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Resistance Profile of *Escherichia coli* Strains Isolated from the Diarrhoeal Faeces of Children Aged 0 to 5 Years in N'Djamena and Sarh (Chad)

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ABSTRACT

Escherichia coli diarrhoea remains a public health problem in Chad. It is a common pathology in developing countries, particularly where hygiene levels are low. In children aged 0-5 years, it remains the leading cause of morbidity and mortality from digestive pathologies. Its management raises questions about its resistance to the drugs used to treat it. The aim of this study is therefore to determine the prevalence of *Escherichia coli* strains and their resistance profiles to the antibiotics commonly used in Chad. Stools from children aged 0-5 years were collected in sterile jars and sent to the Centre Hospitalier Universitaire de la Mère et de l'Enfant for microbiological analysis. The stools were cultured on Eosin Methylene Blue (EMB) agar and incubated in a bacteriological oven for 18 to 24 hours at 37°C. Colonies from this culture, with a metallic sheen, were subcultured onto Muller Hinton agar for purification and antigenic studies. Gram staining and oxidase tests were performed on the different strains. Biochemical identification of the isolates was carried out using the API20E Gallery in accordance with Bio Mérieux recommendations. Antibiotic susceptibility testing of the various strains was carried out using the Kyrbi-Bauer technique and in accordance with the recommendations of the Antibiogram Committee of the French Microbiology Society (CA-SFM). Of the 296 stools treated, 36 strains of *E. coli* were isolated, giving a prevalence of 12.2%. The strains were multi-resistant to the various families of antibiotics used. High resistance to β-lactam antibiotics was observed, with 86.11% for cefotaxime, 83.33% for ceftriaxone, 72.22% for cefepime and 63.60% for ceftaxidime. All strains were resistant to aminopenicillins, with frequencies of 86.11% for amoxicillin-clavunalic acid and 83.33% for amoxicillin. However, imipenem was effective against all the strains studied. Aminoglycosides showed a resistance rate of 77.78% for gentamicin and 80.56% for tobramicin. Amicakacin was the most effective molecule, with a resistance rate of 44.44%. Quinolones and fluorquinolones showed resistance of 69.44% for nalixidic acid, 80.56% for ciprofloxacin and 72.22% for ofloxacin. Cotrimoxazole was the only sulphonamide used, with 88.89% resistance. *Escherichia coli* are still common in children aged 0-5 years. The strains are multi-resistant to the antimicrobials used. The circulation of such strains requires further study to gain a better understanding of their origin and mode of dissemination.

Introduction

The growth of African cities, particularly capital cities, has led to profound changes in their environment in recent years (Bocquier and Traoré 2000). This accelerated increase in population, generating a variety of activities, has led to poor hygiene and environmental sanitation conditions, which could have consequences for public health. The lack of sanitation in urban areas provides favourable bio-ecological conditions for the development of pathogens responsible for numerous diseases, particularly gastroenteritis, the main manifestation of which is diarrhoea (Dongo *et al.,* 2008).

Infantile infectious gastroenteritis is a major public health concern, particularly in developing countries. They are linked to the presence of certain pathogenic bacteria, particularly the enteropathogenic *Escherichia coli* tribe (Titilawo *et al.,* 2015; Lanata *et al.,* 2013). Gastroenteritis affects all age groups in the population. However in infants and young children, enteric diseases are the second leading cause of death in children under 5 years of age with an estimated 2.5 billion cases worldwide and 1.5 million deaths each year (Diawara, 2018). Mortality rates are particularly high in sub-Saharan Africa.

In Chad, 15,900 children under the age of 5 die every year from diarrhoea. 90% of these deaths are directly linked to the consumption of unclean water and the lack of sanitation and hygiene (WSP, 2012).

Escherichia coli is a bacterium commonly found in the digestive tract of humans and warm-blooded organisms (WHO, 2020). It represents almost 80% of the digestive tract and, along with the other components of the microflora, forms a protective barrier for the mucosa (Douyon, 2017). Most strains are harmless. Some, however, can cause food poisoning, leading to serious illness (WHO, 2020); (Mariama *et al.,* 2022).

Antibiotic therapy made a major contribution to the medical revolution at the beginning of the 20ème century. It led to a significant reduction in mortality associated with infectious diseases (Carle, 2009). However, the performance of antibiotic therapy has evolved in parallel with the emergence of bacterial strains resistant to certain antibiotics. Today, this bacterial resistance is a public health problem in many parts of the world (Nascimento, 2000; Kiptoo, 2012). There is an urgent need to find alternative ways of controlling the risk, especially as efforts to halt the situation are hampered by the emergence of virulence and bacterial resistance to antibiotics.

