African Journal of Microbiology Research

Volume 11 Number 40 28 October, 2017

ISSN 1996-0808

Academic Journals

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academicJournals

Vol. 11(40), pp. 1495-1504, 21 October, 2017
DOI: 10.5897/AJMR2017.8698
Article Number: 621753166525
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Diversity and antimicrobial resistance of *Salmonella* strains isolated from different sources in Burkina Faso

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Received 3 September, 2017; Accepted 25 September, 2017

Epidemiologic and traceback evidence of *Salmonella* infection is not much in many developing countries including Burkina Faso. This study investigates the antimicrobial resistance and distribution of *Salmonella* serotypes isolated from diverse sources in Burkina Faso. 615 *Salmonella* serotypes isolated from beef meat, poultry carcasses, poultry, swine, cattle, hedgehog, fish, salad, channel and humans from 2009 to 2011 were analyzed to identify their diversity and distribution among the samples. The *Salmonella* strains were subjected to antimicrobial sensitivity tests using disk diffusion methods, were analyzed and classified into 110 serotypes, with the most prevalent serotype being Derby (91/615) found in beef meat, poultry carcasses, poultry and fish; Muenster (48/615) found in cattle, swine, hedgehog, poultry, human and fish; Chester (38/615) found in poultry carcasses, swine and poultry feces; Hato (32/615) found in beef meat, poultry carcasses, cattle and poultry; Drac (30/615) found in cattle, hedgehog and fish; and Typhimurium (21/615) found in cattle, poultry, human and fish. Among the 615 *Salmonella* strains, 94% (581/615) were resistant to one or more antibiotics; resistance to streptomycin was the most common. The resistance pattern, Str-Sul-Tet, Str-Tet and Str-Sul was dominant and found in 80% of the strains. About 3% of the strains were resistant to 5 or 6 antibiotics; their resistance pattern is amp-str-sul-tet-tmp or amp-chl-str-sul-tet-tmp. One *Salmonella* strain, *S*. Kentucky isolated from human stool was resistant to eight antibiotics; the resistance pattern is amp-str-sul-tet-cip-gen-nal-mec. Findings from this study can help define the guidelines for basic surveillance system of *Salmonella* and other enteropathogenic bacteria circulating among humans, animals, food and environment.

Key words: *Salmonella*, environment, human, risk.

INTRODUCTION

Salmonella spp. especially Non-Typhoid *Salmonella* (NTS) is a common source of foodborne diseases that cause morbidity and mortality worldwide (Smith et al., 2016). It is estimated that *Salmonella* spp. cause 93.8 million cases of gastroenteritis and 155,000 deaths each year worldwide. Approximately 86% of these cases are

the result of foodborne infections (Majowicz et al., 2010). In Burkina Faso, bacteriological results showed that the rate of *Salmonella* contamination remains quite high (Simporé et al., 2009). In fact, the infection progresses mostly periodically and rarely in an epidemic mode, and they are not reported due to lack of food borne pathogens surveillance system.

Salmonella spp. can survive for long periods in natural waters, and the persistence of specific and epidemic strains is a great concern in public health. However, information on the diversity and occurrence of *Salmonella* strains is very scarce (Cui et al., 2008), making the ecology of these species remains unknown. Food animals, including poultry, pigs, and cattle are the key reservoirs for human salmonellosis (Hauser et al., 2011). In developing countries, wild and food animals are the sources of *Salmonella* distribution in water, vegetables, salad and the products derived from these animals due to lack of hygiene (Kagambèga et al., 2013). The emergence of antimicrobial resistant *Salmonella* is mostly associated with the non-therapeutic use of various classes of antimicrobials in large quantities in food animals (Marshall and Levy, 2011; Mir et al., 2015).

Researchers have reported a link between the use of antimicrobials in food animals and the emergence of antimicrobial resistance in pathogenic bacteria (Ungemach et al., 2006; Mir et al., 2015). The increasing number of multidrug-resistant NTS strains is a global concern; this has made some countries and international organizations create surveillance systems which include collaboration between human health, veterinary, and food related sectors to monitor the spread of foodborne bacteria.

Unfortunately, these surveillance systems are missing in many developing countries like Burkina Faso. The absence of controlled reporting of *Salmonella* serotypes through the WHO-GFN program in Burkina Faso inspired the compilation of data on the sporadic reporting of *Salmonella* serotypes isolated from various sources. Therefore, the purpose of the present study is to provide information on the diversity and antimicrobial resistance of *Salmonella* strains isolated from environment, animals, food and humans. These data will allow one to follow the trends in *Salmonella enterica* serotypes that provide information about sources of infection and the efficacy of prevention and control measures.

MATERIALS AND METHODS

Salmonella strains (n= 615) isolated from meat, poultry carcasses, poultry, cattle, pigs, hedgehog, water, salad and humans were collected in Burkina Faso from 2009 to 2011. Sampling was done based on the microbiological conditions. The strains were isolated using standard bacteriological methods and serotyped according to Kauffman White scheme (Kagambega et al., 2013; Bonkoungou et al., 2013; Traoré et al., 2015).

Antimicrobial susceptibility of the isolates was tested by a standard disk diffusion method, and Escherichia coli RHE 6715 (ATCC 25922) was used for validating the antimicrobial test results (CLSI, 2015). The antimicrobial agents used were ampicillin (10 µg), chloramphenicol (30 µg), streptomycin (10 µg), sulphonamides (3 µg), trimethoprim (5 µg), tetracycline (30 µg), gentamicin (10 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), cefotaxime (30 µg), mecillinam (10 µg) and imipenem (10 µg). Minimal inhibitory concentration (MIC) for ciprofloxacin (0.002 to 32 µg/ml) was determined by E-test (AB Biodisk, Solna Sweden) of the isolates resistant to nalidixic acid. MIC breakpoint ≤ 1 µg/ml was interpreted as susceptible (CLSI, 2009).

