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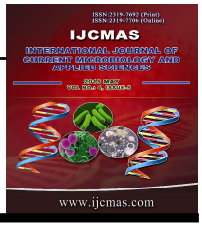

Original Research Article

Carriage of Antibiotic Resistant Commensal *E. coli* in Infants below 5 Months in Ado-Ekiti

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A B S T R A C T

Commensal bacteria are an important reservoir of antibiotic resistant genes because they are capable of facilitating the spread of resistant genes to pathogenic strains. The objective of the study was to investigate the prevalence of antibiotic resistant commensal *E. coli* carried by healthy infants in a community setting in Ado-Ekiti, Ekiti State, Nigeria; and to further examine whether previous use of antibiotics, exclusive breast feeding and infant milk formula feeding were associated with carriage. Faecal swabs collected from healthy infants below 5 months old in Ado-Ekiti were analyzed for presence of antibiotic-resistant *E. coli*. *Escherichia coli* strains were isolated and identified using morphological and biochemical characteristics. A structured questionnaire was administered to mothers to collect information on use of drugs and the practice of exclusive and inclusive breast-feeding. Antibiotic susceptibility to eight commonly prescribed antibiotics was carried out using Kirby Bauer disc diffusion technique. A total of 212 faecal samples were analyzed of which 102 (48.1%) yielded growth of *E. coli*. The *E. coli* isolates exhibited 90.2% and 88.2% resistance to amoxicillin and tetracycline respectively while over 75% were resistant to cotrimoxazole and augmentin; below 20% were resistant to ofloxacin, gentamicin, nalixidic acid and nitrofurantoin. Ninety percent (90%) of *E. coli* isolated had multiple resistance to three or more antibiotics. There was carriage of multiple resistant commensal *E. coli* irrespective of exclusive or inclusive mode of feeding, or previous history of use of antibiotics or other parenteral drugs. Carriage of multiply resistant commensal *E. coli* was present even in infants with no history of use of any drug. We concluded that there were other factors responsible for carriage of resistant commensal *E. coli* in the study area.

Introduction

The largest population of commensal bacteria is that found in the gut, where they are at risk of exposure to orally ingested antibiotics, as well as certain parenteral

preparations. In community settings, young children tend to be the most exposed to antibiotics, and several studies have found that younger children have the highest risk of carrying resistant commensal bacteria [1]. *Escherichia coli*, a near ubiquitous colonizer of the gastrointestinal tract in children and adults has often been used in studies of resistance in commensal bacteria [1, 2, 3]. *Escherichia coli* is a gram-negative rod shaped bacterium that is commonly found in the lower intestine of warm blooded animals and mammals [4]. The non pathogenic strains of *E. coli* referred to as commensal strains are harmless and are useful, not only in digesting and breaking down food, but also in protecting against harmful organisms which may be introduced into the gastrointestinal tract through food and water [5]. However, such commensals can have access to other parts of the body such as urinary tract, blood, or wounds and cause opportunistic infections particularly in immuno-compromised individuals. *Escherichia coli* have been shown to develop resistance in response to antibiotic use, and to be particularly capable of exchanging antibiotic resistance genes with pathogenic bacteria [6]. High resistance rates have often been reported in surveillance studies dealing with clinical isolates and in prevalence studies of commensal bacteria such as *E. coli* [3].

A variety of risk factors have been associated with carriage of resistant commensal organisms particularly in community settings, but the various contributions remain unclear. Kalter *et al*., [3] reported that recent antibiotic use, age and geographical location may contribute to carriage in preschool children.

It has been established that breastfed infants are less prone to a variety of infantile infections including acute otitis media, and gastrointestinal and lower respiratory tract infections than formula fed infants. Exclusive breastfeeding helps protect infants against common infections and lessens the frequency and severity of infectious episodes in developing countries and communities with adequate vaccination coverage and healthcare standards [3]. Partial breast feeding does not seem to provide this protective effect against infections in infancy.

The spread of microbial drug resistance is a global public health challenge, which impairs the efficacy of antimicrobial agents and leads to substantial increase in illness and death rates. The impact of the phenomenon tends to be larger in under-developed and developing countries when compared to that of developed countries. The high antimicrobial drug resistance rates observed in low-resource countries is likely due to combination of several factors, among which irrational antimicrobial drug usage and conditions of poor sanitation play major roles [2].

