

Antibiotic Susceptibility Patterns and Sero-grouping of *Salmonella* Isolated from Clinical Specimens in Eastern Province, Saudi Arabia

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Received: 2 April 2015, Accepted: 5 June 2015

Abstract

The aim of this study was to determine the prevalence and the antibiotic susceptibility patterns of *Salmonella* serogroups isolated from patients attending Saad Specialist Hospital in Eastern Province, Saudi Arabia. A total of 222 *Salmonella* strains were isolated from different clinical specimens (Blood, Stool, Urine and Abscess) collected from children and adults, males and females. All specimens were collected from both in-patients and out-patients hospitalized in different wards at Saad Specialist Hospital in Eastern Province, Saudi Arabia. Microbiological analysis of all clinical specimens was carried out by using standard methods. Slide agglutination tests were performed by using *Salmonella* anti sera (ProLab Diagnostic, UK) to determine serogroups of all the isolated and identified colonies of *Salmonella*. Antibiotic susceptibility patterns of all isolated *Salmonella* were determined by Kirby-Bauer disk diffusion method against 5 antibiotics. Results revealed that 123 (55%) *Salmonella* species were isolated from males and 99 (45%) from female patients of different ages, with no significant differences (P-Value = 0.188) and Stool specimens were the major source of *Salmonella* species 214 isolates (96.4%). A high percentage of *Salmonella* species, 136 (61.3%) was isolated from children aged between 1 to 15 years. The number and percentages were decreased gradually according to age with significant differences for isolates between different age groups. The highest number 104 (46.9%) was isolated from children of the age group 1-3 years old. All isolated *Salmonella* species belong to 8 serogroups (A 1.35%, B 18.9%, C 24.35, D 43.7%, E 9.5%, F 0.45, G 1.35% and R 0.45%). Serogroups B, C and D were isolated in

higher percentages. All *Salmonella* isolates were tested for sensitivity against 5 antibiotics. Results revealed that all *Salmonella* species isolates were highly sensitive (>90%) to Ceftriaxone, Ciprofloxacin and Levofloxacin. Sensitivity to Trimethoprim-Sulfamethoxazole and Ampicillin was 88.3% and 81.5%, respectively.

Keywords antibiotic, bacterial pathogens, *Salmonella*

Introduction

Salmonella is a group of bacteria that normally inhabit the intestines of animals and humans. They are a heterogenous group of the family *Enterobacteriaceae*. The taxonomy and nomenclature of *Salmonella* have changed over the years and are still evolving (CDC, 2005). At present, there are 3 species of *Salmonella* i.e., *S. enterica*, *S. bongori* and *S. subterranean*. There are more than 2,500 serovars and more than 50 serogroups of *Salmonella* classified by using antigenic formulae (Kauffmann-White scheme) (Grimont and Weill, 2007). *Salmonella* infections occur worldwide in both developed and developing countries and are a major contributor to morbidity and economic costs. The contamination of food products with *Salmonella* generates serious health and economic consequences (Ammari et al., 2009). Study of *Salmonella* serogroups and serovars is crucial because it can benefit an outbreak investigation or aid in identifying the source of infection as Salmonellosis is one of the public health problems (Pulsrikarn et al., 2013).

Antimicrobial resistance has complicated the selection of antibiotics for the treatment of enteric bacterial pathogens, particularly to commonly used antimicrobial agents (Asrat, 2008). Until 1960's,

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nearly all *Salmonellae* were sensitive to a wide range of antimicrobial agents but since 1962 emergence of resistance, frequently plasmid mediated, have appeared in *Salmonella* worldwide. The relative importance of antibiotic resistance, and the serotypes in which it occurs, differs from country to country. Knowing the prevalence and local patterns of antimicrobial susceptibility of *Salmonella* species is important for reducing the burden of the diseases (Demissie et al., 2014).

The aim of this study was to determine the prevalence and the antibiotic susceptibility patterns of *Salmonella* serogroups isolated from patients attending Saad Specialist Hospital in Eastern Province, Saudi Arabia.