Ethics approval and consent to participate

Permission to conduct this study was obtained from the slaughterhouse authorities and the study protocol was approved by the Ethical Committee of Burkina Faso.

RESULTS AND DISCUSSION

A total of 615 strains of *Salmonella* isolated from meat, poultry carcasses, poultry, cattle, pigs, hedgehog, water, salad and humans were distributed into 110 different serotypes (Table 1). The six most prevalent serotypes were Derby, Muenster, Chester, Drac, Hato and Typhimurium. *Salmonella* Derby (91/615) was found in beef meat (n=2), poultry carcasses (n= 34), poultry (n= 52) and fish (n = 3). Our findings show that *S.* Derby is the most often isolated serotypes in poultry. This is in contrast with results reported by some authors, showing that this serotype is most common in pig and also the 4 most frequently isolated serotype in humans in Europe (Kerouanton et al., 2013; Hauser et al., 2011).

Salmonella Muenster was the second most common serotype and was found in cattle, swine, hedgehog, poultry, human and fish. This finding shows that this serotype is not restricted to one host but can be found in animals, foods, water or humans. A documented food poisoning outbreak caused by *S*. Muenster occurred in Canada in 1982; it infected cheddar cheese made from unpasteurized milk (Wood et al., 1984). *Salmonella* Chester was found in poultry carcasses, swine and poultry feces; this serotype has been reported in many countries: in Canada, *S.* Chester was responsible for an outbreak associated to frozen meals in 2010 (Taylor et al., 2012); in 2014, six European countries (Belgium, France, Spain, Germany, Sweden and the UK) reported *S*. Chester cases to the European Centre for Disease Prevention and Control (ECDC) associated with travel in Morocco (Whitworth, 2016). *Salmonella* Hato was found

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Table 1. *Salmonella* serotypes and antimicrobials profile.

Salmonella serotypes	Beef meat	Poultry carcasses	cattle	swine	Hedgehog	Poultry	Human	Salad	Fish	Resrvoirs	Channel	Total	Resistance pattern	Number of antibiotic resistered	Number of resistant serotype
S.Abaetetuba	0	0	1	0	0	1	0	0	0	0	0	2	str	1	1
S.Abony	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
S.Adabraka	0	0	0	0	0	0	0	0	0	1	0	1	str	1	1
S.Adelaide	0	1	0	0	0	1	0	0	0	0	0	2	0	0	0
S.Agona	0	4	0	0	0	3	0	0	3	0	0	10	str	1	5
S.Ahmadi	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0
S.Albany	0	0	2	0	0	2	0	0	0	0	0	4	str	1	2
S.Anatum	0	1	0	0	0	1	0	0	0	0	0	2	str	1	2
S.Angers	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
S.Ank	0	0	0	0	4	1	0	0	0	0	0	5	str	1	5
S.Antwepen	0	0	1	0	0	0	0	0	0	0	0	1	str	1	1
S.Apeyeme	0	0	2	0	0	3	0	0	0	0	0	5	str	1	5
S.Banana	0	3	1	0	1	2	1	0	0	0	0	8	str	1	5
S.Bareilly	0	0	1	0	0	0	0	0	0	0	0	1	str	1	1
S.Bargny	0	0	1	0	0	0	0	0	0	0	0	1	str	1	1
S.Binningen	0	0	0	0	0	2	0	0	0	0	0	2	0	0	0
S.Bochum	0	0	0	0	0	0	0	0	2	0	0	2	0	0	0
S.Brancaster	0	1	1	0	0	3	0	0	0	0	0	5	str	1	4
S.Bredeney	1	0	5	0	0	2	0	1	6	0	1	16	str	1	4
S.Brive	0	0	1	0	0	0	0	0	1	0	0	2	str	1	1
S.Carmel	0	0	1	0	0	0	0	0	0	1	0	2	0	0	0
S.Carno	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0
S.Chandans	0	0	2	0	0	0	0	0	0	0	0	2	str	1	1
S.Chester	0	5	1	0	0	31	0	0	0	0	1	38	str	1	37
S.Chomedey	0	0	4	0	0	0	0	0	0	0	0	4	str	1	4
S.Colindale	0	0	1	0	0	0	0	4	0	1	2	8	str	1	2
S.Colobane	0	0	2	0	0	0	0	0	0	0	0	2	str	1	2
S.Cubana	0	0	0	0	0	0	5	0	1	0	0	6	str	1	2
S.Dahra	0	0	2	0	0	0	0	0	0	0	0	2	str	1	1
S.Dakar	0	0	1	0	0	0	0	0	0	0	0	1	str	1	1
S.Derby	2	34	0	0	0	52	0	0	3	0	0	91	str,str-sul,str-tet,str-sul-tet,chl-str,str-cip	5	57
S.Drac	0	0	26	0	1	0	0	0	3	0	0	30	str	1	20
S.Dublin	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0

Table 1. Contd.

