ANTIBIOTIC RESISTANT BACTERIA IN FAECAL SAMPLES OF APPARENTLY HEALTHY INDIVIDUALS IN ADO-EKITI, NIGERIA

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ABSTRACT

The occurrence of antibiotic-resistant bacteria in faeces of apparently healthy individual volunteers was investigated. Faecal samples were collected from 216 individuals comprising 138 adults (70 males and 68 females) and 78 children aged between 4 months and 42 years (mean age was 30.2 months). Individuals on antibiotics were excluded from the study. Faecal samples were examined microbiologically for the presence of bacteria resistant to commonly employed antibiotics. Three hundred and eleven bacterial isolates were recovered; the bacteria detected included Escherichia coli (16.6%), Enterobacter sp. (13.6%), Salmonella sp. (13.2%), Proteus sp. (12.6%), Serratia sp. (11.9%), Klebsiella sp. (9.3%) and Citrobacter sp. (7.9%). Others included Hafnia sp. (3.6%), Providencia sp. (3.0%), Shigella sp. (3.0%), Edwardsiella sp. (2.6%), Yersinia sp. (2.0%) and Pseudomonas sp. (0.7%). Overall resistance to antibiotics was generally high; resistance to streptomycin (59.0%) was highest, while it was least to gentamicin (39.5%). High proportion (96%) of the isolates showed multiple antibiotic resistance (MAR) with 151 phenotypes.

Keywords: *Faecal bacteria, Antibiotic resistance, Antibiotic-resistant faecal bacteria. Healthy individuals*

INTRODUCTION

Emergence and spread of antibiotic-resistant bacteria continues to be an important global problem particularly in developing countries (Lamikanra *et al.*, 1989; Hart and Kariuku, 1998; Okeke *et al.*, 2000; Albrich *et al.*, 2004; Heuer *et al.*, 2005; Maisetta *et al.*, 2006). An association between antibiotic use and resistance in hospitals is well established while high prevalence of antimicrobial-resistant bacteria in commensal flora also contributes to the general increase and spread of bacterial resistance (Livermore, 2003). Intestinal flora are a reservoir for resistance genes; the prevalence of resistance in a commensal bacteria, *Escherichia coli* is therefore, a useful indicator of antibiotic resistance among bacteria in the community. Bacteria efficiently exchange genetic materials particularly among related species and/or groups including the pathogenic ones and the intestine is a suitable environment for this activity.

Multiple antibiotic-resistant bacteria are increasingly being isolated which can be spread from animal, or the environment to the human population and from person to person (London *et al.*, 1993; Levy, 2005). Moreover, resistant bacteria may colonize the human intestine following ingestion of contaminated food while consumption of antibiotic is also known to provide selection pressure which ultimately leads to a higher prevalence of resistant bacteria (Albrich *et al.*, 2004; Schroeder *et al.*, 2004; Bartoloni *et al.* 2006). The availability of antibiotic over the counter without prescription and insufficient adherence of the medical profession to antibiotic policy, have been considered as important factors for the emergence, selection, and dissemination of antibiotic-resistant bacteria in a number of countries (Lansang *et al.*, 1990; Calva *et al.*, 1996; Okeke and Edelman, 2001; Taneja *et al.*, 2004). The opportunity for cross-infection in unsanitary conditions, which exists in most developing countries is enormous and may also compound the situation.

The intestinal tract of human is considered an important reservoir of antibiotic-resistant bacteria in which resistance genes can be transferred from commensal flora to potentially pathogenic microorganisms particularly among related species (Amyes *et al.*, 1992; Okeke and Edelman, 2001; Nys *et al.*, 2004). However, there is paucity of information on the type of antibiotic-resistant bacteria in the healthy human population not subjected to antimicrobial therapy or prophylaxis in Nigeria. This is an important aspect which may assist in monitoring and/or controlling antibiotic usage in the community. We therefore, investigated the profile and prevalence of antibiotic-resistant bacteria in intestinal flora of apparently healthy individuals in Ado-Ekiti, Nigeria.

