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Isolation, Identification and Characterization of Salmonella Shigella Species Obtained from Cattle Dung and Meat Slabs

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Abstract

Globally, Salmonellosis and Shigellosis continues to be major health problem. They also contribute to negative economic impacts due to the cost of surveillance investigation, treatment and prevention of illnesses.

A total of 67 samples were obtained from different abattoirs in all the four local government areas in Ile-Ife (Ife South, Ife East, Ife central and Ife West). Samples collected from the different abattoirs include; table swab and swab of freshly killed cow faecal dung. The samples were transported into the microbiological laboratory and cultured on pre-enrichment media, after which they were incubated at 370C for 18-24 hours. Colonies were identified by basic identification techniques; and susceptibility profile was evaluated on Muller-Hinton agar using agar diffusion method.

Of the 67 samples collected, the total Gram-negative bacteria isolated was 66, the predominant was Shigella spp. (45.45%) followed by Salmonella spp. (31.82%) and Escherichia coli (22.73%). The Salmonella/Shigella isolates were highly resistant to gentamycin (86.67% and 100% respectively), while the isolates were 100% susceptible to Streptomycin, Pefloxacin and Ciprofloxacin.

Therefore, it can be inferred that Streptomycin, Pefloxacin and Ciprofloxacin antibiotic therapeutic agents will be effective in the treatment of cattle infections caused by Salmonella and Shigella species.

Keywords: salmonella; shigella; infections; antibiotic resistant; antibiotics

Introduction

Food-borne pathogens are the leading cause of illness and death in developing countries, costing billions of dollars in medical care with social costs. Contaminated raw meat is one of the main sources of food-borne illnesses. The risk of the transmission of zoonotic infections is also associated with contaminated meat (WHO, 2006). During the slaughtering operations performed in slaughter places with inadequate sanitation and unskilled staff, contamination frequently occurs from dirt, unclean water, and intestinal contents or from dirty knives, hands or clothing of butcher (WHO, 2006). These factors all leads to infection of meat, bacterial multiplication and possible toxin production. Lack of basic facilities, cross-contamination by insects, flies and numerous other unhygienic factors contribute to the danger of consumer infection (WHO, 2013). There is strong evidence that in many developing countries conditions of slaughter houses and current meat handling practices contribute greatly to the spread of zoonotic diseases. Similarly, lack of ante-mortem, postmortem examination cannot identify the diseased animals so that zoonotic diseases transmit easily by handling raw meat, blood and eating diseased meat (WHO, 1984). WHO estimates that cysticercosis affects some 50 million people worldwide and 1in endemic areas, causes some 50000 deaths?

Salmonellaare the leading cause of bacteremia, 28.5% with Salmonella typhi accounting for 20.9% and non-typhi Salmonella 7.6% (Obaro, 2011). Infections due to Salmonella serotypes continue to be a major health problem (Salehi et al., 2005). Worldwide incidence of shigellosis is estimated to be 164.7 million cases per year of which 163.2 million were in developing countries, where 1.1 million deaths occurred. About 60% of all episodes and 61% of all deaths attributable to shigellosis involved cattle and children below five years. The incidence in developing countries may be 20 times greater than that in developed countries (Jaya et al., 2013). Estimated 30% incidences of these infections are caused by S. dysenteriae with case fatality rates reaching 30% (Jaya et al., 2013).

Salmonellosis is one of the most common and widely distributed food-borne diseases. It constitutes a major public health burden and represents a significant cost in many countries (WHO, 2013). Most Salmonella infections in cattle are subclinical, and shedding of the organism can occur for extended periods. Salmonella can also persist in the farm environment (Huston et al., 2002). Shigella species are highly infective, particularly S. dysenteriae considered the most virulent, and can produce a potent cytotoxin known as ―Shiga toxin‖. Shigella dysenteriae type 1 causes severe and sometimes fatal disease. Shigella species have been recently identified to be the most frequently identified agent of laboratory-acquired infections because of their high virulence and low infectious dose (Peng et al., 2011).

These infections are prevalent in developing countries where lack of clean water supply, lack of proper sewage disposal system and flies aggravate the spread of the diseases (Kasper et al., 2005). Epidemiological surveillance is an essential component in controlling Salmonella and Shigella infections (Sharma et al., 2005). Antimicrobial resistance of Salmonella and Shigella are emerging global challenges, especially in developing countries where there is an increased misuse of antimicrobial agents in humans and animals (Kasper et al., 2005). In most developing countries, laboratory investigations of Shigella and Salmonella are diagnostic challenges due to lack of adequate facilities that enable culture and antimicrobial susceptibility testing (Collee et al., 1999).

In this study, we isolated, identified and characterized Salmonella Shigella species obtained from cattle dung and meat slabs Contaminants, Ile-Ife, Osun State.

Materials And Methods

The study area was Ile-Ife, Osun State, Nigeria. Samples tested include fresh cattle dung and meat slab contaminant from different abattoir settings across all regions in Ile-Ife.

