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ENTERIC BACTERIA ASSOCIATED WITH DIARRHOEA AND THEIR SUSCEPTIBILITY PATTERN IN RIVERS STATE

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ABSTRACT

Diarrhoea is an important cause of morbidity and mortality especially in developing countries. Of particular interest in this study are enteric bacteria associated with diarrhoea and their susceptibility pattern in Rivers State. One hundred and twenty stool samples of diarrhoeal patients from the ages of 2 to 60 attending University of Port Harcourt Teaching Hospital and Braithwaite Memorial Specialist Hospital Port Harcourt were collected and inoculated using standard procedures. The result showed *Escherichia coli* constituting the majority of the isolated bacteria forming 50% followed by *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* with 20%, 13.33% and 10% respectively. Antibiotics sensitivity testing of the isolates showed varying degree of resistance and sensitivity. Grouping the antibiotics into those that act against gram positive and negative bacteria, reveal a significantly high level of multidrug resistance (resistance to three or more classes of antimicrobial agents) in 40 (33.33%) isolates, 52 (43.33%) isolates were found to be sensitive to all gram negative antibiotics, 20(16.67%) isolates were found to be sensitive to gram positive antibiotics while 8(6.67%) isolates were found to be sensitive to one of each antibiotics used. The result therefore, proves that *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus and Pseudomonas aeruginosa* are bacteria responsible for diarrhoea disease and there is a high level of multi drug resistance to some commonly used antibiotics for diarrhoea treatment in Rivers State. Public awareness of the dangers of poor hygienic practices, misuse of antibiotics and other causes of diarrhoea should therefore be encouraged to reduce the disease burden.

KEYWORDS: Diarrhoea, Antibiotic resistance, susceptibility pattern, bacteria.

INTRODUCTION

Diarrhoea can be defined as the passage of loose stools three or more times within 24 hour period.[1] It is an important cause of morbidity and mortality in developing countries especially among children less than 5years. It has been estimated globally that there are about 1.7 billion cases yearly resulting in 525,000 deaths in children less than 5years.[2] According to Alabi and his colleagues in 1998, more than 315,000 deaths occurred in pre-school children annually in Nigeria.[3] In 2009, there was an outbreak of cholera, which was an acute form of diarrhoea in 4 Northern States of Nigeria with 260 deaths and in 2010 there were also 2,137 reported new cases and 95 deaths in another cholera outbreak in 9 states in the country of which Rivers State is one of them.[4]

Diarrhoea can be caused by metabolism and organic disturbances, chemical irritations, food and drug intolerance but a vast majority is by infectious pathogens such as bacteria, virus, fungi and parasite but bacterial diarrhoea is more common in tropical and developing countries.[5] In bacteria diarrhoea, the bacteria attaches to the walls of the intestine, producing toxins that attacks, destroys and changes the functions of the mucosal epithelial cells. The causative bacteria are diverse and they include; *Escherichia coli, Salmonella spp, Shigella spp, Camphylobacter jejuni, Vibrio spp, Yersinia spp, Aeromonas spp, Clostridium difficile.*[6]

The common route of infection by these pathogens is through ingestion of food and drinking of water contaminated by faeces and also contact with infected persons. Lack of safe water, basic nutrition and hygiene are major prevailing factors in the disease. World Health Organisation (WHO) in 2015 revealed that water borne disease is a major contributor to the over 4 billion cases of diarrhoea disease annually and 1.8 billion people are currently exposed to sources of water contaminated with faeces.[7]

Symptoms usually appear up to a day after becoming infected, last less than a week and resolves on their own but sometimes last longer when complications occur. The symptoms include fever, abdominal cramps, nausea, headache and bloody or mucus stool, convulsion (seizure) as well as skin rash specific to shigellosis, rice watery diarrhoea (cholera) and foul smelling stool (campylobacter infection).[8] In bloody diarrhoea, suspected cholera or associated sepsis, World Health Organisation recommends the use of antibiotics.[2]

Antimicrobial resistance among enteric Gram negative bacteria is becoming a global public health concern with rapid increase in multidrug resistant microorganisms.[9] There is little information concerning the prevalence of enteric pathogenic bacteria in Rivers State and there has been increasing concerns about the use of three or more antibiotics at different times in diarrhoea treatment without relief.

