IDENTIFICATION OF SOME ENTERIC BACTERIA FROM DIARRHOEIC STOOL SAMPLES OF PATIENTS ATTENDING MURTALA MUHAMMAD SPECIALIST HOSPITAL KANO, NIGERIA

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ABSTRACT

Enteric bacterial pathogens are one of the major causes of food borne gastroenteritis in humans and remain an important health problem worldwide. The study was intended for the identification of some enteric bacterial isolates from diarrheic stool sample of adult male patients attending Murtala Muhammad Specialist Hospital Kano, Nigeria. A total of 10 diarrheic stool samples were collected from Murtala Muhammad specialist hospital from June to November 2015. Using bacteriological, Gram staining and biochemical characterization 42 isolates (6 genera) were recovered. The result obtained from the data shows that the bacterial isolates found in the stool samples were *Escherichia coli, Salmonella spp, Klebsiella spp, Pseudomonas spp, Citrobacter spp and Shigella spp* with the following prevalence 22%, 19%, 14%, 12%, 14%, 19% respectively. The result of the study implies that *Escherichia coli* has the highest prevalence with 22%, followed by *Salmonella* and *Shigella* with 19% each, while *Pseudomonas spp* has the least occurring with prevalence percentage of 12%. The findings of the research indicated that the enteric bacteria are associated with the diarrheic stool infection.

Keywords: Enteric bacteria, isolates, identification, diarrhea, Pathogens

INTRODUCTION

Food borne diseases are an important cause of morbidity and mortality worldwide. There are over 200 different types of illness that may be transmitted by food. The causes of food borne illness are bacteria, viruses, parasites, and chemicals, and bacterial contamination is the most common cause of illness (Lynch *et al.*, 2006). Most food borne bacterial infections cause self-limiting diarrhoea, however, systemic infection and death can occur, particularly in vulnerable groups such as the elderly, people with diminished immunity or infants and young children (2002; Kennedy *et al.*, 2004). Bacteria have accounted for more than 70% of deaths associated with food borne transmission (Lynch *et al.*, 2006; Hughes *et al.*, 2007)

The family Enterobacteriaceae comprises a large group of Gram-negativenon-spore-forming bacteria typically 1-5 μm in length. They are facultativeanaerobes and with the exception of *Saccharobacter fermenters* and some strains of *Yersinia* and *Erwinia*, they share the ability to reduce nitrate to nitrite. These bacteria are generally motile by peritrichous flagella except for *Shigella* and *Tatumella* and some other non-motile members of this family. For example, *Salmonella* are typically motile, notable exceptions being the *Salmonella* serotypes Pullorum and Gallinarum. A common feature of the Enterobacteriaceae, which helps to differentiate them from other closely related bacteria, is the lack of cytochrome Coxidase, although there are exceptions such as *Plesiomonas* spp. Enterobacteriaceae are catalase positive with the exception of *Shigelladysenteriae* and *Xenorhabdus* species. Enterobacteriaceae ferment a variety of carbohydrates, but their ability to produce acid and gas from the fermentation of D-glucose is one characteristic that remains an important diagnostic property and is commonly used as a basis for their detection and enumeration. Some members of the Enterobacteriaceae (e.g., *Enterobacter* spp., *Escherichia coli*, *Citrobacter* spp. and *Klebsiella* spp.) can be recognized using methods that exploit their ability to ferment lactose rapidly (usually within 24-48 h) producing acid and gas (Tortora and Funke, 2009). Enteric bacteria are microbes that reside in the guts of animals and humans. However there are some among them that reside in intestinal tract of animals that can cause diseases and harsh reactions when human become infected with them (Singh *et al*., 2013). They can cause a mild infection, such as food poisoning or severe community-infections like diarrhea. Such examples of enteric bacteria include *Salmonella*, *Escherichiacoli*, *Shigella*, *Klebsiella*, *Campylobacter*, *Enterobacter Yersinia*, *Vibrio* and *Citrobacter* (Kim *et al*., 2015). The human gut is therefore the natural habitat for various bacteria species and majority of them participate in metabolic activities that salvage energy and absorbable nutrients protecting the colonized host against invasion by alien microbes and important atrophic effects on intestinal epithelia and on immune structure and function.

An estimated 9.4 million food borne illness caused by a known pathogen occur annually in United State (Scallan *et al*., 2011). It has been reported that about 2 million diarrhea disease patients die per year throughout the world (Flint *et al*., 2005). Considering the public importance of acute diarrhea disease, laboratory surveillance of acute diarrhea is utilized in many countries for safety and prevention efforts (Kendall *et al*., 2012). In this

study, we isolate and identified enteric bacterial pathogen in diarrheic stool samples of adult male patients attending Murtala Muhammad Specialist Hospital Kano.