Recent studies have highlighted the high or increasing incidence of antibiotic resistance in commensal *E. coli* from healthy children from many countries, but there is no existing data on the carriage of antibiotic resistant commensal *E. coli* in infants in Ado-Ekiti.

The aim of this study is to determine the prevalence of carriage of resistant commensal *E. coli* in infants in Ado-Ekiti; and to further examine the possible roles of exclusive breast feeding and milk formula feeding and early exposure to drugs on carriage of resistant commensal *E. coli*.

Materials and Methods

Sample collection

Rectal swab samples were collected from infants below 5 months attending the immunization clinic in two different centres

in Ado-Ekiti, Ekiti State. A total of two hundred and twelve (212) samples were collected, using sterile swab sticks. The samples were collected with the consent of the infant's parents or guardians who were willing to participate in the study. Each swab stick was named and numbered with the sex of the infant, and the fresh faeces were taken from the diapers of the babies. For rectal swab, the sterile swab sticks were inserted into the anus of the babies and rotated. Relevant information was obtained from the mothers through questionnaires. All collected samples were brought immediately to the laboratory and analyzed within two hours of collection.

Bacteriological analysis: The samples were inoculated directly on Eosin Methylene Blue (EMB) agar and MacConkey agar, and incubated aerobically at 37^{o}C for 24 - 48 hours. All lactose fermenting isolates within 48 hours on MacConkey agar were further identified morphologically and biochemically [7]. To obtain pure strain of inoculum (*E. coli)*, the freshly prepared agar plate was streaked with isolated colonies, incubated at 37°C for 24 h as stated by Cheesborough [8].

Antibiotic susceptibility testing: The *in vitro* antibiotic sensitivity for the *E. coli* isolates was carried out using the disk diffusion technique as recommended by the (CLSI, 2011). The isolates were tested against eight commonly used antibiotics which are; amoxicillin(AMX)- 25µg, ofloxacin(OFL)- 5µg, augmentin (AUG)- 30 µg, gentamicin (GEN)- 10µg, nalidixic acid (NAL)- 30µg, nitrofurantoin (NIT)- 200µg, cotrimoxazole (COT)- 25µg and tetracycline (TET)- 25µg.

Results and Discussion

From the two hundred and twelve infants examined in this study, a higher number of 65 (30.7%) were exclusively breast-fed while only 41 (19.3%) were inclusively fed with infant milk formula; 30 (14.2%) had no previous history of use of any drug, while only 14 (6.6%) had a previous history of antibiotics administration, 62 (29.2%) also had history of administration of other drugs (Table 1).

The ratio of the female and male infants that were used for this study was in ratio 12: 9. The highest prevalence of *Escherichia coli* among these infants were seen within the age range of 2 to 15 weeks (Table 2). However, infants who have no previous exposure to any antibiotics were seen to have lower isolation rate of 36.7% of *Escherichia coli* than those with history of antibiotics use (42.8%) and other drug (54.8%). The *E. coli* isolated from infants in this study exhibited 90.2% and 88.2% resistance to amoxicillin and tetracycline respectively, over 75% of E*scherichia coli* were resistant to cotrimoxazole and augmentin; below 20% were resistant to ofloxacin, gentamicin, nalixidic acid and nitrofurantoin. However, 77 (75.5%) were multiple resistant to 3 or more of the 8 antibiotics tested. The commonest resistant phenotype was amx/tet/cot/aug and this phenotype was isolated from all categories of infants examined irrespective of the mode of feeding and previous history of use of any category of drugs

Out two hundred and twelve infants examined in this study, a higher number of 65 (30.7%) were exclusively breast-fed while only 41 (19.3%) were inclusively fed with infant milk formula. The practice of exclusive breast feeding by mothers in the study area is low compared to that obtained in other Enugu within the country (53.6%). Mathew [9] reported that the practice of exclusive breast feeding was not commensurate with mothers' knowledge of the benefits and protective role.