With a view to comparing data with previous studies, this study was carried out to describe the profile of resistance to commonly used antimicrobials in *Escherichia coli* strains isolated from the diarrhoeal faeces of children aged 0 to 5 years at the Centre Hospitalier Universitaire de la Mère et de l'Enfant (CHUME), Hôpital de Notre Dame des Apôtres (HNDA) and Hôpital Régional de Sarh (HRS) in Chad.

Materials and Methods

Study framework

The study was carried out in two towns in Chad: N'Djamena (Centre Hospitalier Universitaire de la Mère et de l'Enfant and Hôpital Notre Dame des Apôtres) and Sarh (Hôpital Régional). Analyses were carried out at the bacteriology unit of the Centre Hospitalier Universitaire de la Mère et de l'Enfant.

Type, period and study population

This was a descriptive cross-sectional study from July to October 2021. It involved children of both sexes aged between 0 and 5 years who had been seen in paediatric wards where a bacteriological stool examination had been requested.

Inclusion criteria

All children aged between 0 and 5 presenting with gastroenteritis and whose parents agreed to take part in the study were included.

Exclusion criteria

The study did not include children aged over 5 years, children without gastroenteritis or children with gastroenteritis whose parents refused to take part in the study.

Sampling

An exhaustive and consecutive sample was taken of all children aged 0-5 years hospitalised with diarrhoea or suspected diarrhoeal infections seen in paediatric wards.

Collection, culture and isolation of strains

Stool samples were collected from children aged 0 to 5 in sterile, single-use, labelled bottles containing Cary-Blair agar. The samples were transported to the laboratory of the Centre Hospitalier Universitaire de la Mère et de l'Enfant (CHUME) in compliance with the triple packaging transport conditions. Culture and isolation were carried out by plating the stools on EMB (Methylene Blue Eosin) agar and incubating at 37°C for 18 to 24 hours in a bacteriological oven. After 18 to 24 hours, colonies with a metallic sheen on EMB agar, characteristic of *E. Coli, were* transferred to Muller-Hinton (MH) agar for antigenic studies, Gram staining and the oxidase test.

Biochemical identification of germs

Suspect *E. coli* colonies (green with a bright metallic sheen) were plated onto Muller Hinton (MH) agar after 24 hours incubation at 37°C. They were subjected to biochemical tests using the method of Poelma *et al.,* (1984). The oxidase-negative colonies were suspected of being *E. coli* strains. These suspected colonies were subjected to biochemical identification tests, namely the indole test for glucose and lactose metabolism and gas production. H_2 S was determined using Kliger Hajna medium.

Mannitol mobility medium was used to determine mannitol metabolism and bacterial mobility. Simmons citrate medium was used to demonstrate the ability of the bacteria. Citrate was used as the sole carbon source. Readings were taken after 24 hours incubation at 37°C. Biochemical identification of the incriminated strains was carried out after culture of the inoculum on the bio Mérieux API20E gallery in accordance with the instructions of the Société Française de Microbiologie (SFM). Revelation was carried out using TDA, JAMES and VP reagents.

Antibiotic susceptibility test (antibiogram)

The study of antibiotic susceptibility was carried out using the agar diffusion method. This method is suitable for the majority of pathogenic bacteria, including slow-growing bacteria, and allows a variety of antibiotics to be used. No special equipment is required. The reference strain of *E. coli* ATCC25922 was used for quality control.

Methods for performing antibiograms (Kirby-Bauer)

The sensitivity test method used was diffusion in MH agar (disc method or Kirby Bauer technique). The agar was 4mm thick, corresponding to a volume of 22.5 ml for a petri dish (dm 90 mm). The antibiogram was performed using a pure strain isolated on MH agar. This method is based on the fact that when an antibiotic is deposited on the surface of an agar medium at a given point, it diffuses by establishing a concentration gradient inversely proportional to the diameter of the antibiotic.

Preparation of Muller Hinton agar

MH agar was prepared according to the manufacturer's recommendations and sterilised on a hot plate for 15 minutes. After sterilisation, it was autoclaved in a water bath at around 45°C. The agar was 4mm thick, corresponding to a volume of 22.5ml for a petri dish (dm 90 mm). The petri dishes were then placed on a level table for solidification, followed by oven drying.