Methodology

Sample collection

From November 2008 to September 2010, a total of 222 *Salmonella* strains were isolated from different clinical specimens of Blood, Stool, Urine and Abscess collected from children and adults, males and females. All specimens were collected from both in-patients and out-patients hospitalized in different wards (Internal medicine, pediatrics, emergency department (ER), Oncology department and the outpatient clinics OPD) at Saad Specialist Hospital in Eastern Province, Saudi Arabia.

Bacteriological analysis

Microbiological analysis of all clinical specimens was carried out at the Department of Microbiology at Saad Specialist Hospital laboratory. Stool samples were cultured routinely in Xylose lysine deoxycholate (XLD) agar and Selenite broth (SB) and incubated at 37°C for 24 hours, suspected colonies of *Salmonella* (red colonies, most black centre) were separated on Muller Hinton agar to do purity plate and inoculated in Triple Sugar Iron slant (TSI) and UIM (Urea, indole, motility) slant for confirmation (Forbes, 2007). Other urine and abscess samples were cultured on blood and MacConkey agar media, then incubated aerobically at 37°C for 18-24 hours, while blood cultures were analysed by using an automated blood culture system (BACT / ALERT 3D, BioMerieux) to detect bacterial growth. Identification of isolated *Salmonella* was made according to the standard methods mentioned by Forbes (2007) and Cowan and Steels (Barrow and Feltham, 2003). Antibiotic

susceptibility testing was performed by using the Kirby – Bauer disk diffusion technique according to CLSI guidelines on Mueller-Hinton agar media.

Antimicrobial susceptibility test

Invitro antibiotic susceptibility patterns of all isolated *Salmonella* were determined by Kirby-Bauer disk diffusion method according to CLSI recommended against 5 antibiotics: Ampicillin, Ceftriaxone, Ciprofloxacin, Levofloxacin and Trimethoprim-Sulfamethoxazole (Wayne, 2010), purchased from Oxoid Microbiology Products, Thermo Scientific (Hampshire, UK).

Serological tests

Slide agglutination tests were performed by using *Salmonella* anti sera (ProLab Diagnostic, UK) to determine the serogroups of all isolated and identified colonies of *Salmonella*. *Salmonella choleraesuis* ATCC strains (11511, 9270 and 8387) and *Salmonella enteritidis* ATCC strain 13076 used as positive control. *Proteus mirabilis* ATCC strain 25933 used as negative control.

Statistical analysis

Statistical analysis was carried out by using chi square test.

Results and Discussion

The comprehensive data on serogroups is useful for studying the Salmonellosis surveillance and epidemiological distribution of the predominant *Salmonella* (Elhadi et al, 2013; Getachew, 2014).

In this study, a total of 222 *Salmonella* species were isolated from various clinical specimens collected from inpatient and outpatients of different ages and sexes during the study period. All isolated species were confirmed and serotyped by standard manual and automated methods. Total 123 (55%) *Salmonella* species were isolated from males and 99 (45%) from females patients of different ages, with no significant differences (P-Value = 0.188).

Stool specimens were the major source of *Salmonella* species. Total 214, 96.4% and 3.6% were isolated from Blood, Urine and Abscess specimens. The higher percentages of bacteria were isolated from outpatient departments, 46.4% from patients who attend to OPD clinics and 36.5% from patients who attend to Emergency department.

While 17.1% were isolated from inpatients wards (Table 1).

Table 1: Number and percentages of *Salmonella* isolates according to source of specimens and Hospital Wards

Source of specimens	Percentage		Hospital Wards		Percentage	
	Number	(%)	Number	(%)	Number	(%)
Stool	214	96.4	OPD clinics	103	46.4	
Blood	6	2.7	Emergency	81	36.5	
Urine	1	0.45	Pediatrics	30	13.5	
Abscess	1	0.45	Internal	4	1.8	
			Oncology	4	1.8	
Total	222	100		222	100	

As shown in Table 2, a high percentage of *Salmonella* species 136 (61.3%) was isolated from children less than 15 years old. The highest number 104 (46.9%) was isolated from children of the age group 1-3 years old.

