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ORIGINAL ARTICLE

The Correlation between Ciprofloxacin Resistant *Salmonella* Strains and Its Ability to Biofilm Formation

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ABSTRACT

Background: *Salmonellosis is considered one of the most important infectious diseases in the developing countries where there is a significant lack of clean water supplies and poor sanitation in different areas helping the spread of the bacteria. It has a mortality rate of about 2–3% so, is considered a global health burden all over the world. Multidrug-resistant (MDR) strains of* salmonella *have been emerged making the inexpensive and readily available antibiotics including ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole and streptomycin are frequently ineffective. Although resistance to ciprofloxacin, a second-generation fluoroquinolone, is increasing, it is still recommended as a first line therapy for almost all age groups.* ***Objectives:*** *Our study determines ciprofloxacin resistant salmonella strains in patients attending to Beni-Suef University Hospital and Beni-Suef Fever Hospital at Beni-Suef City. Also, this study assesses the capability of these strains to synthesize biofilm.* ***Methodology:*** *A total of 100 patients attended to Beni-Suef University Hospital and Beni-Suef Fever Hospital from December 2022 to March 2023 suspected clinically with salmonellosis were included. Stool samples were taken in suitable transport containers to Medical Microbiology and Immunology Laboratory Faculty of Medicine Beni- Suef University for further processing and identification. Cultures were done on MacConkey's media to detect lactose non fermenting strains and then on salmonella shigella SS agar to identify salmonella strains. Salmonella isolates are further identified by different biochemical tests. Minimum inhibitory concentration (MIC) for ciprofloxacin to the isolated salmonella strains using broth dilution method was determined. Ciprofloxacin resistant salmonella isolates were tested for the presence of biofilm by quantitative microtiter plates.* ***Results:*** *Over a period of five months, about 100 patients attended to Beni-Suef University Hospital and Beni-Suef Fever Hospital with a history and clinical symptoms of salmonella infection were involved in the study. The study included (36 males, 46 females) (54 child & 45 adults) (70 from rural while 30 from urban areas). Stool culture on MacConkey's and SS agar and typing of salmonella strains using API were done. MIC was determined for ciprofloxacin by broth dilution method. Biofilm evaluation of ciprofloxacin resistant salmonella isolates was determined using quantitative microtiter plates.* ***Conclusion:*** *We detected a high prevalence of salmonellosis, so continuous good preventive hygienic measures are needed. Biofilm production by salmonella strains made it more resistant to ciprofloxacin. These bacteria can form biofilm that aggravate the therapeutic problems in human and veterinary medicine, so continuous detection and evaluation is essential.*

INTRODUCTION

Salmonella can be transmitted from animals and food of animal origin to humans[1]. There is a strong correlation between increased antimicrobial resistance and biofilm existence within the bacteria[2].

Salmonellosis especially in patients with immunosuppression is a sever disease. The European Food Safety Authority (EFSA) reports that *Salmonella* continue to be the second most common cause of human infections and food poisoning associated with contaminated food[3].

The aetiological factor of salmonellosis is most often caused by serovars: *Salmonella enterica* ser. Enteritidis (*S. enterica* ser. Enteritidis) and *Salmonella enterica* ser. Typhimurium (*S. enterica* ser. Typhimurium). Host–host transmission usually occurs via the fecal–oral route. Animal products, especially those related to poultry, including meat and eggs are the most common source of infection with *Salmonella*[4].

Salmonellosis infected individuals can be converted to gall bladder or urinary bladder carriers to the organism so, excrete *it* in their faeces and are functioning as a reservoir for this pathogen[5].

METHODOLOGY

This study was conducted over a period of four months on 100 patients who came to to Beni-Suef University Hospital and Beni-Suef Fever Hospital (paediatric and adult) during the period from December 2022 to March 2023.

Stool samples were taken and transported immediately to the Microbiology Laboratory, Faculty of Medicine-Beni Suef University for processing and identification.

Samples were cultured on MacConkey's and SS agar medium (Oxoid, UK) for isolation of *salmonella*. The plates were incubated at 37^{0} C aerobically for 24-48 hours and were examined for growth. Typing of the isolates was done by the API 20E test kit (bioMérieux, Inc., France). (Fig 1).

