



Determination of antibiotics susceptibility pattern of some Enterobacteriaceae associated with acute diarrhea among children in Kano, Nigeria

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Abstract

The study was aimed to determine the antibiotic susceptibility pattern of some enteric bacteria associated with diarrhea among children in Kano, Northern Nigeria. In the study, a total of two hundred and fifty samples (250) from the study subjects were examined. Enteric bacteria were isolated and identified using conventional methods while the identified isolates were screened for antibiotic susceptibility testing using agar disc diffusion method. A total of 523 Enterobacteriaceae isolates identified were subjected to multi drug resistance (MDR) test, of which 27 (5.2%) isolates were resistant to four or more antibiotics tested while 496 (94.8%) of the isolates were resistant to less than four antibiotics. *E. coli* showed high resistance to streptomycin (63.2%) and chloramphenicol (23.8%). *Salmonella* was resistant to streptomycin (78.3%), gentamicin (61.9%) and erythromycin (33.7%) while *Shigella* spp were resistant to streptomycin (74.6%), gentamicin (76.6%) and ampicillin (54%). On the other hand, *E. coli* demonstrated sensitivity to ciprofloxacin (92.8%), tetracycline (92.8%), augmentin (90.7%) and nalidixic acid (94.8%). *Salmonella* spp was highly sensitive to augmentin (96.8%), nalidixic acid (93.5%), tetracycline (91.3%) and ciprofloxacin (93.5%) while *Shigella* spp was sensitive to nalidixic acid (93.7%), chloramphenicol (93.7%) and ciprofloxacin (83%). The antibiotic resistance exists among enteric bacteria associated with diarrhea in children.

Keywords: Acute diarrhea, Antibiotics susceptibility, Children, Enterobacteriaceae.

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Contribution of this paper to the literature

This is one of the studies to determine the how some medicinal plants namely *Sclerocarya birrea* and *Psidium guajava* stem bark and leaf extracts respectively used against enteric bacteria associated with diarrhea among less than 5 years children in Kano. To the best of my knowledge very few studies with regard to this issue have been conducted in the study area. This study contributed to the existing literature by providing new information in Kano State, Northwestern Nigeria.

1. Introduction

Prescription drug abuse, indiscriminate usage, and dispensation in developing nations are the main causes of the rise of antimicrobial resistance worldwide [1]. Drug-resistant bacteria are emerging and spreading far more quickly than new medications are being used in clinical settings [2]. Millions of lives were saved when antibiotics were developed during World War II, marking a breakthrough in therapeutic treatment. The introduction of penicillin in the 1940s marked the beginning of the "golden age" of antibiotic research, and throughout the next three decades, a number of other classes of antimicrobial drugs with various mechanisms of action were brought to market [3]. In the United States, Enterobacteriaceae that produce Extended spectrum Beta Lactamase (ESBL) were responsible for almost 9,000 human deaths in 2017 [4]. The same year, World Health Organization (WHO) ranked these resistant bacteria in the first priority tier, under the characterization 'critical', to guide research, discovery and development of new antibiotics [5].

World health organization defines diarrhea as the passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individual [6]. Worldwide, diarrheal diseases are reported as the leading cause of mortality among children aged five years and below [7]. In some parts of the world, they account for higher mortality rates than all other causes combined [7, 8]. Children aged five and under suffer from childhood diarrhea, which makes up roughly 63% of the world's diarrhea burden [9, 10] and is the second leading cause of infant mortality in developing countries [11, 12] where inadequate potable water supplies and poor sanitation are major contributing factors [13, 14]. In Africa, Asia, and South America, diarrhea accounts for one in eight deaths among children younger than 5 years per annum [12, 15] and an estimated 16% of child deaths in Nigeria annually [16]. In Ogun State, South-West Nigeria, diarrhea is one of the three diseases (the others being typhoid fever and cholera) which together are the second most prevalent water-related disease [17]. Bacterial diarrheal cases are the most prevalent of all diarrheal cases around the globe [18]. Commonly reported enteric bacterial diarrheal diseases and the causative agents are botulism (*Clostridium botulinum*), *Campylobacter* gastroenteritis (*Campylobacter jejuni*), cholera (*Vibrio cholerae*), *Escherichia coli* gastroenteritis, Salmonellosis (various *Salmonella* serovars), *Shigellosis* (*Shigella* spp.), and Staphylococcal food poisoning (*Staphylococcus aureus* enterotoxins) [19, 20].