S.Duisburg	0	0	0	0	0	1	1	0	0	0	0	2	str	1	2
S.Ealing	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
S.Eastbourne	0	1	2	0	0	2	0	0	1	0	3	9	str	1	5
S.Eastglam	0	0	0	0	0	0	0	0	2	0	0	2	0	0	0
S.Elisabethville	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
S.Farakan	0	0	3	0	0	0	0	0	0	0	0	3	str	1	2
S.Freetown	0	0	0	0	0	1	0	0	0	0	0	1	str	1	1
S.Fresno	0	0	1	0	0	4	1	0	1	0	0	7	str	1	3
S.Frintrop	0	0	1	0	0	0	0	0	0	0	0	1	str	1	1
S.Fufu	0	0	1	0	0	0	0	0	0	0	0	1	str	1	1
S.Galiema	0	1	0	0	0	2	0	0	0	0	1	4	str	1	4
S.Gaminara	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
S.Gerland	0	0	0	0	0	0	0	1	0	0	0	1	str	1	1
S.Give	0	0	0	0	0	0	0	0	1	0	1	2	0	0	0
S.Gokul	0	0	1	0	0	0	0	0	0	0	0	1	str	1	1
S.Hato	1	4	5	0	0	22	0	0	0	0	0	32	str, tet,str-tet,sul-tet,amp-str, amp-str-sul-tet-tmp	5	15
S.Havana	0	0	0	0	0	0	0	0	2	0	0	2	0	0	0
S.Hermannswerder	0	0	0	0	0	0	0	0	1	0	0	1	str-sul	2	1
S.Hillingdon	0	0	0	0	0	1	0	0	0	0		1	str	1	1
S.Hvittingfoss	0	0	0	0	0	0	1	0	0	0	0	1	str	1	1
S.Ikeja	0	0	1	0	0	0	0	0	0	0	0	1	str	1	1
S.Ilala	0	0	2	1	0	0	0	0	0	0	0	3	str	1	1
S.Kaapstad	0	0	0	1	0	4	0	0	0	0	0	5	str	1	2
S.Kalamu	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0
S.Kalina	0	0	2	0	0	0	0	0	0	0	0	2	str	1	1
S.Kentucky	0	0	0	0	0	0	1	0	1	0	0	2	amp-str-sul-tet-cip-gen-nal-mec	8	1
S.Kiambu	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
S.Kingston	0	0	2	0	0	3	0	0	0	0	0	5	str	1	2
S.Kokomlemle	0	0	2	0	0	1	0	0	1	0	0	4	str	1	2
S.Korlebu	0	0	2	0	0	0	0	4	1	0	0	7	str	1	5
S.Lagos	0	0	4	0	0	2	0	0	0	0	0	6	str	1	4
S.Llandoff	0	0	0	0	0	0	0	0	1	0	2	3	0	0	0
S.Mbandaka	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0

Table 1. Contd.

S.Minnesota	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
S.Moero	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0
S.Monschaui	2	3	1	0	3	1	0	0	0	0	0	10	str	1	7
S.Montevideo	0	0	0	0	0	0	1	0	1	0	0	2	0	0	0
S.Muenster	0	0	17	3	11	6	4	0	6	0	0	47	str, str-sul, str-tet, str, str-nal, amp-str	5	20
S.Nima	0	1	3	0	0	0	0	0	0	0	0	4	0	0	0
S.Nottingham	0	1	2	0	0	1	0	0	2	0	0	6	str-tet	2	1
S.Offa	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
S.Oranienburg	0	0	1	0	0	0	0	0	0	0	0	1	str	1	1
S.Othmarschen	0	0	1	0	0	0	0	0	0	0	0	1	str	1	1
S.Ouagadougou	0	0	0	0	0	0	0	0	0	0	1	1	str	1	1
S.Ouakam	0	0	0	1	0	0	2	0	0	0	0	3	str	1	2
S.Poona	0	0	2	0	0	1	3	0	2	1	1	10	str	1	5
S.Rissen	0	0	1	0	0	0	0	0	0	0	1	2	0	0	0
S.Rubislaw	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
S.Ruiru	0	0	8	0	0	0	0	0	0	0	0	8	str,str-tet	2	5
S.Group B	0	1	1	0	0	0	2	0	1	0	0	5	str, str-tet, amp-chl--str-sul-tmp	6	4
S.Group C	0	1	1	0	0	9	0	0	0	0	0	11	str, str-sul	2	11
S.Group¤ D	0	0	0	0	0	1	0	0	0	0	0	1	str	1	1
S.Group¤ E	0	0	1	0	0	5	0	0	0	0	0	6	str, str-sul-tet	3	3
S.Group¤ G	0	0	0	0	1	0	0	0	0	0	0	1	str	1	1
S.Group¤ M	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
S.Group¤ O:53	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
S.Saarbruecken	0	1	0	0	0	0	0	0	0	0	0	1	str	1	1
S.Saintpaul	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
S.Salford	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0
S.Schwarzengrund	0	0	1	0	0	3	0	0	1	1	0	6	str	1	3
S.Senftenberg	1	2	0	0	2	8	0	0	0	0	3	16	str, str-tet, str-sul-tet	3	6
S.Shangani	0	0	0	0	0	1	0	0	0	0	0	1	str-sul	2	1
S.Shubra	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
S.Soerenga	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
S.Soumbedioune	0	0	4	0	0	0	0	0	0	0	0	4	str	1	3
S.Stanley	0	0	0	0	0	0	1	0	0	0	0	1	str	1	2

Table 1. Contd.

