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Review Article

Prevalence and Multi-Drug Resistance Pattern of Food Poisoning Enteric Bacteria Associated with Diarrhoea Patients

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Abstract: Diarrhoea continues to be a burden especially in developing countries of Africa. However, the treatment of diarrhoea is complicated due to the increase in resistance of enteric and food poisoning bacteria to commonly utilized antibiotics. This work was focused on the identification of enteric bacteria pathogens implicated in cases of diarrhoea orchestrated by food poisoning by use of specialized identification media and biochemical assays. Eight different bacteria species including; *E. coli, S. typhi,* Non-typhoid *Salmonella, Proteus* sp., *Pseudomonas aeruginosa, Citrobacter* sp., *Klebsiella* sp. and Non-Sorbitol utilizing *E. coli* were isolated from diarrhoea patients in Yenagoa, Nigeria with different frequencies. Of all isolates, *E. coli* had the highest frequency of occurrence (29.9%), followed by *Proteus* sp. (20.8%), Non-typhoid *Salmonella* (19.3%) and *S. typhi* (10.7%). Other isolates had frequencies less than 10% respectively. The isolated enteric bacteria were subjected to antibiotics susceptibility assay by the Kirby-Bauer method using Ofloxacin (5µg), Ciprofloxacin (5µg), Gentamicin (10µg), Ceftazidine (30µg), Nitrofuratoin (300µg), Augmentin (30µg), Cefixime (5µg) and Cefuroxime (30µg). All the bacteria isolates assayed showed 100% resistance to Ceftazidine and Cefuroxime while all isolates with the exception of one *Citrobacter* sp. was resistant to augmentin, thus indicating the unsuitability of these drugs in the treatment of diarrhoea. Majority of the bacteria isolates showed multidrug resistance patterns, with *E. coli, Proteus* sp. and *Klebsiella* sp. showing a 100% resistance to the same six (Ceftazidine, Cefuroxime, Gentamycin, Cefixime, Nitrofuratoin and Augmentin) out of the eight antibiotics assayed. This is of public health significance and shows a growing trend of multidrug resistance to commercially available antibiotics utilized in the management of diarrhoea which can cause an increase in morbidity and mortality associated with acute diarrhoea.

Keywords: Antibiotics, Multi-Drug Resistance, Food Poisoning, Diarrhoea

1. Introduction

Food poisoning and Infections caused by enteric pathogens are an important cause of morbidity and mortality worldwide and have a major impact on public health. Several issues underlie the critical danger that is posed by the rise of Multi Drug Resistance (MDR) bacteria associated with food. First and most importantly, outcomes in patients infected with MDR bacteria tend to be worse as compared to patients infected with more susceptible organisms [1, 2] Available reports in Nigeria indicate that more 315,000 deaths of preschool age children are recorded annually as a result of diarrhoea disease [3]. Diarrhoea is an important cause of under nutrition because patient eat less during diarrhoea and their ability to absorb nutrients is reduced. Moreover, nutrient requirement is increased as a result of infection [4].

Globally, diarrhoea is the second leading cause of death amongst children less than five years of age. Of the estimated 5 million deaths in children less than 5 years of age per year, 0.801 million deaths are caused by diarrhoea [5]. About 80% of these deaths occur in children in the first 2 years of life. Approximately one third of deaths among children less than 5 are caused by diarrhoea [6]. Most diarrhoeal illnesses are acute, usually lasting not more than 3-5 days and are

secondary to infectious causes-bacterial, viral, and parasitic. Infectious agents that causes diarrhoeal diseases are usually spread by the faecal-oral route, specifically by ingestion of contaminated food or water and contact with contaminated hands. [7] The prevalence of diarrhoea is directly related to food hygiene, with most incidences of diarrhoea resulting from the consumption of contaminated food and drinks. A variety of enteric pathogens have been linked to diarrhoea and these have been demonstrated to cause food poisoning such as *Shigella* sp. which is a major cause of gastroenteritis, resulting in annual deaths of three to five million in children under the age of five (5) in developing countries [8] For the reduction of morbidity and mortality in cases of acute diarrhoea, antibiotics treatment plays a very vital role. However, [9]. The causes of antibiotic resistance and MDR organisms though occur in many ways are related to inappropriate antibiotic usage. [10] Although a general increase in the number of resistant microorganisms is being reported worldwide, there is considerable variation in the specific patterns and rates of MDR across many countries [11, 12]. Multidrug resistant (MDR) bacteria are well-recognized to be one of the most important current public health problems. This increase in the resistance of common enteric pathogens implicated in diarrhoea to antibiotics especially in developing countries [12] necessitates focused research on the trend of antimicrobial resistance of these pathogens to facilitate the effective selection of appropriate antibiotics that can be utilized in the treatment of diarrhoea. Thus, this research is focused on the isolation of enteric bacteria pathogens associated with diarrhoea patients suffering from food poisoning and investigation of their resistance/sensitivity to commonly administered antibiotics.