The genetic composition of the bacterium is the foundation of all resistance. Changes in gene expression, modifications to already-existing genes, or the acquisition of additional genes through a process known as "horizontal gene transfers" can all help bacteria develop resistance. Transduction, transformation, and conjugation are the three processes by which horizontal gene transfer occurs. Transformation is the direct intake of genetic material, including plasmids, from the environment by "competent" bacteria, whereas transduction is the transfer of DNA mediated by bacteriophages and transformation is direct uptake of genetic material, including plasmids from the environment by 'competent' bacteria [21, 22]. The study was aimed to determination of antibiotics susceptibility pattern of some Enterobacteriaceae associated with acute diarrhea among children in Kano, Northern Nigeria.

2. Materials and Methods

2.1. Study Sites

The samples for the study were collected from Microbiology Department of Murtala Muhammad Specialist Hospital (MMSH), Danbatta General Hospital, Wudil General Hospital while bacteriological analysis of the samples was conducted at the Laboratory of Microbiology Department of Aminu Kano Teaching Hospital Kano. Kano State is located in the North-western Nigeria, it is coordinated at latitude 11° 30' N and longitude 8° 30' E [23]. It has a total area of 20,131km² (7,777sqm) and estimated population of 13.4 million [24].

2.2. Ethical Consideration

An approval for the study (Reference Number: NHREC/17/03/2030) was obtained from Research and Ethic committee of Ministry of Health Kano State in conjunction with the approval from Ethical Committee of Murtala Muhammad Specialist Hospital (MMSH), Danbatta General Hospital and Wudil General Hospital. The aim of the study was explained clearly to the clients and informed consent obtained before commencing the study [25].

2.3. Determination of Sample Size

Sample size for the study was determined from a standard formula for the calculation of minimum sample size. Sample size was calculated using the formula;

$N = (Z_{1-\alpha})^2 (p) \times (1-p) / d^2$ as described by Omole, et al. [17] where;

N = minimum sample size, $Z_{1-\alpha}$ = Value of standard normal deviate which at 95% confidence interval was found to be 1.96.

p = the best estimate of prevalence obtained from literature review (18.8%) [26] and

d = difference between the true population rate and sample that can be tolerated, this is the absolute precision (in percentage) on either side of the population = 0.05

$$N = (1.96)^2 \times (0.188) \times (1-0.188) / (0.05)^2 = 0.5864 / 0.0025 = 234.56$$

Which is approximately equals to 235, is the minimum number of samples for the study. Therefore, a total of 12 subjects accounted for 5% [27] of the minimum number of subjects were added to the research for attrition, making a total of 250 samples.

2.4. Samples Collection

Two hundred and fifty (250) patients receiving treatment for diarrhea at three major health facilities in Kano State—Murtala Muhammad Specialist Hospital (MMSH), Danbatta General Hospital, and Wudil General Hospital—were included in this study. Samples were collected using a sterile universal container. Patients' diarrhea samples were collected, and using conventional microbiological procedures, they were processed for bacterial isolation and identification [28]. All of the gathered samples were sent right away, in aseptic settings, to the Murtala Muhammad Specialist Hospital's Microbiology Laboratory for identification and isolation.