S.Stanleyville	0	0	0	0	0	1	0	0	0	0	0	1	str-tet	2	1
S.Tamberma	0	0	0	0	0	0	1	0	0	0	0	1	str-tet	2	1
S.Tennessee	0	0	3	0	0	0	0	0	0	0	0	3	str	1	1
S.Teshie	0	0	0	0	0	0	0	0	0	0	1	1	str	1	1
S.Tilene	3	2	1	0	0	0	0	0	1	0	1	8	str	1	7
S.Tounouma	0	0	0	0	0	0	1	0	0	0	0	1	str	1	1
S.Trachau	0	0	1	0	0	1	0	0	0	0	0	2	str	1	2
S.Typhi	0	1	0	0	0	0	1	0	0	0	0	2	str,amp-chl-str-sul-tet-tmp	6	2
S.Typhimurium	0	0	3	0	0	4	13	0	1	0	0	21	str,amp-chl-str-sul-tet-tmp, amp-chl-str-sul-tmp	6	21
S.Umbilo	0	0	1	0	0	0	0	0	0	0	0	1	str	1	1
S.Urbana	0	0	13	2	0	1	0	0	0	0	0	16	str, str-tet, chl-str-tmp	4	11
S.Vilvoorde	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
S.Virchow	0	0	1	0	0	0	2	0	0	0	1	4	str	1	1
S.Waedenswil	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
S.Wagadugu	0	0	0	0	0	0	0	0	0	1	1	2	str	1	2
S.Waycross	0	0	2	0	0	1	0	0	3	0	0	6	str	1	2
S.Yoruba	0	0	1	0	0	0	0	0	0	0	0	1	str	1	1
Salmonella sp.	0	0	1	0	0	0	0	0	0	0	2	3	str	1	2
Total	10	68	161	8	23	193	48	10	56	6	25	608	-	-	359

amp, ampicillin; chl, chloramphenicol; str, streptomycin; sul, sulphonamides; tmp, trimethoprim; tet, tetracycline; nal, nalidixic acid; cip, ciprofloxacin; ftx, cefotaxime; mec, mecillinam.

in beef meat, poultry carcasses, cattle and poultry; *Salmonella* Drac was found in cattle, hedgeghog and fish. S*almonella* Typhimurium was found in cattle, poultry, human and fish. *S*. Typhimurium has been implicated in many outbreaks worldwide (Rayamajhi et al., 2008). This serotype has a well-characterized ability to infect various species (Rabsch et al., 2002) and can survive for a long time in the environment (Baudart et al., 2000); these 2 factors enhance the ability of this serotype to be one of the most common causes of salmonellosis in many geographically diverse regions and has caused many disease outbreaks (Mather et al., 2013; Cui et al., 2008; Galanis et al., 2006).

Although serotype Typhimurium was associated with several foods of animal origin, the most common was chicken (26% of outbreaks), indicating that chicken is a major route of exposure. This corroborates with our present results, where poultry is identified as the primary source of *S*. Typhimurium. In contrast to the report of Foley et al. (2008), Typhimurium was the most common serotype among pork associated with outbreaks. This shows that serotype Typhimurium has emerged as the predominant serotype in swine. After the six most prevalent serotypes, the presence of serotype like Typhi (poultry carcasses and human), Senftenberg, Virchow, Kentucky, Stanley and Kingston which have caused many outbreaks in diverse regions were noted. The presence of *S*. Typhi in poultry carcasses shows that there is a cross-contamination during

carcasses handling since humans are the only reservoir of typhoid *Salmonella*. This is also due to the fact that humans can be a chronic carrier of these *Salmonella* (Eng et al., 2015).

Carriers of *S*. Typhi and *S.* Paratyphi are responsible for the spreading of enteric fever in endemic regions, as the common transmission route is the ingestion of water or food, contaminated with the feces of chronic carriers (Bhan et al., 2005). In this report, *Salmonella* serotypes Drac, Banana, Monschaui, Muenster, Seftenberg are found in wild hedgehogs. This animal has been the source of human salmonellosis in many Europeans countries, used as a pet animal (Nauerby et al., 2000). Although little can be done to prevent the spread of *Salmonella* in the environment amongst wild animals, the control should be done in food handling and drinking water.

Our results show that the same serotype of *Salmonella* is found in diverse sources, meaning that this serotype can be transmitted to humans through many sources. In general, food animals such as swine, poultry and cattle are the prime sources of *Salmonella* infections. The major dissemination routes of the pathogens involve trade in animals and uncooked animal food products. The slaughtering process of food animals at abattoirs is considered one of the important sources of organ and carcass contamination with *Salmonella* (Gillespie et al., 2005). In Burkina Faso, all serotypes of *Salmonella* can circulate between animals, foods, fish, water and humans. This happens as animals shed feces in the environment which rain water carries to rivers, barrages and channel; aquatic animals like fish can be contaminated by diverse microorganisms coming from water. In addition, wastewater and/or untreated animal feces are used to grow salad and other vegetable from gardens. Here, we can see how humans can be contaminated in this closed circle, since animals can contaminate meat, water, fish, vegetables and environment; the consumers are exposed to a high risk of contamination by *Salmonella* and other pathogens.

These findings are critical because Burkina Faso is facing serious problems in the area of water and sanitation due to demographic explosion and poor urban planning. This problem is increasing the risk of environmental and human contamination. In Ouagadougou, the capital city of Burkina Faso, wastewater from channel, where we found many *Salmonella* strains, is used to irrigate vegetables from gardens, and animals' feces are also used in vegetable crops, which is a new source of growth for poor people. Vegetables like salad are not nutritive media for microorganisms but can constitute mechanical vectors for transporting microorganisms to humans if hygienic condition in garden is not maintained well.