MATERIALS AND METHODS

Stool Sample Collection

Ten (10) diarrheic stool samples were collected from adult male patients using clean, dry and leak proof sterile bottle from Microbiology Department of Murtala Muhammad Specialist Hospital Kano, Nigeria. The specimens were then immediately transported to Microbiology Laboratory in the Department of Microbiology, Kano University of Science and Technology Wudil and refrigerated at 4 ^{0}C before bacterial culture and identification.

Isolation of Bacteria

A sterile wire loop was dipped into stool sample to obtain an inoculum which was streaked on to the surface of plate containing MacConkey agar using standard method of Harley and Prescott, (2005). The procedure was applied for each sample and the plates were incubated at 37 ^{0}C for 24 hours. The presumptive colonies of each isolate on agar plates were further sub-cultured to get pure culture. There covered pure isolates were preserved for further bacterial identification.

Identification of Bacterial isolates

The bacterial isolates were identified based on colonial morphology, cultural characteristics and biochemical test. Colonial morphology and cultural characteristics of plates were made and recorded for the different growth on agar. Gram staining was done for each individual isolates according to method described by Holt *et al.*, (1994) and Sherman, (2005). The isolates were also characterized by biochemical tests viz. IMViC reactions i.e. indole test, Methyl Red test, Vogues Proskauer test and Citrate utilization test, as well as Lactose fermentation Reaction test by standard method given by Sherman, (2005) and Holt *et al.*, (1994).

RESULTS

The Cultural characteristics of the recovered isolates on MacConkey agar is presented in Table 1. The result showed how the recovered isolate were characterized on the basis of colony morphology and staining characteristics. It was observed that all the isolates were Gram negative rods i.e. pink colored and morphologically are small rod in shape which are arranged in single or paired under the microscopic examination. The cultural characteristic ranges from mucoid pink, non-mucoid darker pink, colorless colony with jagged edge, transparent colorless colony and pale yellow colony

Table 1: Cultural characteristics of the recovered isolates

S/N	ISOLATE CODE	COLONY MORPHOLOGY (MacConkey agar plate)
1	S_1	Non-mucoid darker pink colony
2	S_2	Transparent colorless smooth colony
3	S_3	Transparent colorless colony with jagged edge
4	S_4	Mucoid pinkish colony
5	S_5	Pale-pinkish smooth rounded colony
6	S_6	Pale yellow smooth colony

The Biochemical characterization of the recovered isolates is presented in Table 2. The result showed how recovered isolates were characterized on the basis of biochemical identification. Biochemical test include Indole, Methyl-red, Vogues-Proskauer, Citrate utilization test, Lactose fermentation, Oxidase and Catalase test.

Table 2: Biochemical characterization of the recovered isolates

S/N	ISOLATE CODE	IN	MR	VP	CI	LF	OX	CA	SUSPECTED ORGANISM
1.	S_1	+	+	-	-	+	-	+	*Escherichia coli*
2.	S_2	-	+	-	+	-	-	+	*Salmonella spp*
3.	S_3	-	+	-	-	-	-	+	*Shigella spp*
4.	S_4	-	-	+	+	+	-	+	*Klebsiella spp*
5.	S_5	-	+	-	+	+	-	+	*Citrobacter spp*
6.	S_6	-	-	-	+	-	+	+	*Pseudomonas spp*

IN = Indole, MR = Methyl-Red, VP = Vogues-Proskauer, CI = Citrate Utilization, LF = Lactose Fermentation, OX = Oxidase, CA = Catalase.

The number of isolates recovered from each stool sample is presented in Table 3. A total of forty-two (42) isolates were recovered from ten (10) diarrheic stool samples with highest number of isolates in sample 1, 5 and 9 (5 isolates each) while least number of isolates was recorded in sample 2 and 6 (3 isolates each).

Table 3: Number and suspected isolates recovered from each stool sample

S/N	Sample code	No. of isolates	Isolates
1	S_1	5	*E.coli, Salmonella, Shigella, Klebsiella,* and *Citrobacter*
2	S_2	3	*E.coli, Salmonella spp* and *Shigella spp*
3	S_3	4	*E.coli, Salmonella, Shigella* and *Citrobacter*
4	S_4	4	*E.coli, Salmonella, Shigella* and *Pseudomonas*
5	S_5	5	*E.coli, Shigella, Klebsiella,Citrobacter* and *Pseudomonas*
6	S_6	3	*Salmonella spp, Shigella spp* and *Pseudomonas spp*
7	S_7	4	*E.coli, Salmonella spp, Klebsiella,* and *Citrobacter spp*
8	S_8	5	*E.coli, Salmonella, Shigella, Klebsiella,* and*Citrobacter*
9	S_9	5	*E.coli, Salmonella, Klebsiella, Citrobacter, Pseudomonas*
10	S_{10}	4	*E.coli, Shigella spp, Klebsiella spp,* and *Pseudomonas spp*

The Number and percentage occurrence of the isolates recovered is presented in Table 4. The results obtained from the data shows that the bacteria found in the stool samples were *Escherichia coli, Salmonella spp, Klebsiella spp, Pseudomonas spp, Citrobacter spp and Shigella spp* and there prevalence was 22%, 19%, 14%, 12%, 14% and 19% respectively.