2. Materials and Methods

2.1. Collection of Samples

A total of 360 stool samples were collected from individuals with diarrhoea resulting from food poisoning within health care centres in Yenagoa. Samples were collected following routine laboratory procedures in universal bottles. A loopful of sample was inoculated into selenite F broth for pre-isolation of *Salmonella* spp and Macconkey broth and analysed in the Department of Medical Microbiology, Federal Medical Centre-Yenagoa, Bayelsa State immediately.

2.2. Stool Analysis

Samples in selenite F broth were incubated for about 18-20hrs for the recovery of isolates after which broth cultures were inoculated onto *Salmonella–Shigella* agar prepared according to manufactures instructions using the streak plate method and incubated for 24hrs to observe for black centred colonies. Samples in Macconkey broth were inoculated onto Mac-Conkey agar prepared according to the manufacturers instruction using the streak plate method and incubated for 24hrs to observe for both lactose fermenters (LF) and non-lactose fermenters (NLF). Suspected colonies from Mac-Conkey plates were sub-cultured onto various differential media including *Salmonella–Shigella* agar for the identification of presumptive *Salmonella, Shigella, Proteus, Citrobacter* and onto Eosine methylene blue (EMB) agar for the presumptive identification of *E. coli*. Further screening for pathogenic strains of *E. coli* was carried out by inoculating on sorbitol Mac-Conkey agar and incubated for about 18-20hrs. After growth on all media, nutrient agar was used to purify isolates before biochemical identification.

2.3. Biochemical Identification

197 Suspected colonies obtained from the above cultures were screened by means of various biochemical tests to differentiate and identify isolated enteric pathogens based on hydrogen sulphide (H_2S) production, gas production, indole test, urease, sugar fermentation and motility test.

2.4. Antimicrobial Susceptibility Testing

The in-vitro antibiotics sensitivity test for the isolates were determined using the Kirby- Bauer Disc Diffusion Technique and interpreted based on the guidelines of the Clinical and Laboratory Standards [13]. The antibiotics (Abtek biological Ltd) Discs used in the study contained the following antibacterial agents: Ofloxacin (5µg), Ciprofloxacin (5µg), Gentamicin (10µg), Ceftazidine (30µg), Nitrofuratoin (300µg), Augmentin (30µg), Cefixime (5µg) and Cefuroxime (30µg). Oxoid sensitest agar plates were swabbed with cells from the bacteria stock solution, preadjusted to the 0.5 McFarland's turbidity standard. The discs were thereafter, carefully layered on the agar and incubated at 37°C for 24Hrs. Interpretation of the strains as sensitive or resistance were based on zones of inhibition according to current NCCLS standards in accordance with WHO requirements [14].

3. Results

3.1. Stool Culture

The study screened Three hundred and sixty stool samples of stool samples, out of which 197 (54.7%) were confirmed to be positive for presumptive food poisoning enteric bacteria. Bacteriological analysis recovered 197 from the 360 stool specimens of which; 59 (29.9%) were strains of *Escherichia coli* others are *Salmonella* Typhi 21 (10.7%), non-typhoid *Salmonellae* spp 38 (19.3%), *Proteus sp.* 41 (20.8%), *Pseudomonas aeruginosa* 13 (6.6%), *Citrobacter* sp. 9 (4.6%), *Klebsiella* sp. 13 (6.6%) and non-sorbitol utilizing *E coli* 3 (1.5%).

Table 1 shows the distribution of enteric bacteria isolated, indicating possible faecal-oral route of transmission of these pathogens. Furthermore, the distribution of bacteria species points towards several routes of carriership of non-inhabitants of the gastrointestinal tract that could likely cause gastroenteritis including through water and food. These bacteria isolated among diarrhoea patients in the study area is threat to public health.