MATERIALS AND METHODS

Sample collection and processing

Of the 248 volunteers who consented to participate in the study, 216 individuals made up of 70 males, 68 females and 78 children attending day care and kindergarten and had not taken any antimicrobial agent or been ill for at least one month prior to commencement, were recruited for the study. They were aged 4 months to 42 years with the mean age being 30.2 months. In the case of the children, informed consent was obtained from parents through the school. Faecal samples were collected using conventional techniques and processed within 1h of collection.

All the samples were plated out onto three solid media: Cystein Lactose Electrolyte Deficient (CLED) agar (Oxoid) and Eosine Methylene Blue (EMB) agar (Oxoid) and MacConkey agar (Oxoid). Isolates were identified as previously described by Barrow and Feltham, (1993).

Susceptibility testing

Susceptibility of isolates to eight most commonly used antibiotics was determined by the disc diffusion method (Bauer *et al.*, 1966), with discs containing (in μg) ampicillin (10), cefotaxime (30), chloramphenicol (30), cotrimoxazole (25), gentamicin (25), nalidixic acid (30), Streptomycin (30) and tetracycline (30). After 18h incubation, the size of the zone of inhibition was measured. The standard disc diffusion method based on the National Committee for Clinical Laboratory Standard (NCCLS) now Clinical Laboratory Standards Institute, guidelines (2004) was used to evaluate the data.

RESULTS

A total of 302 non-duplicate bacterial isolates were recovered from 216 non-repeat faecal samples. The predominant organisms were 50 (16.6%) *Escherichia coli*, 41 (13.6%) *Enterobacter* sp., 40 (13.3%) *Salmonella* sp., 38 (12.6%) *Proteus* sp., 36 (11.9%) *Serratia* sp., 28 (9.3%) *Klebsiella* sp. and 24 (7.9%) *Citrobacter* sp. Others included 11 (3.6%) *Hafnia* sp., 9 (3.0%) *Providencia* and *Shigella* sp., 8 (2.6%) *Edwardsiella* sp., 6 (2.0%) *Yersinia* sp. and 2 (0.7%) *Pseudomonas* sp.

The antibiotic susceptibility testing of the bacteria revealed that all the isolates were resistant to one antibiotic or more (Table 1). Among the antibiotics tested in this study, overall resistance was highest to streptomycin (59.0%) followed by ampicillin (53.1%), tetracycline (52.5%), nalidixic acid (44.9%), cotrimoxazole (43.0%) and cefotaxime (41.6%). Resistance to chloramphenicol was 39.4% while resistance to gentamicin was 35.9%.

Among the bacterial isolates, resistance to antibiotics differed markedly. For instance, 52.0% of *E. coli* were resistant to ampicllin, whereas 10.0% were resistant to gentamicin, 70.7% of *Enterobacter* sp. were resistant to ampicillin, followed by streptomycin (63.4%), tetracycline (56.1%) and the least resistance was to cefotaxime (36.6%). Resistance to antibiotics among *Salmonella* sp. was highest at equal rates to streptomycin and tetracycline (57.5%), followed by chloramphenicol and nalidixic acid (55.0%) and resistance to ampicillin (3.7%) being the lowest and making it the most effective antibiotic. Antibiotic resistance among *Proteus* sp. was highest to streptomycin (86.8%), followed by tetracycline (63.5%) and ampicillin (60,5%). The lowest resistance was to gentamicin (18.4%).

Other notable organisms isolated equally showed varied susceptibility to the antibiotics tested. The resistance to antibiotics among *Serratia* sp. was highest to tetracycline (72.2%), followed by ampicillin and streptomycin (55.6%), chloramphenicol and nalidixic acid (44.4%). Cotrimoxazole was the most active with 25.5% resistance rate. Also resistance among *Klebsiella* sp. was highest to ampicillin (78.6%), followed by cotrimoxazole (60.7%), streptomycin (53.6%) and lowest to gentamicin (17.9%). *Citrobacter* sp. showed the highest resistance to streptomycin (75.0%) followed by chloramphenicol (70.8%) and gentamicin (25.0%) was the most active against the organism.

