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Identification and Evaluation of Antibiotic Sensitivity Pattern of Bacteria Associated with Gastro-Intestinal Disorder in Damaturu Yobe State, 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 aimed to identify and determine antibiotic sensitivity pattern of some bacteria associated with gastro-intestinal disorder among patients attending General Sani Abacha Specialist Hospital in Damaturu Yobe State, Nigeria. A total of thirty (30) sterile swab sticks containing diarrheic stool samples were collected from patients complaining of gastro-intestinal infection attending the hospital were streaked onto the surface of plate containing Nutrient agar using standard method. The bacterial isolates were identified based on Gram staining, cultural characteristics and biochemical test. The bacteria isolates were subjected to antibiotic susceptibility testing using the agar disc diffusion method. The results showed that six different bacteria were isolated and identified namely; *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, Enterobacter, Shigella and Salmonella. The findings of the study on sensitivity pattern of the isolates against antibiotics indicated Sensitivity testing of the isolate against antibiotics indicated that most of the isolates were resistant to rifampicin, streptomycin, gentamicin and chloramphenicol, but sensitive to ciprofloxacin, amoxicillin, norfloxacin and levifloxacin. Findings of this study justify the presence of enteric bacteria as one of the cause of gastric disorder.

Keywords: Bacteria, Identification, Sensitivity pattern, Gastro-intestinal disorder

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 [1]. The causes of food borne illness are bacteria, viruses, parasites and chemicals and bacterial contamination is the most common cause of illness [2]. The human gastrointestinal tract is home to a complex microbial community with more than 100 trillion members of prokaryotic cells. Most food borne bacterial infections cause self-limiting diarrhea, however, systemic infection and death can occur, particularly in vulnerable groups such as the elderly, people with diminished immunity or infants and young children [3]. Bacteria have accounted for more than 70% of deaths associated with food borne transmission [2,4].

Gastrointestinal infections are responsible for a high rate of morbidity in the world, although its highest incidence occurs in the developing countries, according to the World Health Organization (WHO) global health occur [1]. 700 million diarrheal diseases each year and kills 760,000 children under 5 each year for severe dehydration for this reason it is considered the second leading cause of death in the population at this age, even in developed countries, children under 3 years of age have on average 3 diarrhea episodes per year [5]. A broad spectrum of microorganisms may be responsible for the infection and present in common the same clinical manifestations being the most common symptoms diarrhea, vomiting, fever, abdominal pain, malaise, anorexia and dehydration at different levels of severity [6]. The medical diagnosis is usually relatively simple once a correct anamnesis and epidemiological history

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has been made, it is important to perform microbiological laboratory studies to determine the etiological agent responsible for the disease and initiate appropriate treatment [7]. Diarrhea is generally defined as three or more unformed stools evacuations per day and it is often associated with other enteric symptoms [8]. The etiology of diarrhea, abdominal pain, and digestive disorders may be related to more than forty infectious pathogens which could be grouped in viruses, bacteria and parasites [8]. Viral gastroenteritis is a common cause of morbidity and mortality in humans around the world and is considered the most common infectious gastroenteritis. The most common causative agents are Rotavirus, Norovirus, Sapovirus, Adenovirus and Astrovirus [9]. Some bacterial pathogens are able to develop persistent gastrointestinal symptoms and other complications; among the most frequently been identified: *E. coli*, Campylobacter, Salmonella and Shigella [7]. In a study conducted by Nas et al. [10] on identification of some enteric bacterial isolates from diarrheic stool sample of adult male patients attending Murtala Muhammad Specialist Hospital Kano, Nigeria showed the presence of *Escherichia coli*, *Salmonella* spp., Klebsiella spp., *Pseudomonas* spp., *Citrobacter* spp. and *Shigella* spp. with the following prevalence 22%, 19%, 14%, 12%, 14%, 19%, respectively. Considering the public importance of gastro-intestinal infection, laboratory surveillance of such infection is utilized in many countries for safety and prevention efforts [11]. In this, we isolate and identify bacterial associated with gastro-intestinal disorder among patients attending General Sani Abacha Specialist Hospital Damaturu Yobe state Nigeria. The antibiotic sensitivity pattern of the isolates against antibiotics was also evaluated.