2.5. Isolation and Identification of Bacteria

Enteric bacterial isolation was carried out using the Cheesbrough [28]. A sterile wire loop was dipped into the patients' feces sample, streaked onto the Salmonella-Shigella agar (Biomark, India) and MacConkey agar (Life Save Biotech, USA) plates, and then incubated aerobically at 37° C for 24 hours. Bacterial growth was monitored for colony appearance and morphology following incubation. Until a pure colony was achieved, each colony was re-inoculated into newly made agar plates. As explained by Cheesbrough [28] each pure colony was identified by Gram staining it and then undergoing other biochemical tests like indole, methyl-red, Voges Proskauer, citrate utilization, and motility test.

2.6. Multi Drug Resistance (MDR) Screening

The bacteria isolates were subjected to antibiotic susceptibility testing using the agar disk diffusion method, as described by Bauer, et al. [29]. Mueller-Hinton agar plates were inoculated with an overnight culture of each isolate by streak plating. The standard antibiotic sensitivity discs were aseptically placed at equidistance on the plates and allowed to stand for an hour. The plates were incubated at 37°C for 24 hours. Sensitivity pattern of the isolates to different antibiotics belonging to different classes was determined. Isolates were divided into two groups based on the zone of inhibition produced by the antibiotic disc; susceptible, and resistant according to the Clinical and Laboratory Standards Institute guideline; performance standards for antimicrobial susceptibility testing [30]. Isolates that were observed to be resistant to at least four different classes of antibiotics were classified as being multidrug resistant [31].

3. Results

3.1. Distribution of Bacteria Isolated from Study Subjects

The distribution of the bacteria isolated from the stool samples of the subjects is presented in Table 1. The result showed that *E. coli* has the highest frequency with total of 193 occurrence (36.9%), followed by *Salmonella* with total frequency of 92 (17.6%), then *Shigella* with frequency of 63 (12%). The number of isolates recorded by *Citrobacter* and *Serratia* in this study were 28 (5.4%) and 21 (4%) respectively.

Table 1. Distribution of bacteria isolated from study subjects.

S/N	Isolates	No. of isolates	Percentage (%)	P-value
1	<i>E. coli</i>	193	36.9	0.00001*
2	<i>Salmonella</i>	92	17.6	
3	<i>Shigella</i>	63	12.0	
4	<i>Klebsiella</i>	44	8.4	
5	<i>Proteus</i>	39	7.5	
6	<i>Enterobacter</i>	43	8.2	
7	<i>Citrobacter</i>	28	5.4	
8	<i>Serratia</i>	21	4.0	
	Total	523	100	

Note: * = There is statistical significant difference in the number of bacteria isolates isolated in the study. Hence, the result is significant at $p < 0.05$.

3.2. Antibiotic Susceptibility of the Isolate

The resistivity profile of the isolates to some commonly used antibiotics is presented in Table 2. The result showed that *E. coli* showed high resistant to streptomycin (63.2%) and chloramphenicol (23.8%). *Salmonella* was resistant to streptomycin (78.3%), gentamicin (61.9%) and erythromycin (33.7%) while *Shigella* spp were resistant to streptomycin (74.6%), gentamicin (76.6%) and ampicillin (54%). On the other hand, *E. coli* demonstrated sensitivity to ciprofloxacin (92.8%), tetracycline (92.8%), augmentin (90.7%) and nalidixic acid (94.8%). *Salmonella* spp was highly sensitive to augmentin (96.8%), nalidixic acid (93.5%), tetracycline (91.3) and ciprofloxacin (93.5%) while *Shigella* spp was sensitive to nalidixic acid (93.7%), chloramphenicol (93.7%) and ciprofloxacin (83%). From the result, other isolates (*Klebsiella*, *Proteus*, *Enterobacter*, *Citrobacter* and *Serratia*) showed less resistance to the tested antibiotics. Based on the activity of the antibiotics, ciprofloxacin, augmentin and nalidixic acid were the most effective antibiotics while streptomycin, gentamicin and ampicillin were less effective

Table 2. Antibiotic susceptibility of the isolate.