Table 1: Antibiotic susceptibility profiles of bacteria isolated from healthy individuals

Isolates (n)	Antibiotics							
	AMP	CEF	CHL	COT	GEN	NAL	STR	TET
E. coli (50)	26 (52.0)*	18 (36.0)	18 (36.0)	15 (30.0)	5 (10.0)	16 (32.0)	15 (30.0)	16 (32.0)
Klebsiella spp. (28)	22 (78.6)	8 (28.6)	7 (25.0)	17 (60.7)	5 (17.9)	13 (46.4)	15 (53.6)	13 (46.4)
Citrobacter spp. (24)	13 (54.2)	11 (45.8)	17 (70.8)	15 (62.5)	6 (25.0)	15 (62.5)	18 (75.0)	15 (62.5)
Salmonella (40)	3 (7.5)	20 (50.0)	22 (55.0)	19 (47.5)	10 (25.0)	22 (55.0)	23 (57.5)	23 (570.5)
Edwardsiella sp. (8)	4 (50.0)	4 (80.0)	3 (37.5)	3 (37.5)	1 (12.5)	2 (25.0)	5 (62.5)	3 (37.5)
Hafnia spp. (11)	5 (45.5)	10 (90.9)	5 (45.5)	6 (54.5)	5 (45.5)	7 (63.6)	8 (72.6)	7 (63.6)
Serratia spp. (36)	20 (55.6)	15 (41.7)	16 (44.4)	9 (25.0)	14 (38.9)	16 (44.4)	20 (55.6)	26 (72.2)
Proteus spp. (38)	23 (60.5)	16 (42.1)	16 (42.1)	16 (42.1)	7 (18.4)	14 (36.8)	33 (86.8)	24 (63.2)
Shigella spp. (9)	8 (88.9)	2 (22.2)	2 (22.2)	5 (55.6)	6 (66.7)	4 (44.4)	6 (66.7)	3 (33.3)
Pseudomonas spp. (2)	2 (100.0)	1 (50.0)	2 (100.0)	1 (50.0)	1 (50.0)	2 (100.0)	2 (100.0)	1 (50.0)
Enterobacter spp. (41)	29 (70.7)	15 (36.6)	20 (48.8)	18 (43.9)	17 (41.5)	21 (51.2)	26 (63.4)	23 (56.1)
Yersinia spp. (6)	1 (16.7)	3 (50.0)	3 (50.0)	2 (33.3)	1 (16.7)	2 (33.3)	1 (16.7)	1 (16.7)
Providencia spp. (9)	6 (66.7)	4 (44.4)	7 (77.8)	5 (55.6)	1 (11.1)	3 (33.3)	8 (88.9)	5 (55.6)

AMP—Ampicillin, CEF— Cefotaxim, CHL— Chloramphenicol, COT — Cotrimoxazole, GEN — Gentamicin, NAL — Nalidixc acid, STR — Streptomycin, TET — Tetracycline.
(%) Percentage occurrence.*

Table 2: Antibiotic resistant patterns of the bacterial isolates

Isolates (n)	Number of antibiotics to which organisms were resistant							
	1	2	3	4	5	6	7	8
E. coli (50)	4	4	8	12	8	6	2	6
Klebsiella spp. (28)		6	6	6		8		2
Citrobacter spp. (24)			9	3	8	1	3	
Salmonella (40)	4	6	4	9	7	5		5
Edwardsiella spp. (8)		2		2	4			
Hafnia spp. (11)			3	3	2		1	2
Serratia spp. (39)	7	6	7	6	6	4	2	1
Proteus spp. (38)		3	5	9	8	8		3
Shigella spp. (9)				1	4	4		
Pseudomonas spp. (2)				1				1
Enterobacter spp. (41)	5	1	5	15	5	3	1	6
Yersinia spp. (6)	2		1	2	1			
Providencia spp. (9)			4	1	1	3		

