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ANTIBIOTIC SUSCEPTIBILITY OF *SALMONELLA SPP.*: A COMPARISON OF TWO SURVEYS WITH A 5 YEARS INTERVAL

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SUMMARY

Salmonella infections are one of the major global public health problems. During the last decade, antibiotic resistance and multiresistance of *Salmonella* spp. have increased a great deal, especially in developing countries with an increased and indiscriminate use of antibiotics in the treatment of humans and animals.

This study aims to investigate and compare antimicrobial susceptibility patterns of *Salmonella* during 2005 and 2010.

A total of 186 *Salmonella* strain during 2005 and 140 *Salmonella* strain during 2010 were isolated from stool specimens using standard methods. The isolates were confirmed as *Salmonella* by using a battery of biochemical reactions. Specific antisera were used for serologic characterization of *Salmonella* strain. Antimicrobial susceptibility testing was performed by standard disk diffusion method using ampicillin, trimethoprim-sulfamethoxasole, ceftriaxon, chloramphenicol, nalidixic acid and ciprofloxacin.

One hundred eighty (96.8%) of 186 isolated *Salmonella* strains in 2005, and 133 (95%) of 140 isolated *Salmonella* strain in 2010 are recognized as *Salmonella Enteritidis*. Sensitivity of *Salmonella* isolates during 2005 and 2010 were 91.9% and 92.9% to ampicillin, 95.7% and 97.1% to trimethoprim-sulfamethoxasole, 99.5% and 100% to chloramphenicol, 99.5% and 100% to ciprofloxacin, 98.9% and 97.1% to ceftriaxon, 73.1% and 95.7% to nalidixic acid, respectively.

Sensitivity of *Salmonella* isolates to all tested antimicrobial agents except to ceftriaxon was been slightly improved over testing period. Resistance rate to ceftriaxon was higher in 2010 than in 2005, and this fact deserves attention. Significantly increase susceptibility rate to nalidixic acid was observed between the two surveys.

Key words: nontyphoid, salmonella, antibiotic susceptibility

INTRODUCTION

Diarrheal disease is an important global problem that causes high rates of morbidity and mortality in developing countries. Among the bacteria causing diarrhea, *Salmonella* continues to be at first place. Over the last two decades the incidence of nontyphoidal *Salmonella* infections has increased. It is estimated that nontyphoidal *Salmonella* cause between two hundred million and 1.3 billion cases of intestinal disease including 3 million of death each year worldwide [(1)]. Salmonellosis are the second most commonly reported human zoonoses in spite of a decrease in incidence over the last three years in EU [(2)].

Recently, specific serovars were linked with certain foods or exposures. For example, outbreaks of *Salmonella* serovar Enteritidis have been repeatedly associated with raw or undercooked eggs [(3)] and *Salmonella* Marina infection has been associated with exposure to reptiles [(4)].

Infection with nontyphoidal *Salmonella* usually results in a self-limiting gastroenteritis that does not require antibiotic therapy, but sometimes serious sequellae, including systemic infection and death, may occur [(5)].

Examining the antibiotic susceptibility patterns of pathogens is important toward tailoring treatment to the ever changing resistance patterns and distribution of pathogenic bacteria.

During the last decade, antibiotic resistance and multiresistance of *Salmonella* spp. have increased a great deal, especially in developing countries with an increased and indiscriminate use of antibiotics in the treatment of humans and animals [(6)].

Various *Salmonella* serovars resistant to conventional antibiotics such as ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, and other newer antibiotics (quinolones and extended-spectrum cephalosporins) have been reported with increasing frequency in many areas of the world [(5)].

Among the nontyphoidal *Salmonella enterica* serovar Typhimurium has been reported to show multidrug

resistance [7].

This study aims to investigate and compare antimicrobial susceptibility patterns of Salmonella during 2005 and 2010.

MATERIAL AND METHOD

A total of 186 *Salmonella* strain during 2005 and 140 *Salmonella* strain during 2010 were isolated from stool specimens using standard methods.