MATERIALS AND METHODS

Ethical approval

Ethical approval for the study was obtained from Ministry of Health Damaturu, Yobe State based on the consent of General Sani Abacha Specialist Hospital Damaturu Yobe state, Nigeria Ethical Committee.

Samples collection

A total of thirty (30) sterile swab sticks containing diarrheic stool samples were collected from patients complaining of gastro-intestinal infection attending Microbiology Department of General Sani Abacha Specialist Hospital Damaturu Yobe state, Nigeria. The samples were immediately transported to Biology Laboratory in the Department of Biological Science, Yobe State University Damaturu for bacteria isolation and identification.

Isolation of bacteria

The swab sticks containing stool samples were streaked onto the surface of plate containing Nutrient agar (Life save Biotech, USA) using standard method of Harley and Prescott [12]. The procedure was applied for each sample and the plates were incubated at 37°C for 24 h. 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 Gram staining, cultural characteristics and biochemical test. Gram staining was done for each individual isolates according to method described by Holt et al. [13] and Sherman [14]. Colonial morphology and cultural characteristics of plates were made and recorded for the different growth on MacConkey agar (Life save Biotech, USA). The isolates were also characterized by biochemical tests (indole test, Methyl Red test, Vogues Proskauer test and Citrate utilization test) as well as Lactose fermentation Reaction, Nitrate reduction and motility tests by standard method given by Holt et al. [13]; Sherman [14] and Cheesbrough [15].

Gram staining

A drop of normal saline was placed on a well labeled clean grease-free glass slide using a sterile inoculating loop; a colony of an overnight culture of the bacterial isolate was emulsified with the normal saline to make a thin smear. The smear was air dried and then heat fixed. The slide was flooded with primary stain (crystal violet) for 30 s after which the stain was rinsed from the slide with water. The smear was flooded with Lugolr iodine (mordant) to fix the primary stain. The iodine was rinsed with water after 60 s. The slide was then flooded with a decolorizer (acetone) and rinsed off almost immediately. The counter stain; safranin was added and left for 30 s before being rinsed off. The stained smear was air dried and then observed under the microscope using 100x oil immersion objective lens of the microscope [14].

Indole test

Tryptophan broth was inoculated with an isolate of the test organism and incubated at 37°C for 24 h. About 0.5 ml of Kovack's reagents was added to the broth culture.

Methyl red test

MR-VP broth was inoculated with an isolate of the test organism using sterile inoculating loop and incubated at 37°C for 24 h. About 5 drops of Methyl-red reagent was added to the broth culture.

Voges Proskauer

MR-VP broth was inoculated with an isolate of the test organism using sterile inoculating loop and incubated at 37°C for 24 h. Six milliliters (6 ml) of 5% alpha naphthol was added followed by 0.2 ml of KOH. The tube was shaken gently and remained undisturbed for 5 min.

Citrate utilization test

Simmons's citrate agar was streaked back and forth with inoculums of the test organism and incubated aerobically at 37°C for 24 h.

Nitrate reduction test

Nitrate broth was inoculated with an isolate of the test organism using sterile inoculating loop and incubated at 37°C for 24 h. A dropper full of sulfanilic acid and that of α-naphthalamine were added to the broth.

Motility test

A semi solid medium in a test tube was inoculated with an isolate of the test organism using straight sterile wire and making a single stab at the centre of the test tube. The test tube was incubated at 37°C and examined the stab line at various intervals to determine motility.