The results also showed that 21 (about 7.0%) of the 302 isolates were resistant to only one of the antibiotics tested while the remaining 281 showed MAR in varying degrees. An interesting observation of this study is the differences in the distribution of the MAR isolates. It was observed that 27 (9.6%) were resistant to two antibiotics, 51 (18.1%) to three, 70 (24.9%) to four, 54 (19.2%) to five, 42 (14.9%) to six, 11 (3.9%) to seven and 26 (9.3%) to all eight antibiotics tested (Table 2). In all, 151 antibiotic resistance phenotypes were obtained. Resistance to ampicillin, tetracycline and streptomycin was the predominant antibiotype. .

DISCUSSION

Acquisition of resistance to antimicrobial agents among most bacteria, particularly the gram-negative bacilli, presents a mechanism of survival in unfavourable environments. This study shows a relatively high prevalence of resistance among bacterial isolates from healthy individuals to most commonly used antibiotics particularly ampicillin, chloramphenicol, streptomycin and tetracycline indicating a high carriage of resistant bacteria within human population in the studied area. The results showed that all the isolates were resistant to one or more antibiotics of which 93.0% were resistant to 2 or more antibiotics. One of the implications of this phenomenon is that these highly resistant bacteria may be spread into other ecological niches, survive and multiply, leading to further increase in the numbers of antibiotic-resistant bacteria. This agrees with Ramteke *et al.* (1990) who reported that resistant gram-negative bacteria recovered from drinking water sources transferred antibiotic resistance genes in laboratory experiments. Indeed drinking water from natural sources such as rivers, wells and springs (Antai, 1987; Sokari *et al.*, 1988; Ibiebele and Sokari, 1989; Ash *et al.*, 2002) as well as food animals particularly from poultry and cattle have been reported to be reservoir for and sources of antibiotic-resistant bacteria (Iovine and Blaser, 2004; Shiraki *et al.*, 2004; Schroeder *et al.*, 2004) for human that consume

Table 3: Antibiotic resistance types and pattern among the bacterial isolates

Isolates (n)	Single R-Type	Antibiotic	Multiple R-Type	Number of isolate	Antibiotic resistance pattern (Phenotype)
E. coli (50)	2	CHL	2	2	CHL, TET
	2	CEF		2	AMP, CEF
			3	3	CHL, STR, TET
				2	CEF, STR, TET
				2	AMP, CHL, STR
				1	CEF, CHL, COT
			4	2	AMP, CEF, NAL, TET
				4	AMP, CEF, COT, NAL
				2	AMP, CEF, CHL, NAL
				2	AMP, CHL, STR, TET
				2	CEF, COT, STR, TET
			5	4	AMP, CHL, GEN, STR, TET
				2	AMP, CHL, NAL, STR, TET
				2	CEF, CHL, NAL, STR, TET
			6	2	AMP, CEF, CHL, COT, NAL, STR
				2	AMP, CEF, CHL, GEN, NAL, TET
				1	AMP, CEF, CHL, NAL, STR, TET
				1	CEF, CHL, COT, NAL, STR, TET
			7	2	AMP, CEF, CHL, COT, NAL, STR, TET
			8	6	AMP, CEF, CHL, COT, GEN, NAL, STR, TET
Citrobacter freundii (17)	-		3	1	CEF, STR, TET
				1	CHL, COT, TET
				1	CHL, GEN, NAL
			4	1	AMP, STR, TET ?
				1	AMP, COT, GEN, STR
				1	CHL, COT, STR, TET
				1	AMP, CHL, NAL, STR, TET
			5	1	AMP, CEF, CHL, NAL, STR
				1	AMP, CHL, COT, STR, TET
				1	AMP, CHL, NAL, STR, TET
				1	CEF, CHL, COT, NAL, STR
				1	CEF, CHL, NAL, STR, TET
				1	CHL, COT, GEN, STR, TET
			6	1	AMP, CHL, COT, NAL, STR, TET
			7	1	AMP, CEF, CHL, COT, NAL, STR, TET
				1	AMP, CEF, CHL, COT, NAL, STR, TET
				1	CEF, CHL, COT, GEN, NAL, STR, TET
Citrobacter diversie (1)			4	1	AMP, CEF, CHL, COT
Citrobacter sp. (4)			3	1	AMP, CEF, COT
			4	1	AMP, CEF, NAL, STR
			5	1	CHL, GEN, NAL, STR, TET