Based on our findings, as seen in Figure 1, the *Salmonella* transmission routes are based on their diversity and sources. The diversity of possible reservoirs of infection results in significant challenges for public health authorities to control the infections (Dione et al., 2011). Among the 615 strains, 94% (581/615) were resistant to one or more antibiotics, and resistance to streptomycin was the most common (Table 1). The resistance pattern Str-Sul-Tet, Str-Tet and Str-Sul was dominant and found in 80% of the strains. About 3% of the strains were resistant to 5 or 6 antibiotics; their resistance pattern was amp-str-sul-tet-tmp or amp-chl-str-sul-tet-tmp. One *Salmonella* strain, S. Kentucky isolated from human stool was resistant to eight antibiotics with the pattern, amp-str-sul-tet-cip-gen-nal-mec. This serotype was found also in fish in this study. In contrast, Centre for Disease Prevention and Control (CDC) has reported that *S*. Kentucky is typically found in cattle and poultry (CDC, 2011).

In this study, streptomycin resistance was found in 94% of the *Salmonella* serotypes. Majority of aminoglycosides are bactericidal but *Salmonella* use mechanisms to resist their antibiotic families such as expression of plasmid-mediated aminoglycoside modifying enzymes against aminoglycoside (Gebreyes and Altier, 2002). The genes encoding theses enzymes have been found in varieties of *Salmonella* subtypes like: Agona, Typhimurium, Newport, Typhimurium var. Copenhagen, Kentucky, Blockely, Bredeney, Anatum, Derby, Give, Enteritidis, Heidelberg, Saint Paul, London, Saintpaul, Hadar, Heidelberg, and 4,5,12:i: (Levings et al., 2005). In this study, resistance to ampicillin was observed in *Salmonella* serotypes: Hato, Kentucky, Muenster, Typhi and Typhimurium. Beta-lactams are generally considered bactericidal; but *Salmonella* strains are now becoming resistant to ampicillin and methicillin due to their wide clinical use (Angulo et al., 2000). In *Salmonella*, the secretion of a beta-lactamase is the common mechanism of resistance to beta-lactamases. Several authors reported beta-lactamases in a variety of *Salmonella* serotypes including Enteritidis, Dublin, Haadrt, Anatum, Muenchen, Stanley, Panama, Virchow, and Typhimurium (Gebreyes and Thakur, 2005; Batchelor et al., 2005).

In the present study, *Salmonella* serotypes Derby, Hato, Muenster, Nottingham, Ruiru, Senftenberg, Stanleyville, Tamberna, Typhi, Urbana and Typhimurium were resistant to tetracycline. Resistance to tetracycline has been reported in several serotypes including Typhimurium, Saintpaul, Enteritidis, Hadar and Choleraesuis, Agona, Anatum, Blockley, Bredeney, Colorado, Derby, Give, Haardt, Heidelberg, Infantis, Orion, Seftenberg, (Frech and Schwarz, 2000; Pezzella et al., 2004). *Salmonella* serotypes Derby, Hato, Hermannswerder, Kentucky, Muenster, Senftenberg, Shangani, Typhi and Typhimurium were resistant to sulfonamide in the present study. Many authors have been reported resistance to sulfonamide in a wide range of *Salmonella* serotypes such as Enteritidis, Hadar, Heidelberg, Orion, Rissen, Agona, Albany, Derby, Djugu, and Typhimurium (Antunes et al., 2005; Doublet et al.,

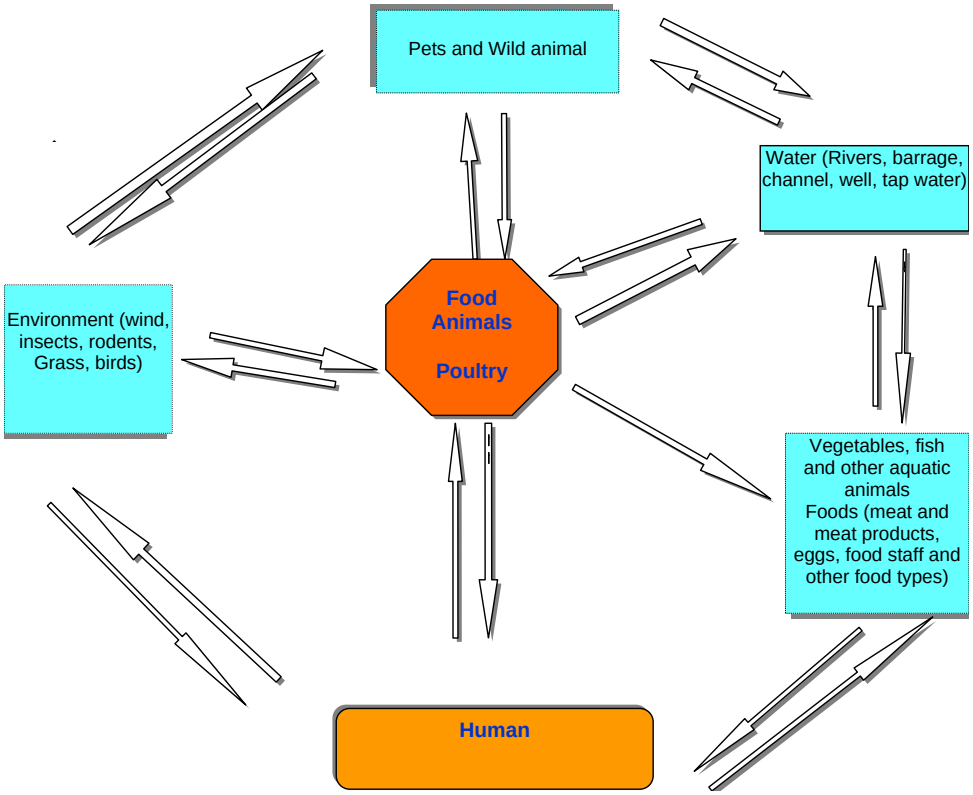

Figure 1. Transmissions routes of Salmonella. Red color = more implicated group in pathogens transmission; Orange color: the second more implicated group.

