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Prevalence and Antimicrobial Resistance in Different Salmonella Spp. Isolates

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***Abstract*— This review summarizes current data on prevalence and antimicrobial resistance of Salmonella spp. isolates worldwide. Salmonella spp. is leading pathogen and its antimicrobial resistance is rising problem in different parts of the world. In this study, in order to see the antimicrobials' percentage of resistance 20 papers were selected published in last 20 years where are used two common methods: E-test and agar disc diffusion method. By analyzing these papers the following results are obtained: ampicillin definitely had the highest percentage of antimicrobial resistance in most samples which is 100% (milk and human/animal feces). On the other hand, the lowest percentage of antimicrobial resistance is shown in chloramphenicol resulting in 1.2% (human feces). So, none of tested antimicrobials showed 0% of resistance to any type of sample which is not giving satisfactory results.**

Keywords- Prevalence, Antimicrobial Resistance, Salmonella, isolates

I. INTRODUCTION

Salmonella is a rod-shaped (bacillus) gram-negative type of bacteria of the Enterobacteriaceae family [1]. Today around 2500 serotypes of Salmonella are found and they are separated into two groups: typhoidal serotypes involving Salmonella Typhi and Salmonella Paratyphi and nontyphoidal Salmonella involving a wide range of serotypes [2,3]. Salmonella spp. are treated with numerous antimicrobial agents (a broad spectrum antibiotics) such as: ampicillin, amoxicillin, tetracycline, cotrimoxazole and fluoroquinolone (ciprofloxacin) [4]. Nowadays, Salmonella strains are starting to resist more, to one or many antibiotics, representing a public health problem [5].

A. Prevalence of Salmonella spp.

Salmonella is regarded as the most prevalent foodborne pathogen in the world between all pathogens [6,7]. It is known as an important zoonotic microorganism of economic importance in animals and humans [8], mostly in the developing countries. Consummation of poisoning food, incorrect storing of food products, low degree of personal hygiene, improper cooling and warming of foods, and extended time between making and using of foods are said to be leading agents to occurrence of salmonellosis in people [9,8]. The prevalence of Salmonellosis is high in less developing countries but not so high in Europe. In one year within 20 million cases more than 200,000 people die [10]. The most vulnerable to infection are kids, commonly until age of five. This is highly expressed in Southeast and South Central Asia, Latin America, and Southern Africa [11,12]. The cases of extracts that resist to treatment are increasing, so they make harder clinical management of this infection [13].

Distribution of antimicrobial drug resistance within foodborne pathogens is higher as it is more often used in human therapy and animal farming for objectives of therapy and prevention [14].

B. Antimicrobial Resistance in Salmonella spp.

Antimicrobial-resistant Salmonella spp. are extracted from different samples (human, animals) [15]. Salmonella strains that are resistant to many antimicrobial agents, especially that resist to fluoroquinolones and cephalosporins, are referred as a huge problem in the world [16], leading to increased morbidity and mortality levels and increased treatment payments. This shows a public health risk by transmitting of resistant Salmonella strains to humans by using contaminated food products.

Infections referred to Salmonella spp. are generally treated by antimicrobial agents such as: ampicillin, amoxicillin, tetracycline, cotrimoxazole, fluoroquinolone (ciprofloxacin) and nalixidic acid [17]. The Salmonella resistance to nalidixic acid is not clinically important but it functions as the marker for the further development of fluoroquinolones resistance [18]. The prevalence of fluoroquinolone-resistant isolates in developing states was discovered to be decreasingly [19]. Salmonella multidrug resistance is related to increased chance of hospitalization and death. Multidrug resistance within Salmonella extracts is because of the acquisition of resistance plasmids [20,21].

To represent the facts in the appearances of antimicrobial resistance (AMR) in different samples, a various countries set national surveillance programs [22]. In United States the National Antimicrobial Resistance Monitoring System (NARMS) was used to monitor antimicrobial resistance within Salmonella bacteria and together with state and local health departments, performs antimicrobial susceptibility testing of extracts originating from humans, meats and food animals. The NARMS collection and testing of bacteria from humans, foods, and food animal sources provide the opportunity to determine emerging resistance issues and potential sources of resistant bacteria. By a good characteristic of this surveillance, NARMS recently recognized emerging resistance in Salmonella enterica serotype Albert [21].