Table 3: Antibiotic resistance types and pattern among the bacterial isolates (Cont'd)

Isolates (n)	Single R-Type	Antibiotic	Multiple R-Type	Number of isolate	Antibiotic resistance pattern (Phenotype)
			7	1	CEF, CHL, COT, GEN, STR, TET
Salmonella spp. (30)	1	AMP	2	1	AMP, CEF
	1	TET		1	CEF, COT
				1	CHL, TET
				1	CHLO, TET
			3	1	AMP, CHL, STR
				1	AMP, CHL, TET
				1	CEF, NAL, STR
			4	2	AMP, CEF, CHL, COT
				1	AMP, CEF, CHL, TET
				1	AMP, CEF, GEN, STR
				1	AMP, CEF, STR, TET
				1	AMP, CHL, STR, TET
				1	AMP, GEN, STR, TET
			5	1	AMP, CEF, CHL, NAL, STR
				1	AMP, CHL, COT, NAL, STE, TET ?
				2	CEF, CHL, COT, STR, TET
				1	CEF, CHL, GEN, NAL, TET
				1	CHL, COT, NAL, STR, TET
Escherichia coli (25)			6	2	AMP, CEF, CHL, NAL, STR, TET
				1	AMP, CHL, COT, NAL, STR, TET
				1	CEF, CHL, COT, NAL, STR, TET
			8	5	AMP, CEF, CHL, COT, GEN, NAL, STR, TET
Edward tarda (5)	-		2	1	COT, STR
				1	CEF, CHL, COT, STR
				2	AMP, CEF, CHL, STR, TET
				1	AMP, CEF, CHL, STR, TET
Hafinia alonni (4)	-		3	1	CEF, NAL, TET
				1	CEF, STR, STR
			4	1	CEF, COT, STR, TET
			8	1	AMP, CEF, CHL, COT, GEN, NAL, STR, TET
Hafinia sp. (7)			3	1	AMP, CEF, COT
			4	1	AMP, CHL, STR, TET
				1	CEF, CHL, NAL, STR
			5	1	AMP, CEF, CHL, NAL, TET
				1	CEF, COT, GEN, NAL, STR
			7	1	CEF, CHL, COT, GEN, NAL, STR, TET
			8	1	AMP, CEF, CHL, COT, GEN, NAL, STR, TET

them. Antibiotic-resistant bacteria have also been reported to be common among children in day-care centres (Reves *et al.*, 1990; Bartoloni *et al.*, 2006).

This study, which is the first of its kind from this area, presents results which are almost congruent with studies on antibiotic resistance from other parts of this country albeit mostly from hospital patients (Montefiero *et al.*, 1989; Oluduro *et al.*, 2003; Oyagade *et al.*, 2004). Okeke *et al.* (2000) reported an increasing antibiotic resistance among *Escherichia coli* isolated from healthy Nigerian students to tetracycline, ampicillicin, chloramphenicol and streptomycin increased from between 9 and 35% in 1986 to 56 and 100% in 1998.