2004). In our report *Salmonella* serotypes Hato, Urbana, Typhi and Typhimurium were resistant to trimethoprim. Martinez et al. (2005) reported trimethoprim resistance in *Salmonella* serotypes Agona, Djugu, Hadar, Neport, Rissen Albany, Derby, and Typhimirium. Chloramphenicol resistance was found in *Salmonella* serotypes, Derby, Typhi, Typhimurium and Urbana.

This finding corroborates with the report of Alcaine et al. (2005), where *Salmonella* Typhi, Agona, Derby, Kiambo, Albany, Newport, Haardy, Enteritidis and Typhimurium isolates have been found to harbor resistant genes for chloramphenicol. There are two mechanisms in which *Salmonella* resistance to chloramphenicol is conferred: (i) by the plasmid-mediated enzymes called chloramphenicol acetyltransferases (CAT) or nonenzymatic chloramphenicol resistance gene cm1A and (ii) Efflux pump in which the antibiotic is pumped out of the cell. In this study, quinolone resistance was observed in *Salmonella* serotypes Derby, Kentucky, Muenster and Typhimurium. The mechanisms of quinolone resistance for *Salmonella* are all chromosomally mediated, so the numbers of quinolone-resistant *Salmonella* can only increase in two ways: (i) the selection of a quinolone-resistant bacterium after exposure to a fluoroquinolone in humans or animals, or (ii) the spread of a quinolone-resistant bacterium to other animals or to humans (Piddock, 2002). According to CDC, outbreaks caused by antimicrobial-resistant *Salmonella* have been associated with an increased rate of hospitalization, and the rate of death was significantly greater in outbreaks caused by resistant strains (CDC, 2011).

Resistance can spread from non-human sources to human by various routes such as animal, water and contaminated foods (Figure 1). Resistance to combinations of many classes of antimicrobial agents in *Salmonella* has led to the re-emergence of multidrug resistance *Salmonella* (MDR) strains (O'Brien, 2002). In this study, *Salmonella* serotypes, Derby, Hato, Hermannswerder, Urbana, Shangani, Tamberma, Kentucky, Muenster, Senftenberg, Shangani, Typhi and Typhimurium were MDR. This is similar to the findings of many authors, who reported that MDR *Salmonella* strains have been found to be of many serotypes such as Agona, Anatum, Pullorum, Schwarzengrund, Choleraesuis, Derby, Dublin, Heidelberg, Kentucky, Newport, Senftenberg, Typhimurium, and Uganda (Chen et al., 2004; Zhao et al., 2008). All S. Typhimurium reported in the present study were MDR and most of them were found to display a phenotype of resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT). These antibiotics are the

most common drug classes used in veterinary medicine (Mulvey et al., 2006).

Conclusion

The genetic make-up of the *Salmonella* strains permits their adaptation in various environments, including human, animal and non-animal hosts. This increases the difficulty in eliminating the bacteria.

Moreover, the emergence of MDR *Salmonella* strains poses a great challenge in terms of effective treatment of the infections caused by these strains. Several preventive measures have been proposed to stop the spread of antimicrobial resistant *Salmonella* infections, and the restriction of indiscriminate use of antibiotics in food animals is by far one of the most effective measures. This report can help international organization to understand *Salmonella* data and trends and to develop more informed solutions for reducing *Salmonella* contamination along the farm to table chain. The report of sporadic data about *Salmonella* serotype distribution will highlight the importance of the potential source of *Salmonella* infection to humans.

The data obtained in this study can be used by the World Health Organization- Global Foodborne Infections Network (WHO-GFN) and public authority to define the guidelines for basic surveillance system of Salmonella and other enteropathogenic bacteria circulating among humans, animals, food and environment.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

This research was supported by the International Foundation for Science (IFS) grant E/5001-2F/4600 to AK. The efforts of all who contributed to the success of the research are highly appreciated, ranging from conducting of strain characterization and drafting of the manuscript, as well as supervision and participation in writing of the manuscript. All authors read, commented on and approved of the final manuscript.

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Vol. 11(40), pp. 1505-1509, 21 October, 2017
DOI: 10.5897/AJMR2017.8672
Article Number: 7A58E9366529
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Milk ring, rose bengal tests and conventional PCR based detection of *Brucella abortus* infected dairy cattle in Bangladesh

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Received 9 August, 2017; Accepted 28 September, 2017

The objective of this study was to detect dairy cattle infected with *Brucella abortus* from Jamalpur, Rangpur districts and Central Cattle Breeding and Dairy Farm (CCBDF), Savar, Dhaka in Bangladesh. Both milk and serum samples of 510 dairy cattle were initially screened by milk ring test (MRT) and Rose Bengal Test (RBT). Twelve samples positive in both MRT and RBT were further confirmed by conventional polymerase chain reaction (PCR). The overall prevalence of brucellosis based on MRT and RBT was 2.7 and 2.4%, respectively. The prevalence of brucellosis was found to be significantly ($p<0.001$) higher in CCBDF than Jamalpur district. Out of 7 MRT and RBT positive samples, 42.9% samples of CCBDF were PCR positive and none of the five samples of Jamalpur and Rangpur districts was PCR positive. These results strongly suggest that the use of MRT, RBT and PCR technique could lead to more reliable diagnosis of brucellosis from dairy cow in Bangladesh.

Key words: Seroprevalence, molecular detection, brucellosis, dairy cattle, Bangladesh.