Isolates	AMP	AUG	NA	ERY	GN	CHL	STR	NOR	TET	CIP
<i>E. coli</i> n = 193	34 (17.6%)	18 (9.3%)	10 (5.2%)	33 (17.1%)	35 (18.1%)	46 (23.8%)	122 (63.2%)	42 (21.7%)	14 (7.2%)	14 (7.2%)
<i>Salmonella</i> n = 92	11 (12%)	3 (3.2%)	6 (6.5%)	31 (33.7%)	57 (61.9%)	28 (30.4%)	72 (78.3%)	28 (30.4%)	8 (8.7%)	6 (6.5%)
<i>Shigella</i> n = 63	34 (54%)	22 (35%)	4 (6.3%)	26 (41.3%)	47 (76.6%)	4 (6.3%)	47 (74.6%)	29 (46%)	24 (38%)	11 (17%)

Isolates	AMP	AUG	NA	ERY	GN	CHL	STR	NOR	TET	CIP
<i>Klebsiella</i> n = 44	4 (9%)	2 (4.5%)	2 (4.5%)	4 (9%)	6 (13.6%)	2 (4%)	3 (6.8%)	3 (6.8%)	3 (6.8%)	2 (4%)
<i>Proteus</i> n = 39	3 (7.7%)	2 (5.1%)	2 (5.1%)	5 (12.8%)	4 (10.3%)	3 (7.7%)	6 (15.4%)	11 (28.2%)	2 (5.1%)	2 (5.1%)
<i>Enterobacter</i> = 43	15 (35%)	5 (11.6%)	11 (25.6%)	6 (14%)	10 (23.3%)	14 (32.6%)	16 (37.2%)	3 (7%)	4 (9.3%)	2 (4.7%)
<i>Citrobacter</i> n = 28	4 (14.3%)	1 (3.6%)	7 (25%)	7 (25%)	3 (10.7%)	6 (21.4%)	10 (35.7%)	6 (21.4%)	4 (14.3%)	2 (7.1%)
<i>Serratia</i> n = 21	5 (23.8%)	1 (4.8%)	3 (14.3%)	3 (14.3%)	4 (19%)	6 (28.6%)	4 (19%)	7 (33.3%)	3 (14.3%)	2 (9.5%)

Note: AMP = Ampicillin (30 µg/disc), AUG = Augmentin (10 µg/disc), NA = Nalidixic acid (30 µg/disc), ERY = Erythromycin (10 µg/disc), GN = Gentamicin (20 µg/disc), CHL = Chloramphenicol (10 µg/disc), STR = Streptomycin (30 µg/disc), NOR = Norfloxacin (30 µg/disc), TET = Tetracycline (10 µg/disc), and CIP = Ciprofloxacin (10 µg/disc).

3.3. Distribution of Multi Drug Resistant (MDR) Isolates from Study Subjects

The determination of Multi Drug Resistant (MDR) isolates is presented in Table 3. The result showed that 523 Enterobacteriaceae isolates identified were subjected to multi drug resistance (MDR) test, of which 27 (5.2%) isolates were resistant to four or more antibiotics tested while 496 (94.8%) of the isolates were resistant to less than four antibiotics.

Table 3. Distribution of multi drug resistant (MDR) isolates from study subjects.

Isolates	No. identified	Percentage (%)	P-value
MDR	27	05.2	0.00001*
NMDR	496	94.8	
Total	523	100	

Note: * = There is statistical significant difference in the number of MDR and NMDR isolates tested in the study. Hence, the result is significant at $p < 0.05$, MDR = Multi-drug resistant, NMDR = Non-multi-drug resistant.