This study was carried out to determine the enteric bacteria associated with diarrhoea and their susceptibility pattern to antibiotics in Rivers State. These enteric bacteria will be isolated and susceptibility pattern determined using standard procedures. The information obtained from this study will be valuable to the general public and health practitioners in diarrhoea treatment in Rivers State.

MATERIALS AND METHODS

Ethical Approval

Ethical approval was given by the Ethics Committee of the university of Port Harcourt Teaching Hospital and Rivers State Hospitals Management Board, Port Harcourt and written informed consents were obtained from each of the participants before stool specimen were collected from them.

Inclusion and Exclusion criteria

Participants who consented to the study that had signs and symptoms of diarrhoea and have 3 or more loose stools within 24hours were selected for the study while healthy persons who did not have any signs and symptoms of diarrhoea were excluded from the study.

Bacterial isolation

A total of 120 Stool samples from individuals between the ages of 2 to 60 who presented with signs and symptoms of diarrhoea were used for the study. The samples were collected from the University of Port Harcourt Teaching Hospital and Braithwaite Memorial Specialist Hospital Port Harcourt from November 2014 to October 2016. These hospitals are the two major tertiary hospitals in the State and are accessible to people of different ethnic groups. The randomly selected individuals are supposed to represent a sub group of individuals in the State. All samples were inoculated onto MacConkey agar, Salmonella Shigella agar, Selenite F broth, Deoxycholate Citrate agar, Nutrient agar, Xylose Lysine deoxycholate and Thioglycholate Citrate Bile Salt agar. The agar plates were incubated aerobically at 37ºC over night for the growth of pure single colonies and suspicious colonies were collated for identification using standard microbiological procedures.[10]

Antimicrobial susceptibility testing

The susceptibility testing of the isolates to antibiotics were examined by agar diffusion method using paper discs according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[11] Antibiotic susceptibility test was carried out on Mueller Hinton agar using the following antimicrobial agents: Ampicillin 30µg, Septrin 30 µg, Nalidixic acid µg, Ceporex 10 µg, Streptomycin 30 µg, Gentamycin 10 µg, Augumentin 30 µg, Ciproflox 10 µg, Perflacine 10 µg, Tarivid 10 µg, Norfloxacin 10 µg, Amoxil 20 µg, Rifampicin 20 µg, Erythromycin 30 µg, Chloramphenicol 30 µg, Ampiclox 20 µg and Levofloxacin 20 µg. Results of resistance (R) and sensitive (S), were recorded according to British Society for Antimicrobial Chemotherapy.[11]

RESULTS

Enteric bacteria isolated

The result showed that 112 out of the 120 diarrhoeic stool samples collected from individuals between the ages of 2 to 60 were positive for enteric pathogenic bacteria. Sixty (50%) isolates were identified to be *Escherichia coli*, 24 (20%) were *Staphylococcus aureus*, 12 (10%*)* were *Klebsiella pneumonia*, 16 (13.33%) were *Pseudomonas aeruginosa* and 8 (6.66%) samples had no significant growth. *Escherichia coli* was found to be the most prevalent enteric bacteria isolated followed by *Staphylococcus aureus* and then *Pseudomonas aeruginosa* while *Klebsiella pneumoniae* was the least prevalent as seen in figure 1.