INTRODUCTION

Brucellosis is an ancient and one of the world's most widespread zoonotic diseases affecting both, public health and livestock production (Ariza et al., 2007), which is caused by Gram-negative, facultative intracellular bacteria of the genus *Brucella.* Bovine brucellosis is caused almost exclusively by *Brucella abortus,* which is associated with abortion during the last trimester of gestation and production of weak newborn calves, and

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infertility in cows and bulls (Xavier et al., 2009). Bovine brucellosis may also be responsible for retention of placenta and metritis and results in 25% reduction in milk production in infected cows (Acha and Szyfres, 2003; Anonymous, 2006). The overall seroprevalence of bovine brucellosis reported in Bangladesh is 5.3% (4.8 to 6.2) (Ahmed et al., 1992; Amin et al., 2004, 2005; Nahar and Ahmed, 2009; Ahasan and Song, 2010; Rahman et al., 2012; Sikder et al., 2012; Belal and Ansari, 2013; Dey et al., 2013; Islam et al., 2013) and overall prevalence of brucellosis in cows based on milk ring test (MRT) is 5.6% (4.8 to 6.3) (Pharo et al., 1981; Rahman and Rahman, 1981; Rahman et al., 1983; Sikder et al., 2012). *B. abortus* DNA has also been detected from bovine milk and serum samples using real time polymerase chain reaction (PCR) assays (Rahman et al., 2014, 2017) in Bangladesh. However, facilities to perform real time PCR are not widely available in Bangladesh. On the other hand, conventional PCR facility is available in most of the research and educational institutions. The organism is shed in the milk of infected cows. Zoonotic transmission occurs most frequently via unpasteurized milk products in urban settings, while occupational exposure of farmers, veterinarians, or laboratory workers can result from direct contact with infected animals or tissues or fluids associated with abortion (Olsen and Palmer, 2014). In Bangladesh, among the high-risk occupationally exposed people, seroprevalence of brucellosis in humans was reported to be the highest in dairy workers (Rahman et al., 2012). Long time direct contact with infected cows may be responsible for such a higher level of brucellosis among dairy workers as consumption of raw milk is unusual in Bangladesh. So, identification of cows, which shed *B. abortus* in milk, is valuable in culling decision. This study describes the Rose Bengal, MRT and conventional PCR based detection of *B. abortus* infected dairy cattle in Bangladesh.

MATERIALS AND METHODS

Both blood and milk samples were collected randomly from 510 dairy cattle, originating from Jamalpur, Rangpur districts and Central Cattle Breeding and Dairy Farm, Savar, Dhaka. The study was conducted between August and October, 2013.

Milk Ring Test (MRT)

Milk ring test (MRT) on individual milk sample was conducted according to Alton et al. (1988). In brief, antigen (Ring Test reagent, Institut Pourquier, Montpellier, France) was kept at room temperature (18 to 23°C) for 1 h before starting the test. After proper mixing, 1.0 ml of milk sample and 50 µl of MRT of antigen were added in each tube. The milk and MRT reagent was mixed with vortex mixture and incubated for 1 h at +37°C and then between +2 and +8°C for 18 to 20 h. The result was read as positive if the ring of cream is equally or more colored than the underlying milk and as negative if the ring of cream is less colored than the underlying milk.

Rose Bengal Test (RBT)

RBT (Rose Bengal, Institut Pourquier, Montpellier, France) was performed following the procedure described by Alton et al. (1988). The detail description of the test procedure can be found in Rahman et al. (2013).

DNA extraction and PCR

DNA was extracted from milk samples by using Wizard Genomic DNA Purification Kit (Promega, USA) according to manufacturer's instruction and then PCR was performed. Briefly, at first, the required number of PCR tubes were labeled and kept on ice. Then 23 µl of reaction mixture was dispensed into each of the PCR tubes and 2 µl of DNA template from each sample was added to the respective tube and mixed well with the help of the micropipette. The tubes were placed in a 24 wells thermo cycler (Eppendorf, Germany). Then the temperature of the thermo cycler was set according to the following thermal profile. Initial denaturation at 95ºC for 10 minutes, denaturation at 94ºC for 15 seconds, annealing at 54ºC for 1 minute, extension at 72ºC for 1 minute and final extension at 72ºC and 40 cycles for 10 minutes. After completion of PCR, the products were separated by electrophoresis in a 1X TAE 1% agarose gel stained by ethidium bromide. The band was then visualized with a medium wavelength UV light (Figure 1). Primer sequence of alkB genes used in this study was 5'-GCGGCTTTTCTACACGGTATTC-3' (F) and 5'-CATGCGCTATGATCTGGTTACG-3' (R) as per Terzi et al. (2010).

RESULTS AND DISCUSSION

The overall prevalence of brucellosis based on MRT and RBT were 2.7 and 2.4% respectively. The prevalence of brucellosis was significantly ($p<0.01$) higher in CCBDF than Jamalpur district (Table 1). Out of seven MRT and RBT positive milk samples of CCBDF, 42.9% were PCR positive, but all of the five samples that originated from Jamalpur and Rangpur districts were PCR negative (Table 2).