Table 4: Number and percentage occurrence of the microorganisms recovered

S/N	MICRO ORGANISM	NO. OF OCCUARANCE	% OCCUARANCE
1	*Escherichia coli*	9	22
2	*Klebsiella spp*	6	14
3	*Salmonella spp*	8	19
4	*Pseudomonas aeruginosa*	5	12
5	*Shigella spp*	8	19
6	*Citrobacter spp*	6	14
	Total	**42**	**100**

DISCUSSION

Enteric bacteria are microbes that reside in the guts of animals and humans. However there are some among them that reside in intestinal tract of animals that can cause diseases and harsh reactions when human become infected with them (Singh *et al*., 2013). They can cause a mild infection, such as food poisoning or severe community-infections like diarrhea. Such examples of enteric bacteria include *Salmonella*, *Escherichia coli*, *Shigella*, *Klebsiella*, *Campylobacter*, *Enterobacter Yersinia*, *Vibrio* and *Citrobacter* (Kim *et al*., 2015). In this study forty two (42) isolates (6 genera) were isolated from 10 diarrhoeic stool samples and

they were characterized on the basis of biochemical identification (Table 3). Biochemical test include Indole, Methyl-red, Vogues-Proskauer, Citrate utilization test, Lactose fermentation, Oxidase and Catalase test. The result obtained from the data shows that the bacteria found in the stool samples were *Escherichia coli, Salmonella spp, Klebsiella spp, Pseudomonas spp, Citrobacter spp and Shigella spp* and there prevalence was 22%, 19%, 14%, 12%, 14%,19% respectively (Table 4). The result of this study also shows that *Escherichia coli* has the highest prevalence with 22%, followed by *Salmonella* and *Shigella* 19% each, while *Pseudomonas spp* has the least prevalence with 12%.

On Gram staining, all the isolates were found to be Gram negative due appearance of red colour. IMViC (Indole, Methyl red, Voges-Proskauer, and Citrate utilization) test was used for identification of enteric bacteria in this study. Bacteriological analysis of the isolates was done using MacConkey agar. MacConkey agar is both selective and differential. The media inhibit the growth of Gram positive bacteria. The medium contain lactose as the sole fermentable source of carbohydrate. Some enteric bacteria ferment lactose while others do not. Colonies of bacteria that ferment lactose produce acid end product and thus the colony will turn pink while non lactose fermenters produce translucent or colourless colony. *E. coli, Klebsiella* and *Citrobacter* were found to be lactose fermenter. *Salmonella, Shigella* and *Pseudomonas* do not ferment lactose.

Similar study was conducted by Obi *et al*. (2007) on enteric bacterial pathogen in stools of residents of urban and rural regions of Nigeria, the results shows the most frequently encountered pathogens in rural area are *E. coli* (28%), followed by *Salmonella* (16%), *Shigella* (14%), *Aeromonas* (9%) and *Campylobacter* (8%). Similarly, the result of this research was inconformity with the study conducted by Kim *et al*., (2015) on enteric bacteria isolated from diarrhea patients in Korea which reveals that *Escherichia coli* was the most prevalent isolated accounted for 22%, this is followed by *Clostridium* 14% and *Salmonella* 13.5%. On the other hand, study conducted by Lopez *et al*., (1996) on Enteropathogenic agents isolated in persistent diarrhea, the result shows that *Salmonella* was the most frequently isolated bacteria, which have similar distribution with *Escherichia coli* and followed by *Shigella*.

LIMITATIONS OF THE STUDY

This study was limited to certain enteric bacterial pathogens and other medically important enteric organisms such as *Campylobacter, Vibrio, Aeromonas* and *Yersinia* were left unidentified due to some constraints.

CONCLUSION AND RECOMMENDATION

Diarrheic stool samples from different patients attending Murtala Muhammad specialist hospital, Kano, Nigeria were tested for identification of enteric bacteria. Isolates were confirmed as *Escherichia coli, Salmonella, Shigella, Klebsiella, Citrobacter and Pseudomonas* by Cultural characteristic, Gram staining and Biochemical tests. This study shows *Escherichia coli* as the most frequent isolate recovered. The findings point out that the enteric bacteria are associated with the infection. We recommended that, health education is

essential to create awareness about food borne infection linked with unhygienic food handling and preparation,

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