The 214 stool samples analysis results of the same patients in all age groups showed normal pus cells (less than 5 cells) in 140 (65.4%) samples, while the pus cells results were higher than normal (More than 5 cells) in 74 (34.6%) samples with significant differences between the two group of results. Nineteen samples of abnormal pus cells results showed positive results for *Entamoeba histolytica* (Table 2).

Table 2: Number and percentages of isolated *Salmonella* according to age groups and number of pus cells in stool analysis for the same patients

Age group	Number	Percentage (%)
1-15	136	61.3
16-30	43	19.4
31-45	22	9.9
46-60	15	6.7
More than 60	6	2.7
p-value= 0.000		
Children		
1-3	104	46.9
4-15	32	14.4
p-value= 0.000		
Stool analysis		
Pus Cells <5	140	65.4
Pus Cells >5	74	34.6
p-value= 0.000		

The higher occurrence of Salmonellosis in children compared to adults suggests a higher vulnerability of children to *Salmonella* infections (Getachew, 2014). Infants and children are at high risk for *Salmonella* infection. In the European Union, more than 100,000 cases of Salmonellosis were reported each year (Pulsrikarn et al., 2013). In this study, most of the isolated *Salmonella* species belong to serogroups D (43.7%), C (24.3%) and B (18.9%) (Table 3) and this was in agreement with Elhadi et al. (2013). Also Lee et al. (2013) in their study in Korea found that Serogroup D was the most common (39.5%), followed by B (32.4%) and C (22.7%).

The prevalence and the local patterns of antimicrobial susceptibility of *Salmonella* species are important for reducing the burden of the disease (Huruy et al, 2008).

Table 3: Number and percentages of isolated *Salmonella* serogroups

Serogroups	A	B	C	D	E	F	G	R	Total
Number	3	42	54	97	21	1	3	1	222
Percentage %	1.3	18.9	24.3	43.7	9.5	0.4	1.3	0.4	100
Age groups									
1--16	1.5	18.4	22.8	46.4	8	0	2.2	0.7	
16-31	0	13.9	23.3	51.2	11.6	0	0	0	
31-46	4.5	13.6	27.3	41	9.1	4.5	0	0	
46-61	0	40	26.7	20	13.3	0	0	0	
More than 61	0	33.3	50	0	16.7	0	0	0	

In this study, all *Salmonella* isolates were tested for sensitivity against 5 antibiotics. Results revealed that all *Salmonella* species isolates were highly sensitive to Ceftriaxone (96.8%), Ciprofloxacin (93.7%) and Levofloxacin (92.8%). Sensitivity to Trimethoprim-Sulfamethoxazole and Ampicillin was 88.3% and 81.5%, respectively (Table 4). This was in agreement with the results obtained by Elhadi et al. (2013) in their study in Saudi Arabia, Demissie et al. (2014) in their study in Ethiopia and Abdullahi et al. (2014) in their study in Nigeria. In contrast Chiu et al. (2004) in their study in Taiwan reported Ciprofloxacin and Ceftriaxone resistance in serogroup D *Salmonella*.

Table 4: Antibiotic susceptibility pattern of isolated *Salmonella* species

Antibiotics	Sensitive	Percentage	
		(%)	(%)
Ampicillin	181	81.5	18.5
Ceftriaxone	215	96.8	3.2
Ciprofloxacin	208	93.7	6.3
Levofloxacin	206	92.8	7.2
Trimethoprim-Sulfamethoxazole	196	88.3	11.7

However, since 1991, outbreaks or cases of infections caused by *Salmonella* resistant to extended-spectrum Cephalosporins and Fluoroquinolones have been increasingly reported (Su et al., 2005).

Conclusion

In our study, the majority of *Salmonella* isolates was found in feces indicating that most of *Salmonella* cases were gastroenteritis and the highest prevalence of Salmonellosis was found in children > 4 years old. Serogroups B, C and D were predominated among 8 serogroups and the most prevalent serogroup was serogroup D. Epidemiological investigations of *Salmonella* species provide useful information for prevention and control. Active surveillance and monitoring of antimicrobial resistance is important for public health and the treatment of the disease.

Acknowledgments

We would like to thank SAAD Specialist Hospital administration for their financial support and all the staff of the department of microbiology for their help and cooperation.

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