Brucellosis has been recognized as an important zoonotic disease as it hampers both animal production and human health. None of the diagnostic tests available in Bangladesh are perfect, so screening results need to be verified by confirmatory test. In this study, milk and serum samples were screened by MRT and RBT and conventional PCR was used as confirmatory test. The MRT is prescribed by OIE for screening of dairy milk samples. It is very easy to perform, cheap and it gives a good reflection of serum antibody (Nielsen, 2002; OIE, 2009). The RBT is also used as the standard screening test followed by confirmatory testing due to its simplicity, cheap consumables, low equipment requirement and standardized assay (Nielsen and Ewalt, 2010). Individual cow milk was screened by both MRT and RBT in parallel to increase the sensitivity of detecting *Brucella* shedding cows. It was possible to test 12 samples by conventional PCR, which were positive in both tests. Only 42.9% of CCBDF samples were PCR positive and none of the five samples that originated from Jamalpur and Dinajpur

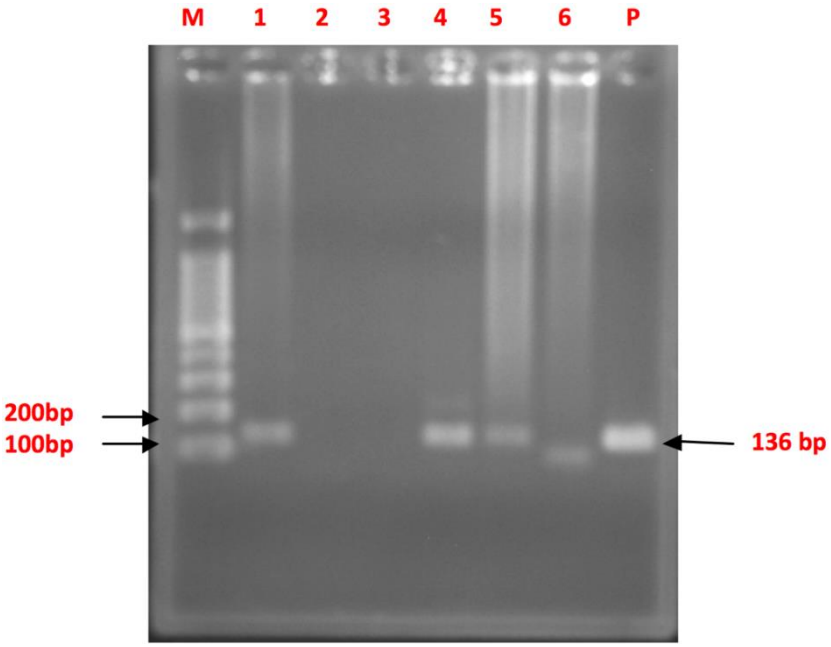

Figure 1. PCR for detection of *B. abortus* from MRT positive milk samples. M, DNA marker (100 bp ladder); 1 to 6 samples; P, positive control. PCR amplicon is analysed in 2% agarose and visualized by Transiiluminator UV Solo, Germany.

Table 1. Breed and district wise prevalence of brucellosis based on MRT and RBT in dairy cattle.

Breed	Positive		Prevalence (%)	
	MRT	RBT	MRT	RBT
Holstein Friesian (n=410)	12	10	2.92	2.43
Sahiwal (n=100)	2	2	2.0	2.0
Fisher's Exact Test P-value	-	-	1	1
District				
Dhaka, CCBDF (n=71)	9	7	12.7	9.9
Rangpur (n=238)	3	3	1.3	1.3
Jamalpur (n=201)	2	2	0.99	0.99
Fisher's Exact Test P-value			<0.001	<0.001
Overall	14	12	2.7	2.4

Table 2. Comparative analysis between MRT, RBT and PCR results.

MRT and RBT positive	Tested	PCR positive	Prevalence (%)
CCBDF	7	3	42.9
Jamalpur and Rangpur districts	5	0	0

districts was positive in PCR. Even being positive in both screening tests, the samples originating from Jamalpur and Rangpur district were negative in conventional PCR, which may be due to low seroprevalence (1.1 to 2.1%) of brucellosis in these areas (Dey et al., 2013). Even if the sensitivity and specificity of a test is very high due to low

prevalence, the positive predictive value of a test may be very low (Rahman, 2015). It is also possible that some milk samples may contain bacteria below the detection limit and failed to be found as positive (O'Leary et al., 2006). Moreover, it is not possible to detect *Brucella* DNA by PCR in majority of the MRT positive samples from cows in their chronic phase of the disease (Terzi et al., 2010). Conversely, the true prevalence and acute infection of brucellosis in CCBDF were reported to be 20.5 and 15.6%, respectively (Rahman, 2015). As a result, more *Brucella* organisms will be shed in milk in this farm increasing the likelihood of detection in PCR. As vaccination against brucellosis in animals was never introduced in Bangladesh, the prevalence indicates natural infection. Identification and culling of acutely infected animals from the population will help to reduce the transmission of the pathogen in animal populations and thereby the zoonotic transmission to humans.

PCR amplification targeting the genus and species-specific genes *alkB* was performed to confirm the presence of *Brucella* DNA in milk samples. Detection of an amplicon of 136 bp confirmed the presence of *B. abortus* DNA. Similar finding was reported by Terzi et al. (2010).

Currently, veterinary diagnostic laboratories utilize MRT for diagnosis of bovine milk samples, which indirectly identifies *Brucella* spp. in the host (Chimana et al., 2010). Just MRT positivity does not indicate acute infection. To declare acute infection evidence of *Brucella* organism or detection of *Brucella* DNA in animal samples is essential (Bricker, 2002; Hamdy and Amin, 2002; Gupta et al., 2006).

The main limitation was that it does not represent diary rich areas like Sirajganj, Chittagong, Satkhira and Munshiganj in Bangladesh. The status of acutely infected dairy cattle of these regions will reveal the importance of this disease in both human and animal health in Bangladesh.

Acute infection of brucellosis in dairy cattle can be determined by MRT, RBT and conventional PCR techniques. This finding will help to cull dairy cattle acutely infected with brucellosis having serious public health hazard.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

Authors are thankful to National Agricultural Technology Project (NATP), Department of Livestock Services, Ministry of Fisheries & Livestock and Ministry of Science & Technology, Government of Peoples Republic of Bangladesh for financial support to first and second authors, respectively.

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