Fig. 1. *Salmonella typhi* reaction of API 20E test kit

Fig. 2. *Salmonella typhimurium* reaction of API 20E test kit

MIC test of ciprofloxacin was done according to interpretative criteria recommended by Clinical and Laboratory Standards **(**CLSI **2020)**[6] (**Table 1).** The ciprofloxacin MICs ranged from <0.015 to 64 **µg/mL**.

Table 1: Interpretive Criteria for Ciprofloxacin (CIP) MIC for *Salmonella* isolates

Interpretation	CIP MIC (µg/mL) Interpretive Criteria *Salmonella*		
	Susceptible	Intermediate	Resistant
CLSI (M100 S21; all *Salmonella*)	≤1.0	2.0	≥4.0

The identified *salmonella* isolates, which were ciprofloxacin resistant, were further processed to evaluate the biofilm existence[7].

Biofilm evaluation:

Commercially available presterilized, polystyrene, flat-bottomed, 96-well microtiter plates (Nunclon; Nalge Nunc International, Roskilde, Denmark) will be used for biofilm formation[8].

The strain optical density (OD) was obtained by the arithmetic mean of the absorbance of three wells and compared with the mean absorbance of negative controls (ODnc). The following classification for the determination of biofilm existence: no biofilm production (ODs = ODnc), weak biofilm production (ODnc < ODs> 2ODnc), moderate biofilm production (2ODnc < ODs> 4ODnc) and strong biofilm production (4ODnc < ODs) Where: OD_C = OD of negative control, OD_S = OD of sample[9].

RESULTS

The 100 patients attended to Beni-Suef University and Beni-Suef Fever Hospital from December 2022 to March 2023 (36 male, 46 female) (54 child & 45 adults) (70 from rural areas while only 30 from urban areas).

Table (2): Isolated organisms causing gastroenteritis during period of the study (*salmonella* strains typed using API):

Isolated strains	Number Total No (100)	%
S. paratyphi A	36	*36%*
S.typhi	*21*	*21%*
S. typhimurium	*16*	*16%*
S. enteritidis	*12*	*12%*
S. paratyphi B	*10*	*10%*
Non salmonella species	*5*	*5%*
TOTAL	100	100%

Table 2 illastrates that the identified *salmonella* strains typed using API as follow: *S. paratyphi A 36%, S. typhi 21%, S. typhimurium 16% S. enteritidis 12%, S. paratyphi B 10% while non salmonella species was 5%.*

Table (3): MIC of ciprofloxacin by dilution method for *Salmonella* isolates:

Isolated *salmonella* strains	Total No		Susceptible to ciprofloxacin		Resistance to ciprofloxacin	
	(No)	%	(No)	%	(No)	%
S. paratyphi A	36	37	*14*	38	*22*	*62*
S. typhi	*21*	*22*	*15*	71	*6*	*29*
S. typhimurium	*16*	*17*	*4*	25	*12*	*75*
S. enteritidis	*12*	*13*	*4*	33	*8*	*67*
S. paratyphi B	*10*	*11*	*6*	60	*4*	*40*
TOTAL	95	100	43	45	52	55

Table 3 shows the resistance of ciprofloxacin was 62 % (22 out of 36) *S. paratyphi A*, 29 % (6 out of 21) *S. typhi*, 75% (12 out of 16) *S. typhimurium*, 67% (8 out of 4) *S. enteritidis* and 40% (4 out of 10) *S. paratyphi B* by MIC values according to CLSI 2023.

Table (4): Biofilm production by *Salmonella* resistant strains:

Isolated *salmonella* strains	Resistance to ciprofloxacin	Biofilm formation among resistant strains		Degree of biofilms formed					
				Strong		Moderate		Weak	
		No	%	No	%	No	%	No	%
S. paratyphi A	*22*	*14*	*63*	*13*	*93*	*1*	*7*	-	*0*
S.typhi	*6*	*5*	*83*	*3*	*60*	*2*	*40*	-	*0*
S. typhimurium	*12*	*12*	*100*	*10*	*80*	*1*	*10*	*1*	*10*
S. enteritidis	*8*	*8*	*100*	*6*	*76*	*1*	*12*	*1*	*12*
S. paratyphi B	*4*	*0*	*0*	-	*0*	-	*0*	-	*0*

Table 4 illustrates that most of *Salmonella* isolates resistant to ciprofloxacin are strong biofilm producer.