The purpose of this study was to perceive the prevalence of Salmonella spp. and to obtain antimicrobials in order to see their percentage of resistance in different types of samples in several countries.

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II. METHODS

Firstly, literature review was obtained by selecting 20 papers published in last 20 years that assessed as qualified in order to obtain relevant results about prevalence and antimicrobial resistance in Salmonella spp. isolates. The criteria, according to which papers were selected, includes the following:

- published in last 20 years,
- full text available,
- the paper must be written in English,
- include prevalence and antimicrobial resistance of Salmonella spp. isolates as key words.

Among 20 papers selected, researchers used two common methods: E-test and agar disc diffusion method.

A. E-test

E-test is a diffusion test that was used to determine the approximate minimal inhibitory concentration (MIC). The MIC was resoluted for antimicrobials such as: amoxicillin, amoxicillin + clavulanic acid, cefotaxime, gentamicin, streptomycin, tetracycline, and sulfamethoxazole + trimethoprim. According to the results of MICs, the strain is selected as sensitive, moderately sensitive or insensitive to each individual antimicrobial.

Sensitivity criteria includes the following: cefotaxim (≥0.5mg/L), nalidixic acid (≥16 mg/L), ciprofloxacin (≥0.125mg/L), ampicillin (≥4mg/L), tetracycline (≥8mg/L), gentamicine (≥4mg/L), chloramphenicol (≥16mg/L), streptomycin (≥32mg/L), trimethoprim (≥2mg/L), sulfamethoxazole (≥256mg/L) [29].

B. Agar disc diffusion method

Antimicrobial susceptibility testing performing the agar disc diff usion method on Mueller-Hinton agar (Oxoid) was used to establish the antibiotic-resistant profiles of each isolate.

Protocol includes the following steps:

1. extracts grow in a shaking water bath at 37^0C while a 0.5 McFarland turbidity standard is gotten,
2. bacterial suspension gets poured on the entire surface of Mueller–Hinton agar plates,
3. antibiotic disks including amoxicillin (20 mg), ampicillin (10 mg), cephalothin (30 mg), cefriaxone (30 mg), gentamycin (10 mg), nalidixic acid (30 mg), nitrofurantoin (30 mg),
4. sulfamethoxazoletrimethoprim (30 mg), and tetracycline (30 mg) are put on the surface of the medium and incubated at 37^0C for 18– 24 hours,
5. following incubation, the diameters of the inhibition zone are measured in millimeters and shown in accordance with CLSI guidelines [30].

Strains are rated as susceptible, intermediate, and resistant. The parameter for resistance of extract is determined if it was resistant to one or more of the antimicrobials. Escherichia coli ATCC 25922 was used as a quality control and standard strain.

III. RESULTS

By analyzing articles [26-35] about antimicrobial resistance in Salmonella spp. isolates results gotten are represented in Table 1.

Antimicrobials were tested on different samples (human feces, bloirel neck skin, chicken, hens, turkey, pork, beef, fish, milk, eggs, bacterial isolates) in last 20 years in following countries: Bosnia and Herzegovina, Serbia, Croatia, Kosovo, Lebanon, China, Ethiopia, Bangladesh, Indonesia, Taiwan and Vietnam. These samples were tested on following antimicrobials: ampicillin, trimethoprim-sulfamethoxazole, ciprofloxacin, ceftazidime, chloramphenicol, nalidixic acid, tetracycline, streptomycin, gentamicin, cefotaxim, amoksicilliin, cephalothin, nitrofurantain, kanamycine, sulfisoxazole, amoxillin-clavulanic acid, ceftiofur, amikacin, levofloxacin, gatifloxacin, ceftriaxone, cefoxitin, minocycline, cloxacyllin and trimethoprim.

Fig. 1. represents antimicrobial resistance of Salmonella spp. on food samples (milk, eggs and raw meat). The highest percentage of resistance for sample of milk showed: ampicillin and streptomycin (100%); for eggs samples: cloxacillin and minocycline (100%) and for raw meat samples: trimethoprim-sulfamethoxazole, streptomycin and chloramphenicol (100%). The lowest percentage of resistance for milk samples showed chloramphenicol (16,7%); for eggs samples cephalothin (4.8%) and for raw meat samples ceftazidime (2%).

