tract infections in primary care settings in New Delhi, India. *Trop Med Int Health* 2014; **19**: 761-8.

235 Table 1 Antibiotic resistance of *Salmonella* isolates from children with gastroenteritis.

Antibiotics	***Salmonella* species, n (%)**									
	Total (n=101)		**Group B/ *S.* Typhimurium (n=46)**		**Group D/ *S.* Enteritidis (n=46)**		**Group C/others (n=9)**		**MDR isolates (n=13)**	
	R	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**
Ampicillin, AM (n=101)	77 (76.2)	24 (23.8)	33 (71.7)	13 (28.3)	40 (87.0)	6 (13.0)	4 (44.4)	5 (55.6)	13 (100)	0 (0)
Cefotaxime, CTX (n=99)	7 (7.1)	92 (92.9)	4 (8.9)	41 (91.1)	2 (4.4)	43 (95.6)	1 (11.1)	8 (88.9)	3 (23.1)	10 (76.9)
Ceftriaxone, CRO (n=2)	-	2 (100)	-	1 (100)	-	1 (100)	-	-	-	-
Meropenem, MEM (n=4)	-	4 (100)	-	4 (100)	-	-	-	-	0 (0)	1 (100)
Cotrimoxazole, SXT (n=101)	24 (23.8)	77 (76.2)	20 (43.5)	26 (56.5)	1 (2.2)	45 (97.8)	3 (33.3)	6 (66.7)	11 (84.6)	2 (15.4)
Azithromycin, AZM (n=52)	1 (1.9)	51 (98.1)	1 (6.7)	14 (93.3)	-	32 (100)	-	5 (100)	1 (100)	0 (0)
Chloramphenicol, CHL (n=49)	13 (26.5)	36 (73.5)	13 (41.9)	18 (58.1)	-	14 (100)	-	4 (100)	10 (83.3)	2 (16.7)
Ciprofloxacin, MCIP (n=50)	27 (54.0)	23 (46.0)	13 (40.6)	19 (59.4)	14 (100)	-	-	4 (100)	10 (83.3)	2 (16.7)
Tetracycline, T (n=49)	30 (61.2)	19 (38.8)	28 (90.3)	3 (9.7)	-	14 (100)	2 (50.0)	2 (50.0)	12 (100)	0 (0)

236 R, resistant; S, susceptible; MDR, multidrug-resistant; -, not tested.

237 Table 2 Antibiotic resistance patterns and MARI of *Salmonella* species.

***Salmonella* species**	**No. antibiotic tested**	**No. antibiotic resistance**	**MAR index**	**Resistance phenotypes**	**No of resistant species n (%)**	**Total n (%)**
Group B/ *S.* Typhimurium (n=46)	4	0	-	-	4 (8.7)	15 (32.6)
		1	0.25	AM	2 (4.3)	
		1	0.25	SXT	1 (2.2)	
		2	0.50	AM, SXT	6 (13.0)	
		3	0.75	AM, CTX, SXT	1 (2.2)	
		4	1.00	AM, AZM, CTX, SXT	1 (2.2)	
	6	0	-	-	2 (4.3)	27 (58.7)
				T	1 (2.2)	
		2	0.33	AM, T	8 (17.4)	
				CHL, T	1 (2.2)	
		3	0.50	AM, MCIP, T	2 (4.3)	
				CHL, MCIP, T	1 (2.2)	
				CHL, SXT, T	1 (2.2)	
		4	0.67	AM, CHL, SXT, T	2 (4.3)	
				AM, CHL, MCIP, T	1 (2.2)	
				AM, CTX, MCIP, T	1 (2.2)	
		5	0.83	AM, CHL, MCIP, SXT, T	7 (15.2)	
	7	0	-	-	1 (2.6)	4 (8.7)
		1	0.14	T	1 (2.6)	
		2	0.29	AM, T	1 (2.6)	
		5	0.71	AM, CTX, MCIP, SXT, T	1 (2.6)	
Group D/ *S.* Enteritidis (n=46)	4	0	-	-	4 (8.7)	32 (69.6)
		1	0.25	AM	27 (58.7)	
		2	0.50	AM, CTX	1 (2.2)	

	6	1	0.16	MCIP	2 (4.3)	
		2	0.33	AM, MCIP	10 (21.7)	14 (30.4)
		3	0.50	AM, CTX, MCIP	1 (2.2)	
				AM, MCIP, SXT	1 (2.2)	
Group C/others (n=9)	4	0	-	-	2 (22.2)	
		2	0.50	AM, CTX	1 (11.1)	5 (55.6)
				AM, SXT	2 (22.2)	
	6	0	-	-	2 (22.2)	
		1	0.17	T	1 (11.1)	4 (44.4)
		3	0.50	AM, SXT, T	1 (11.1)	
MDR isolates (n=13)	4	4	1.00	AM AZM CTX SXT	1 (7.7)	1 (7.7)
	6	4	0.67	AM CHL SXT T	2 (15.4)	4 (30.8)
				AM CTX MCIP T	1 (7.7)	
				AM CHL MCIP T	1 (7.7)	
		5	0.83	AM CHL MCIP SXT T	7 (53.8)	7 (53.8)
	7	5	0.83	AM CTX MCIP SXT T	1 (7.7)	1 (7.7)

238 AM, ampicillin; AZM, azithromycin; CHL, chloramphenicol; CIP, ciprofloxacin; CTX, cefotaxime; CRO-ceftriaxone; MEM, meropenem; SXT,
239 trimethoprim-sulfamethoxazole; T, tetracycline.