Antibiotic sensitivity test

The bacteria isolates were subjected to antibiotic susceptibility testing using the agar disc diffusion method as described by Bauer et al. [16]. Mueller Hilton agar (MHA) (Life save Biotech, USA) plates were inoculated with overnight culture of each isolate by streak plating method. The standard antibiotic sensitivity discs were then aseptically placed at equidistance on the plates and allowed to stand for 1 h. The plates were then incubated at 37°C for 24 h. Sensitivity pattern of the isolates to Augmentin (30 µg/disc), Taravid (10 µg/disc), Streptomycin (30 µg/disc), Ampicillin (30 µg/disc), Gentamicin (20 µg/disc), Reflacin (10 µg/disc), Ceporex (30 µg/disc), Nalixidic acid (20 µg/disc), Ciprofloxacin (10 µg/disc) and Septrin (30 µg/disc), produced by Abtek pharmaceutical limited, were determined. Isolates were divided into three groups based on the zone of inhibition produced by the antibiotic disc; susceptible, intermediately susceptible and resistant according to the Clinical and Laboratory Standards Institute (CLSI) guideline; Performance Standards for Antimicrobial Susceptibility Testing [17]. The experiment was conducted in triplicate and the average value was calculated.

RESULTS

Cultural characteristics of the isolates

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. Colony morphology ranges from mucoid pink, non-mucoid darker pink, colorless colony with jagged edge, transparent colorless colony.

Table 1. Biochemical characterization of bacteria isolated from stool samples.

Isolate code	Gram staining	Cultural characteristics on MacConkey agar
IS_1	Negative/rod	Non-mucoid, dry, flat and darker pink colony
IS_2	Negative/rod	Mucoid pinkish colony
IS_3	Negative/rod	Colorless colony with characteristic foul smell
IS_4	Negative/rod	Produce pinkish colony
IS_5	Negative/rod	Colorless colony with jagged edge
IS_6	Negative/rod	Transparent colorless colony

Biochemical characteristics of the isolates

The biochemical characteristic of the isolates is presented in **Table 2**. The isolates were characterized based on indole, methyl-red, Voges Proskauer, citrate utilization and motility tests. Nitrate reduction test and lactose and fermentation tests were also conducted. The results showed that six different bacteria species were isolated and identified namely; *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, Enterobacter, Shigella and Salmonella.

Table 2. Biochemical characterization of bacteria isolated from stool samples.

Code	IN	MR	VP	CI	LF	NR	MO	CA	Isolates
IS_1	+	+	-	-	+	+	+	+	*Escherichia coli*
IS_2	-	-	+	+	+	+	-	+	*Klebsiella pneumoniae*
IS_3	-	+	-	+	-	+	+	+	*Proteus mirabilis*
IS_4	-	-	+	+	+	+	+	+	*Enterobacter* spp.
IS_5	+	+	-	-	-	+	-	-	*Shigella dysenteriae*
IS_6	-	+	-	-	-	+	+	+	*Salmonella typhi*

Key: IN: Indole; MR: Methyl Red; VP: Voges Proskauer; CI: Citrate; LF: Lactose Fermentation; NR: Nitrate Reduction; MO: Motility Test; CA: Catalase Test

Antibiotic sensitivity test

The zone of inhibition of antibiotic sensitivity disc against the bacterial isolates is presented in **Table 3**. Most of the antibiotics are active against the isolates. *E. coli* is sensitive to norfloxacin, gentamicin, erythromycin, levifloxacin and chloramphenicol. Klebsiella is resistant gentamicin, amoxicillin, rifampicin, ampicillin and chloramphenicol. Proteus mirabilis is resistant to ciproloxacin rifampicin, streptomycin, ampicillin and erythromycin. Enterobacter is sensitive to all antibiotics but resistant streptomycin and rifampicin. Salmonella and Shigella were resistant to most antibiotic tested but sensitive to ciprofloxacin, Amoxicillin, ampicillin and levofloxacin.

Table 3. Mean zone of inhibition of antibiotics against bacterial isolates.