Fig. 2. represents antimicrobial resistance of Salmonella spp. on clinical samples (human and animal feces, bloirel neck skin and bacterial isolates). The highest percentage of resistance for samples of human and animal feces samples showed ampicillin (100%); for bloirel neck skin samples: ampicillin and nalidixic acid (95.5%) and for bacterial isolate samples nalidixic acid (81.6%). The lowest percentage of resistance for human and animal feces samples showed chloramphenicol (1.2% and 6.7%); for bloirel nesk skin samples cefotaxim (68.2%) and for bacterial isolates samples: ampicillin, gentamicin and trimethoprim (1.8%).

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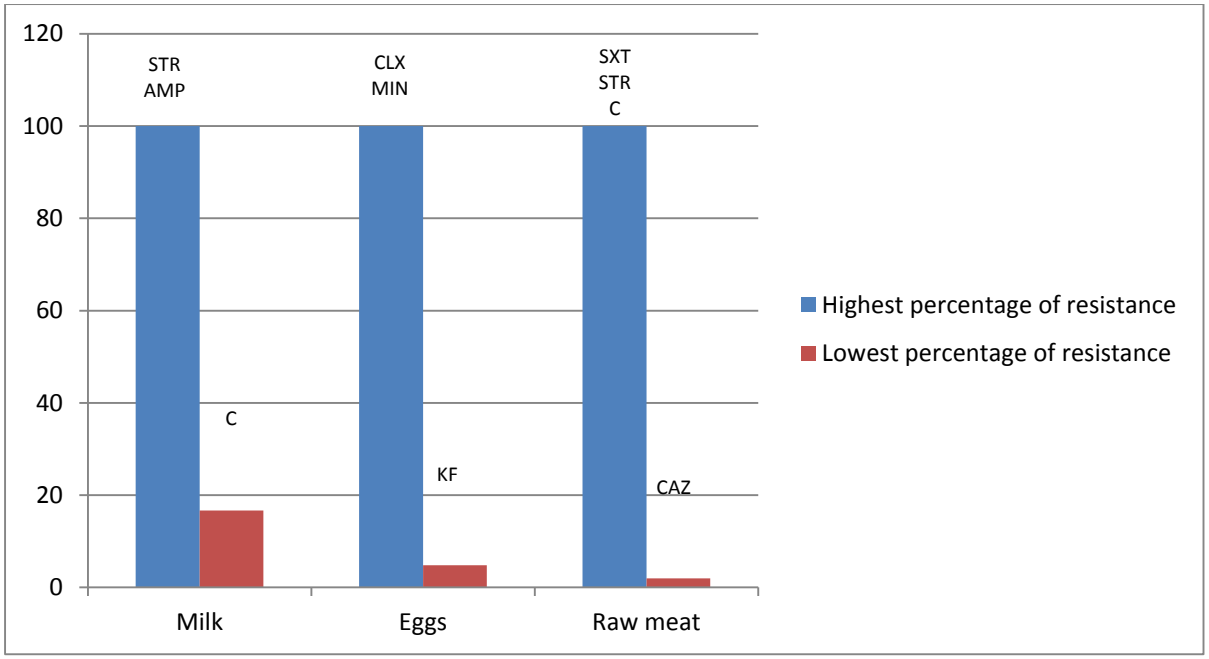