DISCUSSION

Salmonella is enteric Gram-negative organisms that are widely dispersed in nature. It present as a common commensal in the gastrointestinal tracts of animals[10].

This organism can cause substantial economic loss resulting from its high mortality, morbidity and major complications of infected patients. Also, it's hazardous to cause gastroenteritis which represents a serious problem for the food processing and industry[11].

Approximately 600 million become infected with salmonellosis each year. *It* is characterized by an acute onset of fever, abdominal pain, and diarrhea. It is considered one of the most prevalent foodborne pathogens causing hospitalizations and deaths. Different strains of *Salmonellae* have been present in poultry meat and the environment[12].

In our study, 54 child (2-13 years) were the most vulnerable age group with males being more affected

than females. It was noticed by Davidson [13] found also that the majority of food poisoning patients were in the 2-10 years age group accounting for 79% of recorded cases but with no significant difference between males and females. This finding may be explained by the attitude of this age group to eat outdoor especially male gender. Moreover, the unclean hands of food workers are the main source of pathogens in the food, increasing the risk of finger contamination by *salmonella*[14]. *Salmonella* is currently a global problem. So, it is critical for further development of effective control measures[15, 28].

Ninty - five *Salmonella* isolates were isolated with the most common serovar was *S. paratyphi A (37%*) and *S. typhi* (22%). Admassu[4] who found that the overall prevalence of *Salmonella enterica* serovar *Typhi and Salmonella enterica serovar Paratyphi* was 11%. The prevalence of *Salmonella enterica serovar Typhi* (7%) was higher than *Salmonella enterica* serovar *Paratyphi* (4%). Another research by Roza [16] in Ethiopia reported about 1.6% only prevalence of *S.typhi* at Adare General Hospital.

Higher prevalence of *Para typhi A* in our study may be explained by that the most cases are due to food borne organisms while *Para typhi A* is mainly transmitted by water-contaminated supllies[17].

A fluoroquinolones (FQs) recommended by the World Health Organization for treatment of *salmonella*, whether caused by MDR or fully susceptible organisms. The FQs ciprofloxacin (CIP) and ofloxacin (OFO) are often better to use due to they are available in oral forms and are less expensive than ceftriaxone[18].

Ciprofloxacin for all *Enterobacteriaceae*, with a MIC >2 µg/mL means a poor response for systemic *Salmonella* infection treatment[24]. In Europe and Asian countries, a decrease in ciprofloxacin susceptibility was observed among *Salmonella enterica* strains[19].

We reported a higher resistance to ciprofloxacin in *Salmonella para typhi A* which is similar to that reported by another workers Majtán[20] who estimated that most *S. typhi* isolates were resistant to ciprofloxacin.

Also anoher study by Fernández[21] stated that there is elevated resistance of *Salmonella* to ciprofloxacin. Moreover, *Salmonella* is able to exhibit a reduced susceptibility to ciprofloxacin; physicians still prescribe ciprofloxacin as an early treatment *Salmonella* infections.

Biofilm production confers resistance to bacteria against antimicrobials; increased survival of pathogen within host and the environment; such are associated with the virulence properties[22]. *Salmonella enterica* are capable of adhering and forming biofilm on different kinds of materials during their life cycle. Biofilms survive *Salmonella* in unsuitable environmental conditions, such as the poultry farm environment[23].

There was a significant positive linear correlation between the strength of biofilm and the strength of resistance to ciprofloxacin. A similar finding done by Tabak [24] who stated that bacteria biofilms are able to tolerate harsh unsuitable conditions and resist antibiotics treatments as a result of a unique biofilm matrix components. Microbe may sense the environment and cause the cellular responses triggering biofilm synthesis. Biofilm matrices act as both physical and chemical barriers reducing concentration of antimicrobials at their targets in microbes increasing resistance. Besides this, the depletion of nutrient resources and development of biofilm resistant phenotypes in microorganism have been proved as mechanisms that aggravate the antibiotic resistance of pathogens.

Also Sharma [25] explained this strong correlation between biofilm synthesis and ciprofloxacin resistance due to the great chances of gene transfer with the help of virulence factors and antibiotic-resistant genes from resistant to susceptible Gram-negative bacteria, which leads to emergence of antibiotic resistance in the bacteria.