Furthermore, the isolation rate of *E. coli* was found to be higher in those with inclusive (82.9%) than exclusive (26.2%) type of feeding. These findings therefore corroborate findings from previous studies regarding the protective role of breast feeding against bacterial infection. Pronounced exposure of infants to environmental bacteria and changes in diet occurs very early in developing countries [10]. Thus, the pattern and level of exposure to bacteria during the neonatal period is likely to influence the microbial succession and colonization in the gastrointestinal tract. In this study, infants with no history of use of antibiotics or other drugs also had a lower isolation rate (36.7%) of *E. coli* than those with history of antibiotic use (42.8%) and other drugs (54.8%). The early exposure of infants to antibiotics exerts a selective pressure whereby sensitive bacteria would have been destroyed while selecting resistant strains. This study also shows high prevalence of antibiotic resistance *E. coli* in healthy infants, living in Ado-Ekiti Metropolis. The *E. coli* isolated from infants in this study exhibited 90.2% and 88.2% resistance to amoxicillin and tetracycline respectively over 75% were resistant to cotrimoxazole and augmentin; below 20% were resistant to ofloxacin, gentamicin, nalixidic acid and nitrofurantoin. These values are higher compared to similar studies reported by Djie-maletz [11] who reported 35% and 37% resistance to tetracycline and Cotrimoxazole respectively. This trend was observed among all the *E. coli* isolates from infants in this study irrespective of the parameters vis á vis previous exposure to antibiotics and or other drugs, inclusive breast feeding and exclusive breast feeding (Table 2).

Worldwide, antibiotics are the most commonly prescribed drug for children, especially for acute respiratory illness and diarrhea [12, 13]. Increasingly, antibiotic resistance is usually attributed to overuse and misuse of antibiotics. It has been estimated that use is unnecessary in 20-50% of the cases. Unfortunately, this misuse of antibiotics is causing emergence of resistant pathogens early in life, especially in the developing world where antibiotics are available without prescription.

Furthermore, 77 (75.5%) were multiple resistant to 3 or more of the 8 antibiotics tested. The commonest resistant phenotype was amx/tet/cot/aug and this phenotype was isolated from all categories of infants examined irrespective of the mode of feeding and previous history of use of any category of drugs even though the incidence of this pattern was lower in *E. coli* strains isolated from those with no history of use of antibiotics. At the time this research was conducted, all the infants were healthy, yet it is alarming to discover such a high prevalence (75.5%) of carriage of multiple resistant strains of *E. coli* in infants at such an early stage of life. This trend further confirms the high level of multiply resistant organisms in the environment of infants in the study area. The growing antibiotics resistance may be due to irrational use of antibiotics and the transfer of resistance genes by plasmids, bacteriophages, transposons and integrons. At times, a plasmid or transposon can carry several resistance indexes, simultaneous resistance to multiple antimicrobial agents leading to multiply antibiotic resistance organisms. For instance, it has been reported that resistance to cotrimaxazole is usually accompanied by resistance to ampicillin, cephalosporin and tetracycline [14].

Our result further confirms the fact that antibiotic resistant bacteria are common in the environment, as healthy breast-fed infants who had no previous exposure to antibiotics have been found with the resistant strains of commensal *E. coli* [15].

Seventy one percent (71%) of the infants received medications on antibiotics such as amoxicillin, tetracycline, and ampiclox during their first year of life. This indicates a high prevalence of multidrug resistant despite the exclusive mode of feeding. Both infants and aged children in developed and developing countries are at risk of diarrhoea infection and some infection that are yet to be noticed due to poor hygienic practices by mothers, change in dietary, environmental exposure, irrational use of antibiotic, household animals and commercial/household poultry. It is reported that even commensal *E. coli* is now recognized as a very important agent of diarrhoea infection in healthy infants globally [16].

In addition, incidence of *E. coli* in infants can be traced primarily to faulty weaning practices, poor personal hygiene, exposure to environmental bacteria and indiscriminate antibiotic usage. Healthy children get infected immediately after birth through exposure to mother faecal flora, other environmental factors and during weaning. Therefore, training in the use/prescription of drugs, mother's personal hygiene in handling infant should be improved among nursing mothers. There should be effective legislation by the government to control the indiscriminate purchase of antibiotics to prevent its abuse. Also, mothers should be encouraged to practice good personal hygiene. Patterns of greater resistance to antibiotics necessitates the use of newer and more expensive drugs in order to control infections and may contribute to higher risk or morbidity and mortality in small infants treated for more serious infections in the developing world.