Figure 1. Antimicrobial resistance of Salmonella spp. on food samples

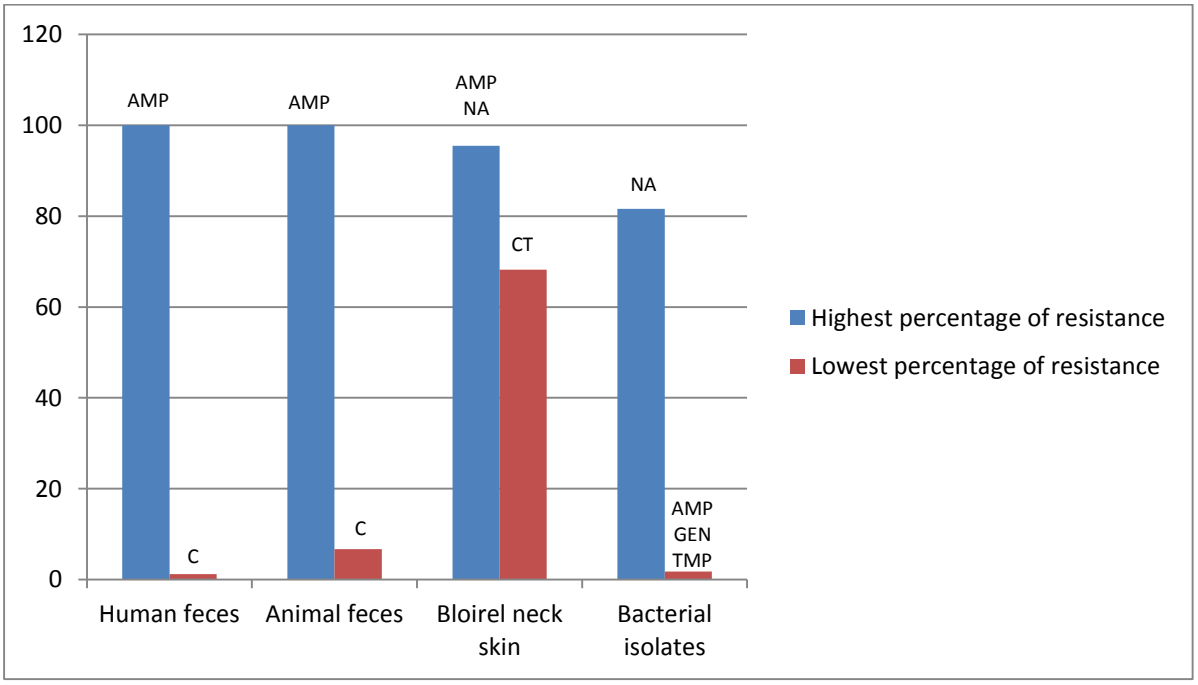

Figure 2. Antimicrobial resistance of Salmonella spp. on clinical samples

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TABLE I. RESULTS OF ANTIMICROBIALS TESTED

AMP-ampicillin; SXT-trimethoprim-sulfamethoxazole; CIP-ciproflaxin; CAZ-ceftazidime; C-chloramphenicol; NA-nalidixic acid; TCY-tetracycline; STR-streptomycin; GEN-gentamicin; CT-cefotaxim; AML-amoxicillin; KF-cephalothin; F-nitrofurantain; KAN-kanamycine; SIX-sulfisoxazole; AMC-amoxillin-clavulanic acid; TIO-ceftiofur; AMK-amikacin; LEVO-levofloxacin; GAT-gatifloxacin; CRO-ceftriaxone; CFX-cefoxitin; MIN-minocycline; CLX-cloxacillin; TMP-trimethoprim.

COUNTRY	PERIOD	SAMPLE TYPE	ANTIMICROBIALS TESTED Percentage of resistance (%) AMP SXT CIP CAZ C NA TCY STR GEN CT AML KF F KAN SIX AMC TIO AM K LEVO GAT CRO CFX MIN CLX TMP D
Bosnia and Herzegovina	1998-2000.	sporadic	22.6 6.7 - - 2.7 7.2 16 - - - - 10.6 40 - - 15 - - - - - - - - - 44
		human feces	23.3 7.1 - - 1.2 8 16.5 - - - - 10.1 36.4 - - 13.3 - - - - - - - - - 46.3
Serbia	2010.	bloirel neck skin and human feces	95.5 - - - - 95.5 91.0 - - 68.2 - - - - - - - - - - - - - -
roatia	2010.	chicks, hens, turkey	95.5 95.5 85.4 - 100 58 97.5 100 99.3 98.7 - - - - - - - - - - - - -
Kosovo	2012.	feces, dust, eggs	- - - - - - - 90 - - 86 - - - - - - - - - - - 100 100 - -
Lebanon	2011-2014	clinical	23.4 7.9 3.8 2.4 -
		food	20.4 100 10.2 2.0 -
China	2007-2012.	retail chicken, pork, beef, fish, dumplings, cold dishes	53.2 68.0 22.2 - 40.3 63.4 70.6 34.9 31.3 - - - - 36.9 78.1 42.6 28.6 20.0 18.7 17.9 17.7 13.2 - - - -
Ethiopia	2014-2015.	meat, eggs, milk	14.3 28.6 - - - 9.5 42.6 - 9.5 - 9.5 4.8 9.5 - - - - - - - - - - - - -
	2014.	human stool	100 5.2 - - 5.2 26.3 47.4 - 5.2 - - - - - - - - - - - - - - -
	2010.	milk (cows)	100 - - - 16.7 - 50 100 33.3 - - - 66.7 66.7 - - - - - - - - - - -
		feces (cows)	100 - - - 6.7 - 26.7 66.7 13.3 - - - 40 26.7 - - - - - - - - - - -
		human stool	100 - - - - - 33.3 - - - - - - - - - - - - - - - - - - -
Bangladesh Indonesia Taiwan Vietnam	2007-2009.	bacterial isolates	68.4 68.4 39.5 - 57.9 81.6 21.1 60.5 - - - - - - - - - - - - - - - - - 57.9 -
			1.8 3.6 - - 3.6 1.8 3.6 3.6 1.8 - - - - - - - - - - - - - - - - 1.8 -
			2.8 2.8 - - - - 2.8 - - - - - - - - - - - - - - - - - 2.8 -
			80.4 80.4 8.3 - 80.4 19.6 84.3 80.4 - - - - - - - - - - - - - - - - 80.4 -