The high rate of multiple resistance observed in this study may be mediated either by genetic elements or natural selection of resistance by the bacteria due to antibiotic abuse. The high resistance of the organisms to streptomycin, tetracycline and ampicillin may not be surprising; this is a phenomenon, which has been associated with living patterns and life styles (Amyes *et al.*, 1992; Okeke *et al.*, 1999). The socio-economic and behavioural factors that favour the development and spread of antibiotic resistance particularly in developing countries have been reported (Young and Jesudason, 1990; Amyes *et al.*, 1992; Okeke *et al.*, 1999; Walson *et al.*, 2001; Taneja *et al.*, 2004). Besides, the daily ingestion of high numbers of faecal bacteria in contaminated water supplies (Oluyege and Famurewa, 2005), wrong prescription and unrestricted access to antibiotics (Obaseiki-Ebor, *et al.*, 1987; Lamikanra *et al.*, 1989; Walson *et al.*, 2001) may as well be responsible for the initiation and maintenance of the high resistance rate observed in this study.

Grenet *et al.* (2004) reported that antimicrobial resistant bacteria can be spread in persons not previously exposed to antibacterial agents; once resistance elements have been introduced into a population, moderate use of antimicrobial drugs in that particular environment is enough to maintain them in intestinal bacteria particularly in poor sanitary conditions as ours. Watson *et al.* (2001) and Grigoryan *et al.* (2006) also attributed this phenomenon to indiscriminate use, self-medication, sub-optimal quality of the antimicrobial drugs and poor hygiene. Likewise, Miller *et al.* (1996), Walson *et al.* (2001) and Levy (2002; 2005) associated this with what has been described as "societal effect" among those individuals occupying the same environment. The use of antimicrobial drugs in companion animals of man as a potential source of emergence of resistant bacteria has been suggested recently (Heuer *et al.*, 2005; Paauw *et al.*, 2006). Muniesa *et al.* (2004) reported that bacteriophages may contribute to the spread of some b-lactamase genes in the environment. This is a potential danger for the emergence of plasmid-linked antimicrobial-drug resistance which may accelerate transfer to individuals in the immediate and remote environments.

Expectedly, *E. coli* were the predominant isolates recovered and showing varying resistance to the antibiotics. Some extra-intestinal pathogenic *E. coli* have been reported to have greater prevalence of antimicrobial resistance, such as to ampicillin, tetracycline, chloramphenicol and sulphonamide, express significantly fewer virulence factors and more commonly invade compromised host (Branger *et al.*, 2005).

The four antibiotics to which a considerable rise in resistance was shown, i.e. ampicillin, chloramphenicol, streptomycin and tetracycline, are used extensively in Nigeria like other developing countries (Hart and Kariuku, 1998; Okeke *et al.*, 1999); they are available unrestricted and so widely used. However, due to the special administration by injection, gentamicin and streptomycin are not as widely used, yet resistance was highest to streptomycin. Surprisingly, however, resistance to cotrimoxazole was lower (43.0%) in spite of its unrestricted and widespread use, whereas resistance to gentamicin was the lowest owing to the generally low con-

sumption as it is only available in liquid form for *intra venous* application. This may confirm the belief that exposure results in selective pressure of antibiotic resistance.

The subjects recruited in this study were apparently healthy and were not on antibiotic medication prior to the commencement of this study. Thus, the possible sources of the resistant bacteria in the volunteers could be food, water supplies and person-to-person transfer, particularly, in unhygienic and or overcrowded environment with poor sanitary conditions.

It is noteworthy that most of the isolates recovered in this study are presumably mostly environmental organisms; they are not only able to cause infections, but also could serve as potential reservoirs of resistance genes that could be transferred to other pathogens as antibiotic resistance now pervades all communities. There is therefore, the urgent need to curtail the spread of antimicrobial resistance through adoption of some stringent measures including educating people on the appropriate use of antibiotics, improved resistance surveillance through routine monitoring and data collection of antibiotic resistance to provide data for therapy and resistance control, judicious use of antibiotics in non-human settings and as supplements for advocating compliance with good hygiene practises in Nigeria.

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