Table 1. *Distribution of enteric bacteria isolated from asymptomatic careers in Wilberforce Island.*

Organism	Number of positives per bacteria isolated	Frequency (%)
E coli	59	29.9
S. typhi	21	10.7
Non-typhoid Salmonella spp	38	19.3
Proteus sp.	41	20.8
Pseudomonas aeruginosa	13	6.6
Citrobacter sp.	9	4.6
Klebsiella sp.	13	6.6
Non-Sorbitol utilisation E coli	3	1.5
Total	197	100

3.2. Antibiotics Susceptibility Pattern of Enteric Bacteria Isolated

The antibiotic susceptibility assay of the isolated bacteria revealed that all the bacteria were completely resistant to Ceftazidine and Cefuroxime, implying that these antibiotics are not relevant in the management and treatment of infectious diarrhoea diseases caused by these organisms. Other antibiotics show varying degree of susceptibility with ciprofloxacin showing the highest (Table 2).

Table 2. *Antibiotic susceptibility pattern of isolated enteric organism.*

ORGANISM	SENSITIVITY	ANTIBIOTICS							
		CAZ	CRX	GEN	CXM	OFL	NIT	CPR	AUG
S Typhi	Resistant	100	100	100	60	20	100	0	100
	Susceptible	0	0	0	40	80	0	100	0
Non typhi Salmonella	Resistant	100	100	90	80	40	100	40	100
	Susceptible	0	0	10	20	60	0	60	0
E. coli	Resistant	100	100	100	100	65	100	0	100
	Susceptible	0	0	0	0	35	0	100	0
Proteus spp.	Resistant	100	100	100	100	65	100	50	100
	Susceptible	0	0	0	0	35	0	50	0
Pseudomonas spp.	Resistant	100	100	50	100	50	50	100	100
	Susceptible	0	0	50	0	50	50	0	0
Citrobacter spp.	Resistant	100	100	0	100	0	0	0	93
	Susceptible	0	0	100	0	100	100	100	7
Klebsiella spp.	Resistant	100	100	100	100	0	100	50	100
	Susceptible	0	0	0	0	100	0	50	0
Non-Sorbitol utilizing E. coli	Resistant	100	100	0	100	33	100	0	100
	Susceptible	0	0	100	0	67	0	100	0

KEY:
CAZ-Ceftazidine, CRX-Cefuroxime, GEN-Gentamycin, CXM-Cefixime, OFL Ofloxacin,
NIT-Nitrofuratoin, CPR-Ciprofloxacin, AUG-Augmentin S-Sensitivity, I-Intermediate, R- Resistant.

3.3. Multi-Drug Resistance of Isolated Bacteria

Multidrug resistance pattern of food poisoning bacteria isolated from diarrhoea patients as shown in Table 3. Indicates that all the bacteria species isolated were resistant to augmentin, with the exception of one *Citrobacter* isolate which showed limited sensitivity. However, all bacteria isolates without exception were resistant to ceftazidine and cefuroxime. Similarly, all isolates with the exception of *S. typhi* (8) and Non-typhi *Salmonella* (8) were resistant to cefixime.

The highest sensitivity to antibiotics was registered by *Citrobacter* sp. which showed a 100% sensitivity to four out of the eight antibiotics assayed and 100% resistance to only three antibiotics. While the highest resistance to antibiotics was recorded by *E. coli, Proteus* sp. and *Klebsiella* sp. which showed 100% resistance to the same six out of the eight antibiotics assayed. This is followed by *S. typhi* and *Pseudomonas* sp. which recorded 100% resistance to five of the antibiotics assayed.

Table 3. *Multi-drug resistance pattern of bacteria isolates.*

Bacteria	No. of Resistant Isolates	No of Antibiotics Resistant To	Antibiotics
S. typhi	21	5	CAZ, CRX, GEN, NIT, AUG
Non-typhi Salmonella	38	4	CAZ, CRX, NIT, AUG
E. coli	59	6	CAZ, CRX, GEN, CXM, NIT, AUG
Proteus sp.	41	6	CAZ, CRX, GEN, CXM, NIT, AUG
Pseudomonas sp.	13	5	CAZ, CRX, CXM, CPR, AUG
Citrobacter sp.	9	3	CAZ, CRX, CXM
Klebsiella sp.	13	6	CAZ, CRX, GEN, CXM, NIT, AUG
Non-sorbitol utilizing E. coli	3	4	CAZ, CRX, CXM, NIT