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IV. DISCUSSION AND CONCLUSION

In this study 20 articles published in last 20 years were analyzed that are related to prevalence and antimicrobial resistance of Salmonella species isolates. In Bosnia and Herzegovina antimicrobial resistance of sporadic samples and samples from hospitalized patients was tested on 16 antimicrobials in period between 1998-2000. According to the results they got doxycycline showed the highest percentage of resistance for both sample types followed by nitrofurantoin [27].

In Serbia in 2010. antimicrobial resistance of bloirel neck skin and human feces sample types was tested on 7 antimicrobials. Their results represent that ampicillin and nalidixic acid have the highest percentage of resistance followed by tetracycline [28].

In Croatia in 2010. chicken, hens and turkey sample types were tested on 9 antimicrobials to obtain the percentage of antimicrobial resistance. According to their results chloramphenicol and streptomycin showed the highest percentage of resistance for all sample types, followed by gentamicin [29] and the result for chicken sample does not agree with the result gotten in China in period between 2007-2012 [34].

In Kosovo in 2012. feces, dust and egg sample types were tested on 4 antimicrobials. Results they got showed that minocycline and cloxacillin have the highest percentage of resistance [33].

Trimethoprim-sulfamethoxazole showed the highest percentage of resistance for food samples and ampicillin for clinical samples, tested in period between 2011-2014. in Lebanon [26].

In China in period between 2007-2012. samples including retail chicken, pork, beef, fish, dumplings and cold dishes were tested on 17 antimicrobials. According to their results sulfisoxazole showed the highest percentage of resistance for all sample types [34].

On the other hand in Ethiopia in period between 2014-2015. meat, egg and milk sample types were tested on 8 antimicrobials. The results they got showed that tetracycline has the highest percentage of resistance for all samples [30]. In 2014. human stool was tested on 6 antimicrobials and results showed that ampicillin has the highest percentage of resistance [31] that agreed with the result gotten for the same sample type in 2010. [32]. In 2010. milk (cows) and feces (cows and human) sample types were also tested and results showed that ampicillin has the highest percentage of resistance for all samples, followed by streptomycin for milk (cows) [32].

Finally, in period between 2007-2009. bacterial isolates were tested on different antimicrobials in Bangladesh, Indonesia, Taiwan and Vietnam. Results gotten in Bangladesh showed that nalidixic acid has the highest percentage of resistance for tested sample type, in Indonesia trimethoprim-sulfamethoxazole, chloramphenicol, tetracycline and streptomycin, in Taiwan ampicillin, trimethoprim-sulfamethoxazole and tetracycline and in Vietnam tetracycline [35].

Salmonella species are the leading pathogens worldwide. Beside the concern on quality of healthcare [36-38] and diagnosis of chronical diseases [39-45], antimicrobial resistance is rising problem becoming more frequent in different parts of the world. From obtained results ampicillin definitely had the highest percentage of antimicrobial resistance in most samples which is 100%. On the other hand, the lowest percentage of antimicrobial resistance is shown in chloramphenicol resulting in 1.2%. So, none of tested antimicrobials showed 0% of resistance to any type of sample which is not giving satisfactory results.

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