Map Of Osun State Indicating the Study Area

Sample Collection

A total of 67 samples were collected from meat slabs and dung of freshly killed cows using sterile swab sticks and sterile plastic containers. 17 samples were collected from Ife Central L.G.A, 20 from Ife East L.G.A, 15 from Ife North L.G.A, and 15 from Ife South L.G.A. The samples were transported to the Microbiology Laboratory of Obafemi Awolowo University, IleIfe within an hour of collection for bacteriological analysis. The samples were examined immediately upon arrival at the laboratory.

Bacterial isolation, identification and characterisation.

The different bacterial cultures were purified using streak plate method on nutrient agar medium. Using sterilized inoculating loop, a colony was picked up from the spread plate and the loop was dragged over the surface of another plate in a zigzag motion. The loop was sterilized over the flame, the plate was turned 90% and the loop was dragged over the area streaked before in similar manner. The loop was sterilized again, and the same streaking process was repeated. All the plates were incubated at 370C for 24 hours. The heaviest growth was seen in the first sector, and the isolated colonies were in the third sector. This method was repeated several times until purified colony were obtained. The purified bacterial cultures were maintained over Nutrient agar slant. Gram staining, morphological identification, catalase and coagulase tests, sugar fermentation analysis and antimicrobial susceptibility trends of the isolate were conducted.

Results

A total of thirty (30) Shigella isolates and twenty-one (21) Salmonella isolates were isolated from sixty-seven (67) samples

obtained from the abattoir (i.e., dung of freshly killed cow, meat slabs and meat vendor aprons).

Biochemical Characterization of *Salmonella* spp.

	Biochemical tests

Sample code	Catalase	Citrate	Urease	Indole	Gram staining	Triple Sugar Ion				
						Glucose	Sucrose	Lactose	H_2S	Gas
Kc4	+	+	-	-	-	+	-	-	+	-
Mc22	+	+	-	-	-	+	-	-	+	+
Ac4	+	+	-	-	-	+	-	-	-	-
Ac2	+	-	-	-	-	+	+	+	+	-
Hc2	+	+	-	-	-	+	-	-	+	-
Hc4	+	+	-	-	-	+	-	-	-	-
Gc4	+	+	-	-	-	+	-	-	+	-
Ic2	+	+	-	-	-	+	-	-	+	-
Ic4	+	+	-	-	-	+	-	-	+	-
Jc2	+	+	-	-	-	+	-	-	+	-
Hb	+	+	-	-	-	+	-	-	+	-
Fb2	+	+	-	-	-	+	-	-	+	-
Fb3	+	+	-	-	-	+	-	-	+	-
Gb1	+	+	-	-	-	+	-	-	+	-
Ga	+	+	-	-	-	+	-	-	-	-
Ia1	+	+	-	-	-	+	-	-	+	-
Ia2	+	+	-	-	-	+	-	-	+	+
Ma	+	+	-	-	-	+	-	-	-	+
Fa	+	+	-	-	-	+	-	-	+	+
Ea2	+	+	-	-	-	+	-	-	+	-
Ea1	+	+	-	-	-	+	-	-	+	-

Biochemical Characterization of *Shigella* spp.

Sample code	Biochemical tests									
	Catalase	Citrate	Urease	Indole	Gram staining	Triple Sugar Ion				
						Glucose	Sucrose	Lactose	H_2S	Gas
Lc41	+	+	-	-	-	+	-	-	-	-
Lc42	+	+	-	-	-	+	-	-	+	-
Mc21	+	+	-	-	-	+	-	-	+	-
Fc2	+	+	-	-	-	+	-	-	+	-
Fc4	+	+	-	-	-	+	-	-	-	-
Nc2	+	+	-	-	-	+	-	-	+	-
Nc4	+	+	-	-	-	+	+	+	+	-
Ib	+	+	-	-	-	+	-	-	-	-
Cb3	+	+	-	-	-	+	-	-	-	+
Fb1	+	+	-	-	-	+	-	-	+	-
Bb	+	+	-	-	-	+	-	-	+	-
Gb4	+	+	-	-	-	+	-	-	+	-
Cb1	+	+	-	-	-	+	-	-	+	-
Cb2	+	+	-	-	-	+	-	-	+	-
Kb	+	+	-	-	-	+	-	-	+	-
Ha	+	+	-	-	-	+	-	-	+	-
La	+	+	-	-	-	+	-	-	-	-
Na	-	+	-	-	-	+	-	-	-	+
Ba	+	+	-	-	-	+	-	-	-	-
Aa2	+	+	-	-	-	+	-	-	+	+
Gb3	+	+	-	-	-	+	-	-	-	-
Aa4	+	+	-	-	-	+	-	-	+	-

Antibiotic Susceptibility index of Salmonella

Antibiotics	Total isolates	Susceptible n (%)	Intermediate n (%)	Resistant n (%)	Total n (%)
Chloranphenicol	15	14(93.33)	1(6.67)	0(0)	15(100)
Ciprofloxacin	15	15(100)	0(0)	0(0)	15(100)