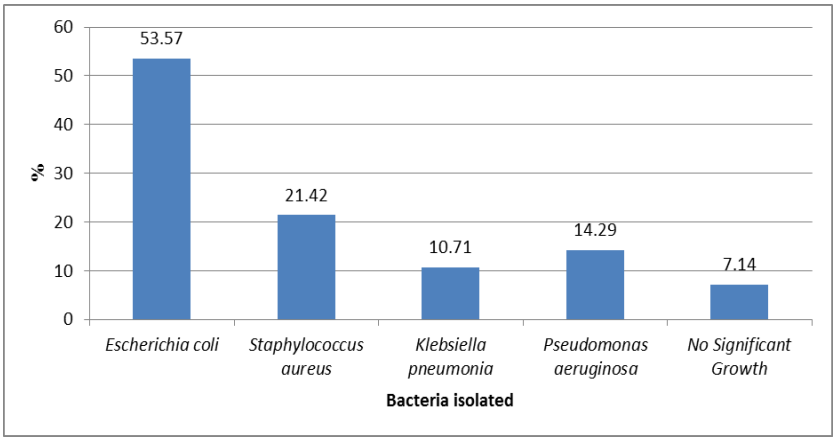

Figure 1: Enteric bacteria isolated using conventional methods.

Susceptibility pattern of bacteria isolated

Antibiotics sensitivity testing of the isolates to commonly used antibiotics revealed that the isolates showed varying degree of resistance to various types of antimicrobial agents used in this study (Table 1). *Escherichia coli* isolates had the highest resistance to erythromycin (60%). *Klebsiella pneumoniae* isolates had 100% resistance to augumentin and ampiclox respectively; *Pseudomonas aeruginosa* isolates had highest resistance to Nalidixic acid (100%) while in *Staphylococcus aureus* isolates had highest resistance to Amoxil and Norfloxacin with 87.5% respectively. *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* isolated from this study were known to be gram negative microorganisms while *Staphylococcus aureus* was the gram positive microorganism. In grouping the antibiotics into those that can act against gram positive bacteria and those that can act against gram negative bacteria (Table 2), a significantly high level of multidrug resistance (resistance to three or more classes of antimicrobial agents) was detected in 40 (33.33%) isolates. Fifty-two (43.33%) isolates were found to be sensitive to all gram negative antibiotics and 20(16.67%) isolates were found to be sensitive to gram positive antibiotics while 8(6.67%) isolates were found to be sensitive to one of each antibiotic used (Figure 2).

Table 1: Antimicrobial susceptibility testing of the isolates.

Antimicrobial agent	*Escherichia coli* (n=60)		*Klebsiella pneumonia* (n=12)		*Pseudomonas aeruginosa* (n=16)		*Staphylococcus aureus* (n=24)	
	S	R (%)	S	R (%)	S	R (%)	S	R (%)
Ciproflox 10µg	28	8(13.3)	12	0(0)	15	12(75)	14	11(45.8)
Norfloxacin 10µg	16	15(25)	0	10(83.3)	9	14(87.5)	10	21(87.5)
Gentamycin 10µg	20	16(26.7)	5	0(0)	13	0(0)	30	14(58.3)
Amoxil 20µg	21	29(48.3)	0	7(53.8)	12	14(87.5)	8	21(87.5)
Streptomycin 30µg	30	7(11.7)	0	11(91.7)	10	2(12.5)	18	3(12.5)
Rifampicin 20µg	2	13(21.7)	0	3(25)	2	9(56.3)	6	12(50)
Erythromycin 30µg	12	36(60)	0	8(66.7)	8	12(75)	5	15(62.5)
Chloramphenicol 30µg	11	31(51.7)	0	7(58.3)	14	15(93.8)	13	17(70.8)
Ampiclox 20µg	13	27(45)	0	12(100)	3	13(81.3)	2	11(45.8)
Levofloxacin 20µg	17	13(21.7)	0	10(83.3)	5	12(75)	23	13(54.2)
Tarivid 10µg	12	25(41.7)	9	0(0)	16	10(62.5)	21	11(45.8)
Rrflacine 10µg	8	6(10)	2	1(8.3)	5	2(12.5)	13	8(33.3)
Augumentin 30µg	30	14(23.3)	11	12(100)	14	15(93.8)	12	8(33.3)
Ceporex 10µg	27	3(5)	8	5(41.7)	10	8(50)	9	9(37.5)
Nalidixic acid 30µg	18	17(28.3)	11	10(83.3)	5	16(100)	15	16(66.7)
Septrin 30µg	26	15(25)	10	11(91.7)	12	12(75)	30	15(62.5)
Amplicin 30µg	30	13(21.7)	12	9((75)	12	15(93.8)	9	19(79.2)

Key:
S – Sensitive
R – Resistant

Table 2: Antibiotics that acted against gram positive bacteria and gram negative bacteria.