Antibiotics (µg/disc)/Average mean zone of inhibition (mm)										
Isolates	**CIP (10)**	**NOR (10)**	**GEN (10)**	**AMO (20)**	**STR (30)**	**RIF (20)**	**ERY (30)**	**AMP (20)**	**LEV (20)**	**CHL (20)**
Escherichia coli	16	22	21	10	17	10	21	10	20	19
Klebsiella	20	21	10	10	18	10	20	10	19	10
Proteus mirabilis	10	19	10	21	15	10	13	10	18	22
Enterobacter spp.	21	19	22	22	15	15	22	21	20	21
Shigella spp.	20	21	10	20	10	10	10	22	21	10
Salmonella typhi	21	10	10	21	10	10	10	20	19	10

Key: CIP: Ciprofloxacin; NOR: Norfloxacin; GEN: Gentamicin; AMO: Amoxil; STR: Streptomycin; RIF: Rifampicin; ERY: Erythromycin; AMP: Ampicillin; LEV: Levifloxacin; CHL: Chloramphenicol

DISCUSSION

The human gut is natural habitat for various bacteria species and majority of them participate in metabolic activities. 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 [18]. 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 [19].

In the presence study 6 different bacteria were isolated from stool samples of patients complaining of gastro-intestinal disorder. The bacterial isolate include *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, Enterobacter, Shigella and Salmonella. Bacterial were tested for gram staining and biochemical test **(Table 2)**. Bacteriological analysis of the isolates was done using MacConkey agar. MacConkey agar is both selective and differential. 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 colorless colony. *E. coli*, Klebsiella and Enterobacter were found to be lactose fermenter. Salmonella, Shigella and Proteus mirabilis do not ferment lactose. Several studies were conducted to characterize bacteria associated with gastro-intestinal infection.

The finding of this study was in conformity with that of Nas et al. [10] on identification of some enteric bacterial isolates from diarrheic stool sample of adult male patients attending Murtala Muhammad Specialist Hospital Kano, Nigeria which found the presence of *Escherichia coli*, *Salmonella* spp., *Klebsiella* spp., *Pseudomonas* spp., *Citrobacter* spp. and *Shigella* spp. with the following prevalence 22%, 19%, 14%, 12%, 14%, 19%, respectively. Similar study was conducted by Obi et al. [19] 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*, followed by Salmonella, Shigella, Enterobacter and Campylobacter. Similarly, the result of this study correspond to the one conducted others on bacteria isolated from diarrhea patients in Korea which reveals that *Escherichia coli* as one of the most prevalent bacteria, then followed by Clostridium and Salmonella. A study conducted by Lopez et al. [20] on Entero-pathogenic agents isolated in persistent diarrhea, the result shows that Salmonella, *Escherichia coli* and Shigella as the major enteric bacteria responsible for gastric disorder. This finding supported the present study.

On the sensitivity pattern of the isolates against the antibiotics used, most of the antibiotics are active against the isolates. Most of the antibiotics are active against the isolates. *E. coli* is sensitive to norfloxacin, gentamicin, erythromycin, levifloxacin and chloramphenicol. Klebsiella

is resistant gentamicin, amoxicillin, rifampicin, ampicillin and chloramphenicol. *Proteus mirabilis* is resistant to ciproloxacin rifampicin, streptomycin and erythromycin. Enterobacter is sensitive to all antibiotics but resistant streptomycin and rifampicin. Salmonella and Shigella were resistant to most antibiotic tested but sensitive to ciprofloxacin, Amoxicillin, ampicillin and levifloxacin. The result of this study supported the result of a study conducted by Dessalegn et al. [21]. They found that *E. coli* were resistant to ampicillin (100%), Proteus was resistant to ampicillin (100%) and Salmonella isolates were susceptible to ciprofloxacin.

LIMITATIONS OF THE STUDY

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

CONCLUSION

The finding of the present study showed that *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, Enterobacter, Shigella and Salmonella represent some bacteria associated with gastro-intestinal disorder because they reside in intestinal tract of animals that can cause diseases and harsh reactions when human become infected with them. Sensitivity testing of the isolate against antibiotics indicated that most of the isolates were resistant to rifampicin, streptomycin, gentamicin and chloramphenicol, but sensitive to ciprofloxacin, amoxicillin, norfloxacin and levifloxacin. Findings of this study justify the presence of enteric bacteria as one of the cause of gastric disorder.

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