3.4. Antibiotic Resistance of Isolates to Specific Number of Antibiotics

The antibiotic resistivity pattern of the isolates is presented in the Table 4 and 5. The result showed that a total of 12 isolates were resistant to 4 different antibiotics, while 7 were resistant to 5 different antibiotics, four (4) isolates were resistant to 6 different antibiotics while 2 were resistant to 7 different antibiotics. From the result, only 1 isolate was resistant to 8 and 9 antibiotics respectively. Most of the isolated showed resistant to ampicillin, erythromycin, gentamicin and streptomycin.

Table 4. Antibiotic resistance of isolates to specific number of antibiotics.

S/N	Antibiotic resistant to (n)	Number of isolates (n)	Percentage (%)
1	9	1	3.7
2	8	1	3.7
3	7	2	7.4
4	6	4	14.8
5	5	7	26.0
6	4	12	44.4
	Total	27	100

Table 5. Antibiotic resistance pattern of the isolated bacteria.

Isolate code	Antibiotics resistant to (n)	Resistance pattern
EC ₁	4	ERY, GN, CHL, STR
EC ₄	4	CHL, STR, NOR, TET
EC ₇	4	NA, ERY, GN, CHL
EC ₉	4	ERY, GN, CHL, NOR
EC ₁₀	4	ERY, GN, CHL, STR
EC ₁₁	4	NA, ERY, GN, CHL
EC ₃	5	AMP, ERY, GN, CHL, STR
EC ₆	5	NA, ERY, CHL, STR, TET
EC ₂	6	AMP, ERY, GN, CHL, STR, NOR
EC ₈	6	AMP, ERY, GN, STR, NOR, CIP
EC ₅	7	AMP, NA, ERY, GN, CHL, STR, NOR
KL ₁	5	AMP, ERY, GN, CHL, STR
SA ₂	4	ERY, GN, CHL, NOR
SA ₅	4	GN, CHL, STR, NOR
SA ₇	4	GN, CHL, STR, NOR
SA ₈	4	CHL, STR, NOR, TET
SA ₄	5	NA, ERY, GN, CHL, TET
SA ₆	5	AMP, AUG, GN, CHL, STR
SA ₁	6	AMP, AUG, GN, CHL, STR, TET
SA ₃	7	AMP, ERY, GN, CHL, STR, NOR, TET
SH ₁	4	ERY, GN, CHL, STR
SH ₃	4	ERY, GN, CHL, STR
SH ₅	5	ERY, GN, CHL, STR, TET
SH ₇	5	NA, ERY, GN, CHL, NOR
SH ₆	6	AMP, NA, ERY, GN, CHL, STR,
SH ₂	8	AMP, NA, ERY, GN, CHL, STR, NOR, TET
SH ₄	9	AMP, AUG, NA, ERY, GN, CHL, STR, NOR, TET

Note: EC = *E. coli*, KL = *Klebsiella* spp, SA = *Salmonella* spp, SH = *Shigella* spp, AMP = Ampicillin, AUG = Augmentin, NA = Nalidixic acid, ERY = Erythromycin, GN = Gentamicin, CHL = Chloramphenicol, STR = Streptomycin, NOR = Norfloxacin, TET = Tetracycline, and CIP = Ciprofloxacin.

4. Discussion

The result showed that *E. coli* showed high resistant to streptomycin and chloramphenicol. *Salmonella* was resistant to streptomycin, gentamicin and erythromycin. On the other hand, *Shigella* spp were resistant to streptomycin, gentamicin and ampicillin. From the result, other isolates (*Klebsiella*, *Proteus*, *Enterobacter*, *Citrobacter* and *Serratia*) showed less resistance to the tested antibiotics. Based on the activity of the antibiotics, ciprofloxacin, augmentin and nalidixic acid were the most effective antibiotics while streptomycin, gentamicin and ampicillin were less effective. In the present study, *E. coli* was sensitive to ciprofloxacin, tetracycline, augmentin and nalidixic acid. Finding of this study was in conformity with the finding of Ansari, et al. [32] who reported the sensitivity of *E. coli* (isolated from stool samples of children infected with diarrhea) to tetracycline and resistivity to ampicillin. The result of this study was also in agreement with the study of Rathaur, et al. [33] who reported ciprofloxacin was highly effective against *E. coli* and most of isolates were resistant to Ampicillin and Al-Gallas, et al. [34] found 71.4% isolates sensitive to Tetracycline and 20.4% isolates resistant to Ampicillin. More than half of all Enterobacteriaceae were resistant to ampicillin [35].