Gram positive antibiotics	Gram negative antibiotics
Ciproflox 10µg	Tarivid 10µg
Norfloxacin 10µg	Rrflacine 10µg
Gentamycin 10µg	Ciproflox 10µg
Amoxil 20µg	Augumentin 30µg
Streptomycin 30µg	Gentamycin 10µg
Rifampicin 20µg	Streptomycin 30µg
Erythromycin 30µg	Ceporex 10µg
Chloramphenicol 30µg	Nalidixic acid 30µg
Ampiclox 20µg	Septrin 30µg
Levofloxacin 20µg	Amplicin 30µg

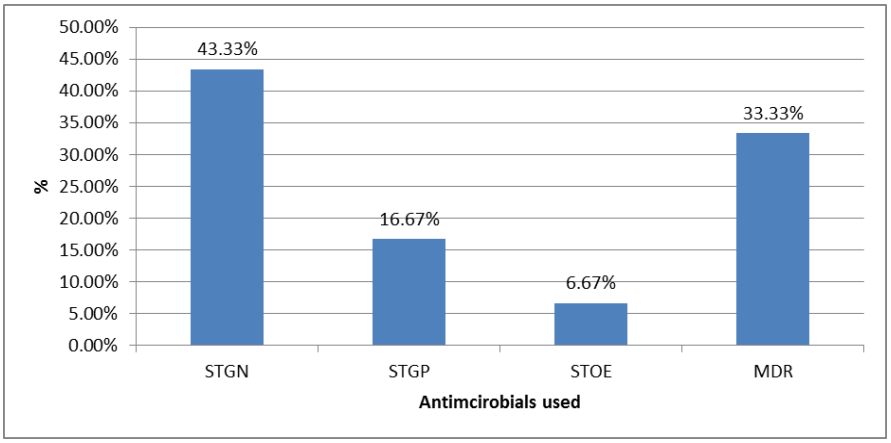

Figure 2: Antibiotic susceptibility pattern of isolates.

Key:
STGP - Sensitive to gram positive.
STGN – Sensitive to gram negative.
STOE – Sensitive to one of each antimicrobial used.
MDR – Multi drug resistant.

DISCUSSION

The primary goal of this study was to isolate the enteric bacteria associated with diarrhoea in Rivers State and their susceptibility pattern. Four types of enteric bacteria were isolated namely; *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The finding showed that *Escherichia coli* 60(50%) were the predominant pathogenic enteric bacteria while *Klebsiella pneumoniae* 12 (10%) were the least isolated in Rivers State. Other bacteria isolated were 24 (20%) of *Staphylococcus aureus*, 16 (13.33%) of *Pseudomonas aeruginosa* and 8 (6.66%) samples had no significant growth. These isolated bacteria have been reported by other researchers. Studies have shown that *Escherichia coli* have been isolated from stool samples of diarrhoeal patients.[12] Also, Chuang and his co-researchers reported *Pseudomonas aeruginosa* to be associated with diarrhoeal disease in children.[13] The report of Claassen-Weitz and his colleagues confirms faecal carriage of *Staphylococcus aureus* in hospital and community settings and *Klebsiella Pneumoniae* has also been reported to be an occasional cause of bloody diarrhoea in humans.[14,15] The result of this work is also supported with that of a study carried out in Nigeria in 2010, which reveals *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus and Pseudomonas species as* highly prevalent bacteria isolated in diarrhoeic stool samples.[16] The high prevalence of *Escherichia coli* may be due to the fact that it is the predominant facultative flora of the human intestine and it is easy to identify to species level. This is in agreement with the statement by Nataro and Kaper in 1998.[17]