Amoxicillin	15	10(66.67)	1(6.67)	4(26.67)	15(100)
Augmentin	15	9(60)	2(13.33)	4(26.67)	15(100)
Gentamycin	15	2(13.33)	0(0)	13(86.67)	15(100)
Pefloxacin	15	15(100)	0(0)	0(0)	15(100)
Streptomycin	15	15(100)	0(0)	0(0)	15(100)

Antibiotic Susceptibility Index of Shigella

Antibiotics	Total isolates	Susceptible n (%)	Intermediate n (%)	Resistant n (%)	Total n (%)
Chloranphenicol	19	17(89.47)	2(10.53)	0(0)	19(100)
Ciprofloxacin	19	19(100)	0(0)	0(0)	19(100)
Amoxicillin	19	14(73.68)	3(15.79)	2(10.53)	19(100)
Augmentin	19	14(73.68)	3(15.79)	2(10.53)	19(100)
Gentamycin	19	0(0)	0(0)	19(100)	19(100)
Pefloxacin	19	19(100)	0(0)	0(0)	19(100)
Streptomycin	19	19(100)	0(0)	0(0)	19(100)

Discussion

It has been established that contaminated food is the main source of transmission for many pathogenic bacteria. It is the major cause of enteric diseases in developing countries. Several studies have demonstrated that meat handlers harbour microorganisms especially bacteria asymptomatically (Senthilkumar et al., 2005; Archana et al., 2013). Meat handlers with poor personal hygiene and inadequate knowledge working in establishments like abattoir could be potential sources of infections of many enteropathogenic bacteria (Akagha et al., 2015). Likewise, meat handlers who harbour pathogenic bacteria may contaminate foods with their fingers/aprons during food processing, and finally to infection of consumers (Elhadi, 2014). In this study, cow dung and meat slab culture from meat vendors were investigated for the presence of food-borne Gram-negative bacteria. The result showed positive for public health important bacteria such as Salmonella and Shigella species. Presence of these public health important bacteria from food vendors may pose significant risk on the consumers.

S. typhi is one of the principal causes of food and water borne gastroenteritis in human. The WHO had estimated that 16 million new cases of typhoid fever are recorded annually worldwide. Salmonellosis and shigellosis remain a major public health problem across the globe

(WHO, 2003; Tsen et al., 2000). Similarly, the Centers for Disease Control and Prevention (CDC) estimates that 48 million cases of food-borne illnesses occur in the United States annually. Many of these illnesses are caused by Salmonella spp. and E. coli. The situation is worsened by the production of toxins by E. Coli (Archana et al., 2013; Saleh et al., 2009; Pui et al., 2011).

The high isolation rate of Shigella as compared to salmonella among the bacterial isolates points to the fact that the sanitary practices among the meat vendors are not satisfactory. This is because Shigella organisms do not have any natural reservoirs in animals and can only spread from person to person (Dagnew et al., 2013). In addition, Salmonella species which are flora of intestinal tract of animals and humans, expectedly would have recorded a higher carriage among food handlers.

The antibiotic sensitivity profiles of the bacterial isolates are presented in Table 4.4.1 and 4.4.2. This study shows that all the isolates had strains that were susceptible to Streptomycin, Pefloxacin and Ciprofloxacin. Thus Streptomycin, Pefloxacin and Ciprofloxacin are the most active of all the evaluated antibiotics. The Salmonella/Shigella isolates were highly resistant to gentamycin (86.67% and 100% respectively), while the isolates were 100% susceptible to Streptomycin, Pefloxacin and Ciprofloxacin, which is in agreement with the report of Omololu-Aso et al., (2017) on Salmonellosis and Shigellosis Associated with Cattle Dung Contaminant from Indigenous Abattoirs, Osun State, Nigeria. In their study, they found out that streptomycin and ciprofloxacin antibiotic therapeutic agents will be effective in the treatment of cattle infections caused by Salmonella and Shigella species.

It can therefore be inferred that Streptomycin, Pefloxacin and Ciprofloxacin antibiotic therapeutic agents will be effective in the treatment of cattle infections caused by Salmonella and Shigella species, which is in variance with the work of Niyogi (Niyogi et al., 2010) on changing pattern of serotypes on antimicrobial susceptibility of Shigella sp. isolated from children in Calcutta.

The multi-antibiotic resistance profile of the isolated bacteria is suggestive that they can overcome the likely sanitary norms practiced by the vendors and might originate from the vendors body as frequent antibiotic use might favor development of resistance in bacteria Eruteya et al., 2012.

Conclusion And Recommendation

Isolation of multi-drug resistant public health important bacteria, Salmonella and Shigella from cow dung and meat slabs poses significant risk on the consumers. There is thus, urgent need for applying proper hygienic practices among the food vendors. The use disposable covers for meat slabs is highly recommended as these will restrict transfer and the spread of food borne pathogens.

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