Table.1 Rate of isolation of E. coli isolated from infants below 5 months

Category	**Male**		**Female**		**Total**	
	No of infants	**Growth of *E. Coli***	**No of infants**	**Growth of *E. Coli***	**Total No of infants (%)**	**Total no of Growth of *E. Coli* (%)**
Exclusive Breast feeding	29	8(27.6%)	36	9(25.0%)	65(30.6)	17(26.2)
Inclusive breast feeding	17	22(47.8%)	24	12(50.0%)	41(19.3)	34(82.9)
Use of Antibiotics	5	1(20.0%)	9	5(55.5%)	14(6.6)	6(42.8)
Other drugs	32	19(59.4%)	30	15(50.0%)	62(29.3)	34(54.8)
No drug	9	3(33.3%)	21	(38.1%)	30(14.2)	11(36.7)
Total	**92**	**53(57.6%)**	**120**	**49(40.8%)**	**212(100)**	**102(48.1%)**

Table.2 Age range of infants examined and the rate of isolation of E. coli

Age range	**Sex**				
	Male	Female	Total	Number of *E. coli* isolated	**(%)**
1 - 13 days	02	10	12	10	83.3
2 – 4 wks	28	28	56	26	46.4
5 – 10 weeks	36	36	72	34	47.2
11 – 15 weeks	24	44	68	32	47.1
16 - 25 weeks	02	02	04	0	0
Total	92	120	212	102	48.1

Table.3 Percentage antibiotic resistance among E. coli isolated from infants with or without previous use of drugs, exclusive and inclusive breast feedings

Parameters	AMX	TET	COT	AUG	NAL	NIT	GEN	OFL
Use of Antibiotics(n=6)	6 (100%)	6 (100%)	4 (66.6%)	3 (50%)	(16.6%)	0(0%)	0(0%)	0 (0%)
Use of Other Drugs (n=34)	29 (85.3%)	31 (91.2%)	27(79.4%)	23(67.6%)	6(17.6%)	6 (17.6%)	2 (5.9%)	2 (5.9%)
No drug (n=11)	11 (100%)	8 (72.7%)	7(63.6%)	10(90.9%)	2 (18.2)	1 (9.1)	1 (9.1%)	0 (0%)
Exclusive breast Feeding (n=170)	16 (94.1%)	13 (76.5%)	11(64.7%)	12(70.6%)	3(17.6%)	4 (23.5%)	1 (5.9%)	1 (5.9%)
Inclusive breast feeding(n=34)	30 (88.2%)	32 (94.1%)	27(79.4%)	24(70.6%)	6(17.6%)	3 (8.8%)	2 (5.9%)	1 (2.9%)
Total n=102	**92 (90.2%)**	**90 (88.2%)**	**76(74.5%)**	**72(70.6%)**	**18(17.6%)**	**14 (13.7%)**	**6(5.9%)**	**4(3.9%)**

AMX=Amoxicillin, TET=Tetracycline, COT=Cotrimoxazole, AUG= Augmentin, NAL=Nalidixic acid, GEN=Gentamycin, OFL=Ofloxacin

Table.4 Percentage multiple resistance patterns (Phenotype) of E. coli isolated from faeces of infants below 5 months

Phonotypic patterns of Resistance of Antibiotics	No of Classes	Use of Antibiotics	Other Drugs	No Drug	Exclusive Breast-Feeding	Inclusive Breast Feeding	Total	(%)
AMX/TET/COT/AUG/NAL/NIT/GEN/OFL	8	0	2	0	1	1	4	5.2
AMX/TET/COT/AUG/NAL/NIT/GEN	7	0	0	0	0	1	1	1.3
AMX/TET/COT/AUG/NAL/NIT	6	0	4	0	2	1	7	9.1
AMX/TET/COT/AUG/NAL	5	1	0	1	0	3	5	6.5
AMX/TET/COT/AUG/NIT	5	0	0	0	1	0	1	1.3
AMX/TET/COT/AUG	4	2	17	5	7	18	49	63.6
AMX/TET/COT	3	1	4	0	0	3	08	10.4
AMX/TET/AUG	3	0	0	1	1	0	2	2.6
Total		4	27	07	12	27	**77**	100

AMX=Amoxicillin, TET=Tetracycline, COT=Cotrimoxazole, AUG=Augmentin, NAL=Nalidixic acid, GEN=Gentamycin, OFL=Ofloxacin

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