In the area of food and water safety, studies have shown that up to 70% of diarrhoea episode are actually caused by water and food contamination with pathogens.[18] Similarly, in 2010, Curtis and his colleagues stated that there is an association between food vending practices and transmission of enteric bacterial pathogens.[19]

Poor sanitation and hygienic practices at home which includes, lack of hand washing and poor disposal of waste generated such as child faeces and domestic waste can also contaminate food and water supply leading to transmission of these enteric bacteria. Studies showed significant positive association between the availability of hand washing facility with reduction of childhood diarrhoea.[20]

Our findings also showed varying degree of resistance to various types of antimicrobial agents used in this study. *Escherichia coli* isolates had the highest resistance to erythromycin (60%), this is in agreement with other studies conducted in different parts of the world.[21-23] *Klebsiella pneumoniae* isolates had 100% resistance to augumentin and ampiclox and *Pseudomonas aeruginosa* isolates had also had 100% to Nalidixic acid while in *Staphylococcus aureus* isolates had highest resistance to Amoxil and Norfloxacin with 87.5% respectively. There have also been reported cases of *Klebsiella pneumoniae* resistance to ampiclox, multiple resistance pattern in *Pseudomonas aeruginosa* and Staphylococcus aureus in other studies.[24-26]

There was a significantly high level of multidrug resistance (resistance to three or more classes of antimicrobial agents) in 40 (33.33%) isolates in this study. Fifty-two (43.33%) isolates were found to be sensitive to all gram negative antibiotics and 20(16.67%) isolates were found to be sensitive to gram positive antibiotics while 8(6.67%) isolates were found to be sensitive to one of each antibiotic used frequently used antibiotics to treat diarrhoea in Rivers state. In Nigeria, high levels of Multidrug Resistance *E. coli* Isolated from Diarrhoeal Stools and Surface Waters has also been reported.[27]

A study by Rupp and Fey in 2003 revealed that some bacteria gain resistance by producing some enzymes known as beta-lactamases that provide multi resistance to beta lactam antibiotics such as penicillin, broad-spectrum cephalosporins and monobactams.[28] ESBL-producing organisms are now increasing among clinical isolates worldwide.

In this study, the gram negative organisms isolated were *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* Of all these organisms isolated, *E.coli* and *Klebsiella pneumoniae* had the highest level of resistance to the gram negative antibiotics. This is in agreement with a report by Kim and his colleagues which reveal that the majority of ESBL producing strains are *Escherichia coli* and *Klebsiella pneumonia.*[29]

The high level of resistance could be as a result of the presence of plasmids that carry some resistance genes in them. According to a report by Hudson and his colleagues in 2014, plasmids are said to be the primary source of resistance genes.[30] Antibiotic resistance is a global public health problem because new resistance mechanisms are emerging and spreading globally threatening the ability to threat common infectious diseases which can result in prolonged illness and death. It also increases the cost of health care and makes therapy ineffective.

The high level of multidrug resistance found among the isolates might be as a result of the hospitals where the stool samples were collected and indiscriminate use of antibiotics by residents of Rivers State. Resistance is selected for the environment where antimicrobial are present in high concentration such as in hospitals and widespread indiscriminate prescribing of antibiotics favours resistance to all common drugs.[31]

Cultural practices and misconceptions to diseases may increase the transmission of the pathogens associated with the disease. Examples of such misconceptions are seeing diarrhoea as a sign of early teeth development, unclean breast milk from the mother and antibiotics are taken to clean the body system. However, these might be the cause of the presence of the enteric pathogens and the high level of multi drug resistance from the diarrhoeic stool in this study.

CONCLUSION

From this study, it can be concluded that *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus and Pseudomonas species* are bacteria responsible for diarrhoea in Rivers State with higher prevalence of the ESBL carrying *E. coli* and *Klebsiella pneumoniae* in our community. Our findings also demonstrated high level of multi drug resistance to the commonly used antibiotics in diarrhoea treatment in Rivers State. That is why it is now of importance that the authorities concerned should improve strategies on infectious disease control in Rivers State through education and proper awareness of transmission of diarrhoea.

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