Strong biofilm formation in *Salmonella* was explained by the presence of higher amount of protease and lipolytic activity in the *salmonella* species and all factors needed for biofilm formation[26, 27].

CONCLUSIONS

The results from this study indicated that *Salmonella* can tolerate antimicrobials due to its ability to produce biofilms and the presence of resistance genes. Sanitation of food-contact surfaces must be efficient for complete removal of all foodborne pathogens. Multidrug resistance phenotypes were observed in some isolates in our study. *Salmonella* antimicrobial resistance lead to challenges in antibiotic treatment of salmonellosis.

N.B: On behalf of all authors, there is no conflict of interest in the article.

In addition, a written consent was obtained from the patients included in our study.

The above-mentioned manuscript has not been published, accepted for publication or under editorial review for publication elsewhere. All authors have seen and approved the content of the manuscript and have contributed significantly in the work.

The study was approved for ethical point of view by **Approval No: FMBSUREC/0612 2022/ Radi**

REFERENCES

1. Gerstel and Römling, U. Gerstel, U. Römling a control unit for biofilm formation in *Salmonella* Res. Microbiol., 154 (2003), pp. 659-667

2. Harichandran D, Dinesh KR Antimicrobial susceptibility profile, treatment outcome and serotype distribution of clinical isolates of *Salmonella enterica* subspecies *enterica*: a 2-year study from Kerala, South India. .Infect Drug Resist. 2017 Mar 14;10:97-101.
3. Maharjan A, Dhungel B, Bastola A, Thapa Shrestha U, Adhikari N, Banjara MR, Lekhak B, Ghimire P, Rijal KR Antimicrobial Susceptibility Pattern of *Salmonella* spp. Isolated from Enteric Fever Patients in Nepal. .Infect Dis Rep. 2021 Apr 21;13(2):388-400.
4. Admassu D, Egata G, Teklemariam Z. Prevalence and antimicrobial susceptibility pattern of *Salmonella enterica serovar* Typhi and *Salmonella enterica serovar* Paratyphi among febrile patients at Karamara Hospital, Jigjiga, eastern Ethiopia. SAGE Open Med. 2019 Mar 15;7:205.
5. Chen S, Zhao DG, White CM. Schroeder R, Lu H, Yang PF, McDermott S, Ayers J. Meng Characterization of multiple-antimicrobial-resistant Salmonella serovars isolated from retail meats and carriers 2004Appl. Environ. Microbiol., 70 (2004), pp. 1-7.
6. CLSI Performance Standards for Antimicrobial Susceptability Testing. 30th Edition.CLSI guideline M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.
7. Shin JH, Kee SJ, Shin MG, et al. Biofilm production by isolates of Candida species recovered from nonneutropenic patients: comparison of bloodstream isolates with isolates from other sources. J Clin Microbiol, 2002; 40, 1244-1248.
8. Baillie GS, Douglas LJ. Effect of growth rate on resistance of *salmonella* biofilms to antifungal agents. Antimicrob.Agents Chemother. 1998; 42, 1900-1905.
9. Peeters E, Nelis HJ, Coenye T. Comparison of multiple methods for quantification of microbial biofilms grown in microtiter plates. J Microbiol Methods, 2008; 72:157-165.
10. Chuanchuen P, PadungtodPathanasophon S, Khemt ong, W. Wannaprasat, P. Padungtod Antimicrobial resistance genes in Salmonella enterica isolates from poultry and swine in Thailand J. Ved. Med. Sci., 71 (2009), pp. 1349-1355
11. Collao B, Morales EH, Gil F, Polanco R, Calderón IL, Saavedra CP. Differential expression of the transcription factors MarA, Rob, and SoxS of Salmonella Typhimurium in response to sodium hypochlorite: down-regulation of rob by MarA and SoxS Arch. Microbiol., 194 (2012), pp. 933-942.
12. Rodrigues, LB, Santos LR, Tagliari VZ, Rizzo NN, Trenhago G, Oliveira AP, Goetz F, Nascimento VP. Quantification of biofilm production on polystyrene by *Listeria, Escherichia coli, salmonella and Staphylococcus* aureus isolated from a poultry slaughterhouse. Braz. J. Microbiol., 2010; 41, 1082–1085.
13. Davidson and Harrison PM, Davidson MA. Harrison Resistance and adaptation to food antimicrobials, sanitizers, and other process controls Food Tech, 56 (2002), pp. 69-78.
14. CDC. (Centers for Disease Control and Prevention) Attribution of Foodborne Illnesses, Hospitalizations, and Deaths to Food Commodities by Using Outbreak Data, United States, 1998-2008 (2011),
15. Fàbrega A, Vila J. *Salmonella enterica* skills to succeed in the host: virulence and regulation Clin. Microbiol Rev., 26 (2013), pp. 308-341.
16. Roza Nasir Awol, Dawit Yihdego Reda , Deresse Daka Gidebo Prevalence of Salmonella enterica serovar Typhi infection, its associated factors and antimicrobial susceptibility patterns among febrile patients at Adare general hospital, Hawassa, southern Ethiopia BMC Infect Dis. 2021 Jan 7;21(1):30.
17. Kirtika Gautam1,* Kathmandu, Nepal P Prevalence of Typhoid and Paratyphoid fever in a tertiary care hospital of Kathmandu valley J. Microbiol., 55 (2009), pp. 163-178.
18. Papavasileiou, K. et al. Comparative antimicrobial susceptibility of bioflm versus planktonic forms of *Salmonella enterica* strains isolated from children with gastroenteritis. Eur J Clin Microbiol Infect Dis 29, 1401–1405 (2010).
19. Nik Yusnoraini Yusofm Nur Iffah Izzati Norazzmanm Nur Fatihah Mohd Zaidi Prevalence of Antimicrobial Resistance Genes in *Salmonella Typhi*: A Systematic Review and Meta-Analysis by Institute for Research in Molecular. Trop. Med. Infect. Dis. 2022, *7*(10), 271;
20. Majtán J, Majtánová L, Xu M, Majtán V. In vitro efect of subinhibitory concentrations of antibiotics on bioflm formation by clinical strains of *Salmonella enterica* isolated in Slovakia. Journal of Applied Microbiology 104, 1294–1301 (2008).
21. Fernández Maria Luisa Márquez, M. José Grande Burgos, R.P. Pulido, A. Gálvez, R. Lucas López Biocide tolerance and antibiotic resistance in *Salmonella* isolates from hen eggshells
22. Foodborne Pathog. Dis., 14 (2017), pp. 89-95.
23. Raza A., Yasra Sarwar , Aamir Ali et al. Efect of bioflm formation on the excretion of Salmonella