Using sterile swabs, the stool samples were directly inoculated onto plates of SS agar (Himedia, HIMedia Laboratiries Pvt.Ltd. India). The same samples were then plated onto Selenite F broth (BIOTEC, BIOTEC Laboratories Ltd, UK) and incubated as aforestated for enrichment. Agar plates and enrichment broth were incubated aerobically at 37єC for 20-24 hours. Following the incubation of Selenite F broth, a loopful was streaked onto SS agar plates and incubated at 37°C for 24 h. One to three colonies suspected to be *Salmonella* (small colorless colonies sometimes with black spot) were selected and characterized biochemically using Kligler Iron Agar (KIA) and Urease tests.

Specific antisera were used for serologic characterization of *Salmonella* strain. Antimicrobial susceptibility testing was performed by standard disk diffusion method (NCCLS, 1997; 2001) using ampicillin, trimethoprim-sulfamethoxasole, ceftriaxon, chloramphenicol, nalidixic acid and ciprofloxacin (Beckton Dickinson, MD).

RESULTS

Salmonella Enteritidis was the most common isolated serovar during 2005 and 2010 (96.8%; 95%, respectively). Salmonella gr. B with 3.2% isolation rate was the second most isolated serovar in 2005. Serovars distribution is shown in table 1.

Table 1. Distribution of *Salmonella* serovars during 2005 and 2010

Salmonella SEROVAR	YEAR				TOTAL	
	2005		2010			
	No	%	No	%	No	%
Salmonella Enteritidis	180	96.8	133	95	313	96
Salmonella gr.B	6	3.2	2	1.4	8	2.5
Salmonella Paratyphi B	0	0	1	0.7	1	0.3
Salmonella gr. C	0	0	1	0.7	1	0.3
Salmonella Infantis	0	0	1	0.7	1	0.3
Salmonella Wirchow	0	0	1	0.7	1	0.3
Salmonella gr.A	0	0	1	0.7	1	0.3
TOTAL	186	100	140	100	326	100

Three strain *Salmonella* gr. C and one strain *Salmonella* gr. A were isolated in 2010.

The antibiotic susceptibility of *Salmonella* isolates is shown in table 2.

Table 2. Antibiotic susceptibility of salmonella isolates

ANTIBIOTICS	2005				2010				Total			
	Sensitive		Resistant		Sensitive		Resistant		Sensitive		Resistant	
	No	%	No	%	No	%	No	%	No	%	No	%
Ampicillin	171	91.9	15	8.1	130	92.9	10	7.1	301	92.3	25	7.7
Trim/sulfameth.	178	95.7	8	4.3	136	97.1	4	2.9	314	96.3	12	3.7
Ceftriaxon	184	98.9	2	1.1	136	97.1	4	2.9	320	98.2	6	1.8
Nalidixic acid	136	73.1	50	26.9	134	95.7	6	4.3	270	82.8	56	17.2
Ciprofloxacin	185	99.5	1	0.5	140	100	0	0	325	99.7	1	0.3
Chloramphenicol	185	99.5	1	0.5	140	100	0	0	325	99.7	1	0.3

Thirty-seven percent of Salmonella isolates were resistant to at least one antimicrobial in 2005, and 10% in 2010. It was significant difference ($p=0.00< 0.05$) (table 3).

Table 3. Total susceptibility to antimicrobial according to years

Susceptibility	YEAR				TOTAL	
	2005		2010			
	No	%	No	%	No	%
Susceptible to all antimicrobial	118	63.4	126	90	244	74.8
Resistant to at least one antimicrobial	68	36.6	14	10	82	25.2
TOTAL	186	100	140	100	326	100

The incidence of multidrug resistance (MDR - resistance to three or more antibiotics) in *Salmonella* strains was 1.6% in 2005 and 2.1% in 2010 (table 4).

Table 4. The incidence of multidrug resistance *Salmonella* strain

Year	No of isolates	No of MDR *Salmonella*	% of MDR *Salmonella*
2005	186	3	1.6
2010	140	3	2.1
TOTAL	326	6	1.8

Multiresistant strains of *Salmonella* were isolated in higher percent in 2010 (2.1%) than in 2005 (1.6%), but the difference was not significant (p=0.725>0.05). All multiresistant strains were *Salmonella* Enteritidis.