4. Discussion

Infectious diarrhoea caused by bacteria is an important cause of morbidity and mortality in infants, young children and patient with compromising immune system in most developing countries including Nigeria [15]. Clarification of the entero-pathogens involved in diarrhoeal disease in the country is an essential step towards the implementation of effective primary health care activities against the disease [16]. This study found eight bacteria species (*Escherichia coli, Salmonella typhi*, non-typhoid Salmonellae*, Proteus* sp., *Pseudomonas aeruginosa*, *Citrobacter* sp., *Klebsiella* sp. and non-sorbitol utilizing *E coli*) which were isolated from diarrhoeagenic subjects in Wilberforce Island, Bayelsa state Nigeria. of the 360 samples taken, the prevalence of isolation of enteric bacteria in Wilberforce island is 54.7% (197 isolates). This study though in contrast to similar study in Abakaliki, south-eastern Nigeria [17] is consistent with the reports of 63.3%-71.83% in Tanzania and 50-60% in other developing countries [18] [3] The prevalence of this enteric pathogens is attributed to the low socioeconomic indices and it could likely increase the incidence of diarrhoea, typhoid and several community-acquired infections especially among pre-school children and those with compromising immune system. The spectrum of bacteria isolated in this study shows *E. coli* and *Proteus* sp. having the highest frequency. But this is not worrisome because they are normal flora of GIT. However, *E. coli* 0157 and *S typhi* which are not normal flora of the GIT could be implicated in gastroenteritis and other food borne infections. Furthermore, inadequate potable water supplies pose a serious socioeconomic problem in many sub urban communities closely linked to Yenagoa metropolis such as Wilberforce island, where inhabitant resort to polluted river water for domestic water consumption [19]. Another study by [20] shows vended food harbours these bacteria, including *E. coli* 0157, and may likely be shed by asymptomatic carriers who are vendors of food and water.

The study went further to evaluate the antibiotic susceptibility of all bacteria strains isolated. The results obtained show varying degrees of resistant to commonly administered antibiotics. Data shows a prevalence of antimicrobial resistance by these strains to the β-lactam class of antibiotics (Amoxycillin-clavulanic acid (Augmentin) which are frequently used empirically for the treatment of diarrhoea. However, all the strains had varying percentage susceptibility to quinolones (Ciprofloxacin, Nitrofurantoin, ofloxacin) and aminoglycoside (gentamycin). With 4 (50%) of isolated bacteria completely resistant to gentamycin and 8(100%) resistant to second and third generation cephalosporins (ceftazidine and cefuroxime). However, the fourth generation has a better susceptibility pattern compared to antibiotics belonging to its class (cephalosporin or beta-lactams). This means that these antibiotics are fairly effective in the treatment of diarrhoea caused by these pathogens but the use of Ciprofloxacin in young children has grave risks. It is noteworthy that the arbitrary empirical use of antibiotics might be responsible for the emerging resistance pattern as shown by this study.

5. Conclusion

Infectious Diarrhoeal diseases are common in socioeconomically poor nations of the world due to favourable environment that promotes the growth of infectious organisms. Practices such as improper waste disposal and unhygienic sanitary practices are rampant leading to the proliferation of pathogenic organisms. These practices can lead to severe water pollution, instigating large–scale outbreaks of disease. Improvement in hygienic conditions would play a significant role in reducing the incidence of diarrhoea causing bacteria infection which can be life-threatening in patients with an underlying disease. The presence of *S typhi,* the causative agent of typhoid fever and non-sorbitol fermenting *E. coli* among diarrhoea patients in Yenagoa is an indication of pathogenic strains of *E. coli* which can result to widespread disease outbreaks of public health significance This study has also further drawn attention to the growing concern of the spread of Multi Drug Resistant strains of enteric bacteria in Yenagoa where there are no restrictions of antibiotic sale and use. The antimicrobial sensitivity pattern of isolates from within Yenagoa is in line with the current trend reported in literature from various parts of the world. Therefore, necessary interventions that will help to control this threat of antibiotic resistance including the control and monitoring of antibiotics usage, provision of potable water, accurate laboratory diagnosis, health education, and detailed epidemiological investigation in Yenagoa and other areas in the state should be of paramount concern.

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