Salmonella spp was highly sensitive to augmentin, nalidixic acid, tetracycline and ciprofloxacin while *Shigella* spp was sensitive to nalidixic acid, chloramphenicol and ciprofloxacin. The result of the present study was in conformity with the finding of Karambu, et al. [36] who reported sensitivity to Nalidixic acid and ciprofloxacin and resistivity to ampicillin by enteric isolated from diarrhea infected children in Kenya. Similar resistance to amoxicillin by enteric bacteria associated with diarrhea in children was revealed in a study done in Abuja, Nigeria by Ifeanyi, et al. [37]. Mandomando, et al. [38] reported 85% isolates were sensitive to Tetracycline and 62% isolates of *Salmonella* spp. were resistant to Ampicillin. According to Patel, et al. [39] 10% of the *Salmonella* isolates were resistant to Ampicillin and 7.1% were resistant to Nalidixic acid. The resistance to ampicillin in this finding is much lower than our study. This may be due to empirical use of these antibiotics and development of resistance.

This finding also confirmed the work of Bodhidatta, et al. [40] in Thailand, who reported high susceptibility of *Shigella* spp. to ciprofloxacin. According to a similar study conducted by Abdullahi [41] in Kano, Nigeria, *Shigella* spp. were highly susceptible to nalidixic acid, which is consistent with our study. However, this conclusion is contrary to that of Ansari, et al. [32] and Das, et al. [42] who both reported increased resistance of *Shigella* spp to nalidixic acid. The resistance of some isolates to antibiotics may be attributed to their indiscriminate use in the population due to the broad-spectrum antimicrobials, which are more commonly used for various infections. Resistance of enteric pathogens to currently used antimicrobial agents has increased worldwide due to the widespread use of antimicrobials [43].

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The overall resistance of the isolates to the antibiotics present was very low, as only 5.2% (27 out of 523) of the isolates were multidrug resistant (MDR) isolates, i.e. resistant to four or more antibiotics tested, while 496 (94.8%) isolates were resistant to fewer than four antibiotics. This figure (5.2%) in this study was lower than that found in Bangladesh [44] which reported 15%, and Iran [45] which reported less than 30% MDR among enteric bacteria associated with diarrhea in children. The lower prevalence of MDR among enteric bacteria in this study may be attributed to the fact that most children did not have a history of antibiotic use. The prevalence of MDR strains of pathogenic bacteria may be an important cause of serious and prolonged health problems in children with diarrhea. The rapid and uncontrolled increase in the use of antibiotics as treatment for humans and as feed and treatment options for animals has significantly contributed to the growth and spread of MDR, which is confirmed by previous studies [44, 46].

5. Conclusion

Findings of the study reveal that 523 Enterobacteriaceae isolates identified were subjected to multi drug resistance (MDR) test, of which 27 (5.2%) isolates were resistant to four or more antibiotics tested while 496 (94.8%) of the isolates were resistant to less than four antibiotics. Total of 12 isolates were resistant to 4 different antibiotics, while 7 were resistant to 5 different antibiotics, four (4) isolates were resistant to 6 different antibiotics while 2 were resistant to 7 different antibiotics. From the result, only 1 isolate was resistant to 8 and 9 antibiotics respectively. It is recommended that monitoring and regular surveillance are necessary especially among the physician's knowledge on the updated and more effective empirical treatment of the disease.

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