Preparing the inoculum

From a culture of 18 to 24 hours on MH agar, pure colonies of the strain to be studied were suspended directly in saline solution (5ml physiological water) and then homogenised using a vortex. The density of the mixture was adjusted using a densitometer to obtain a turbidity equivalent to Mc-Farland's 0.5 gram standard, which corresponded to an inoculum of approximately 10^5 CFU/ml for *Escherichia coli*. The inoculum was adjusted by adding a colony if the bacterial suspension did not reach a turbidity of 0.5 McFarland or by adding sterile physiological water if the suspension was denser.

Inoculum seeding

A sterile swab was dipped into the bacterial suspension, then pressed against the inside wall of the tube. Spreading was done by rubbing the swab over the entire surface of the agar from top to bottom in tight striations and was repeated 2 times by turning the petri dish at a 60° angle on each side of the dish in order to obtain a homogeneous distribution of the inoculum (CLSI, 2010). The disc was applied 5 to 10 minutes at room temperature (25°C) after inoculation. Petri dishes were dried in an oven at 37°C for 24 hours.

Choice of discs

In this study, 14 antibiotics were tested. These antibiotics are generally active against Enterobacteriaceae and are commonly used in probabilistic antibiotic therapy of Enterobacteriaceae infections, particularly gastroenteritis. These antibiotics were: Amoxicillin (25µg), Amoxicillin+clavunalic acid (20/10µg), Ceftriaxone (30µg), Ceftazidime (30µg), Cefepime (30µg), Cefotaxime (CTZ), Imipenem (10µg), Gentamicin (10µg), Tobromycin (10µg), Amikacin (30µg), Nalixidic acid (30µg), Ciprofloxacin (5µg), Ofloxacin (5µg) Cotrimoxaxole (25µg).

Application of antibiotic-impregnated discs

The antibiotics used for antibiotic susceptibility testing came from Bio Mérieux and Bio Rad discs. They were placed individually with sterile princes. The number of discs per petri dish had to be such that the inhibition zones did not intersect, so that the diameters could be read in several directions. The number of discs chosen varied between 6 and 7 per 90 mm dish.

Reading and interpretation

After 24 hours of incubation, the circular zones of inhibition around each disc were measured using a graduated ruler and compared with the standard zones of inhibition. The critical diameters were proposed by the manufacturer in accordance with NCCLS (Performance standards for Antimicrobiol succeptibillity testing).

Results and Discussion

Frequency of *E.* coli

A total of 296 stools from children aged 0-5 years were received during the study period, from which 36 strains of enteropathogenic *E. coli were* isolated, representing a prevalence of 12.2%.

Strain resistance to β-lactam antibiotics

Susceptibility testing of the 36 strains to the β-lactam family showed resistance to cepholosporins: cefotaxime (86.11%), ceftriaxone (83.33%), cefepime (72.22%) and ceftaxidime (63.60%). Most strains were resistant to aminopenicillins, with frequencies of 86.11% for amoxicillin-clavunalic acid and 83.33% for amoxicillin. Imipenem remained effective against all strains (100%).

Resistance of strains to the aminoglycoside family

Table II shows the resistance of strains to the aminoglycoside family: amicakacin showed resistance of 44.44%, gentamicin (77.78%) and tobramicin (80.56%).

Resistance of strains to the quinolone and fluor quinolone family

Table III shows that nalixidic acid showed resistance of 69.44%, ciprofloxacin (80.56%) and ofloxacin (72.22%).

Resistance of strains to the sulphonamides family

Cotrimoxazole was the only sulphonamide used in this study: the disk load of this antibiotic was 25 µg, with a critical value of 10-16. Resistance was 88.89% and sensitivity 11.11% for the 36 strains tested.

In developing countries, gastroenteritis is endemic and a major public health problem, particularly in children aged 0-5 years. The first-line antibiotics for their treatment are phenicoles (chloramphenicol), β-lactams (amoxicillins, ceftriaxones) and sulphonamides (sulphametaxazole-trimethoprim) (Aubry and Güzère, 2015). In recent years, particularly in tropical areas, the incidence of antibiotic-resistant *E. coli* has increased rapidly.