DISCUSSION

Over 2500 serovars of *S. enterica* have been identified belonging to six subspecies. *S. enterica* species are typically orally acquired pathogens which represent more than 99.5% of the *Salmonella* strains isolated from humans and other warm-blooded animals [8,9].

In our investigation there was one isolate *Salmonella* Paratyphi B in 2010. *Salmonella* gr. B was the second most isolated serovar in 2005, but in 2010 there was greater diversity of isolated serovars than in 2005. *Salmonella* gr. B and *Salmonella* gr. C were isolated with the same frequency in 2010. *Salmonella* Enteritidis has been the most common isolated serovar in both years of survey. *Salmonella* strains without identified serovars were rarely serovars and laboratory did not have appropriate antisera for its identification.

There is a disturbing general trend in *Salmonella* serovars being resistant to commonly used antimicrobials. Antimicrobial resistance among human *Salmonella* isolates is increasing worldwide and is likely due to the widespread use of antimicrobial agents for the empiric treatment of febrile syndromes and as growth enhancers in animal production [10]. High rates of resistance to ampicillin, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole have been reported from many areas of the world [5]. Also resistance of *Salmonella* to newer antibiotics (quinolones and extended spectrum cephalosporins) has been demonstrated in developing as well as developed countries with increasing frequency [5, 11, 12, 13].

Results in our study show that the frequency of *Salmonella* strains susceptible to all tested antibiotic was significantly higher in 2010 than in 2005. Our data show that the majority of isolates (90%) in 2010 were susceptible to all tested antibiotics.

Although the rate of sensitivity to individual antibiotics was higher in 2010 than in 2005, a slight increase of multi-resistant strains was marked in 2010 compared to 2005.

Multidrug-resistant (MDR) strains of *Salmonella* are now encountered frequently worldwide and the rates of multidrug-resistance have increased considerably in recent years. Even worse, some variants of *Salmonella* have developed multidrug-resistance as an integral part of the genetic material of the organism, and are therefore likely to retain their drug-resistant genes even when antimicrobial drugs are no longer used, a situation where other resistant strains would typically lose their resistance [14].

Out of all tested antibiotics *Salmonella* isolates had the lowest level of susceptibility to nalidixic acid in 2005 and ampicillin in 2010. *Salmonella* isolates had the second lowest level of susceptibility to ampicilin (91.9%) in 2005 and nalidixic acid (95.7%) in 2010. We detected a significant increasing susceptibility to nalidixic acid in 2010 (95.7%) compared to 2005 (73.1%). Nalidixic acid has not been on the registered drug list for several years in Montenegro.

The highest level of susceptibility was detected to chloramphenicol and ciprofloxacin in both years of our investigation.

The investigation of sensitivity of *Salmonella* Enteritidis strains in Europe in 2006 shows results similar to our once concerning the resistance to ampicillin (8.1%), chloramphenicol (0.3%) and ciprofloxacin (0.6%). The rate of resistance to nalidixic acid was higher in 2005 than those in Europe in 2006 (14.8%) (The Community Summary Report).

CONCLUSION

The sensitivity of *Salmonella* strains to antibiotics in Montenegro does not differ significantly from the sensitivity of *Salmonella* strains in Europe. Sensitivity of *Salmonella* isolates to all tested antimicrobial agents except to ceftriaxon was been slightly improved over testing period. Resistance rate to ceftriaxon was higher in 2010 than in 2005, and this fact deserves attention. Although the rate of sensitivity to individual antibiotics was higher in 2010 than in 2005, a slight increase of multi-resistant strains was marked in 2010 compared to 2005. Significantly increase susceptibility rate to nalidixic acid was observed between the two surveys. The surveillance of antimicrobial resistance in *Salmonella* spp. is very important. Also, it is important to maintain salmonella active surveillance of resistance on an international and intersectoral level.

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