enterica serovar *Typhi* in feces. Int. J. Infect. Dis. 15, e747–52 (2011).

24. González
J.F. González, H. Alberts, J. Lee, L. Doolittle, J. Gu nn
25. Biofilm formation protects *Salmonella* from the antibiotic ciprofloxacin in vitro and in vivo of chronic carriage Sci. rep., 8 (2018), p. 22.
26. Tabak, M., Scher, K., Chikindas, M. L. & Yaron, S. Te synergistic activity of triclosan and ciprofoxacin on bioflms of Salmonella. FEMS Microbiology Letters 301, 69–76 (2009).
27. Sharma D, Misba L, Khan AU. Antibiotics versus biofilm: an emerging battleground in microbial communities. Antimicrob Resist In. 2019. Dec;8(76):1–10.
28. Frees D, Brøndsted L, Ingmer H. Regulated Proteolysis in Microorganisms. Springer; Berlin/Heidelberg, Germany: 2013. Bacterial proteases and virulence; pp. 161–192.
29. Rosenau F, Isenhardt S, Gdynia A, Tielker D, Schmidt E, Tielen P, Schobert M., Jahn D, Wilhelm S, Jaeger K-E. Lipase LipC affects motility, biofilm formation and rhamnolipid production in Gram-negative. FEMS Microbiol. Lett. 2010;309:25–34.
30. Condell C, Iversen S, Cooney KA, Power C, Walsh C. Burgess, S. Fanning Efficacy of biocides used in the modern food industry to control *Salmonella enterica*, and links between biocide tolerance and resistance to clinically relevant antimicrobial compounds Appl. Environ. Microbiol., 78 (2012), pp. 3087-3097.