The β-lactams (aminopenicillins, cephalosporins, monobactam and carbapenem) are the most widely used family of antibiotics (Ouédraogo *et al.,* 2017). The misuse of these antibiotics has rapidly led to the growth of increased resistance to these antibiotics. Regarding *E. coli* resistance to aminopenicillins, the present study showed resistance to amoxicillin 83.33%. These results are comparable to those obtained by Mamadou (2020) which were 85.94% and those of Konaté (2018) of 74.4%. However, the resistance rate of 86.11% was observed in the present study with amoxicillin-clavunalic acid.

Similar resistance of *E. coli* to amoxicillin-clavunalic acid (73.3%) was observed in a previous study in Burkina Faso (Dembélé *et al.,* 2015). The study by Fissou *et al.,* (2019) in Chad observed a low resistance of 30% and another previous study carried out in 2011 in Dar es Salaam, Tanzania, noted an *E. coli* resistance to amoxicillin-clavunalic acid of 7.8% (Mayo *et al.,* 2011). This shows a geographical disparity and an increase in

the rate of resistance to aminopenicillins. These resistances could appear naturally over time, generally as a result of genetic changes. However, the over-prescription or over-use of these antibiotics, as well as non-compliance with treatment times, could be the cause of the acceleration of this resistance process. Resistance could also be due to self-medication.

Resistance of *E. coli* strains was also observed with third generation cephalosporins (CTX, CAZ, CRO) with respective rates of 86.11%, 63.60%, 83.33%. Similar results have been reported in some studies such as Warjri *et al.,* (2015) carried out in India where the resistance rates to cefotaxime and ceftriaxone were 97.2% and 94.9% respectively. Mathlouthi *et al.,* (2016) also observed cefotaxime and ceftriaxone resistance rates of 97% and 84% in research carried out in hospital wards in Algeria. The results obtained in this study are similar to those reported by Gadou (2019) who obtained a resistance rate of 95.6% and 96.7%. The resistance observed is thought to be due to the production of a penicillinase associated with a cephalosnase or an extended-spectrum β-lactamase capable of resisting all β-lactam antibiotics with the exception of imipenem (Zeba *et al.,* 2007; Duval, 1999; Haukkak, 2007; Cavallo, 2004). Similar work by Yandai *et al.,* (2014) in Chad reports a digestive carriage of 25.57% of cefotaxime (C3G) resistant strains.

The results obtained in this study show a high sensitivity of *E. coli* to Imipenem (100%). Other similar studies carried out by certain authors (Labombardi *et al.,* 2006; Endimiani and Paterson 2007; Nordemann *et al.,* 2009; Messai *et al.,* 2006) in Algeria, (Tamayo *et al.,* 2007) in Spain, (Mamadou, 2020) in Mali, (Gadou, 2019) in Côte d'Ivoire and (Hassanie and Boulanoir, 2019) also confirmed that imipenem was the most active molecule on all strains. This shows the absence of carbapenemase-producing *E. coli* strains. In fact, *E. coli* are naturally sensitive to Imipenem, which justifies its place as the first choice in the treatment of severe infections caused by multi-resistant bacteria.

In this study, *E. coli* strains showed fairly high resistance to aminoglycosides, with rates of 80.56% for tobramycin and 77.78% for gentamicin, while amikacin had a resistance rate of 44.44%. In Ghana, a study by Obeng-Nkrumah *et al.,* (2013) reported high levels of resistance to gentamicin (90%) and amikacin (45%). Research by Anago *et al.,* (2015) in Benin found that 82.7% of strains were resistant to gentamicin and 72.4% showed resistance to amikacin. Similarly, in Burkina Faso, work by Ouédraogo *et al.,* (2016) indicated high rates of resistance, with 89% of strains resistant to gentamicin and 86% to tobramycin. Other studies carried out in Sudan revealed that 4.3% of *E.coli* strains were resistant to Amikacin (Ibrahim *et al.,* 2013), 25% in Israel (Bishara *et al.,* 2005) and 38.46% in Chad (Yandai *et al.,* 2014). The results of this research highlight the efficacy of amikacin compared with tobramycin and gentamicin in the treatment of bacterial infections caused by *E.coli*. These findings highlight the scale of the problem of resistance to aminoglycosides in *E.coli on an* international scale.

With regard to fluroquinolones, the rate of resistance to ciprofloxacin in the present study was 80.56%. The results of the present study are similar to those obtained by some authors in Africa. In Burkina Faso, Ouédraogo *et al.,* (2016) had reported that 80% of strains were resistant to ciprofloxacin. Similarly Mathlouthi *et al.,* (2016) showed in their work that 80% of strains were resistant to ciprofloxacin. Rafai *et al., (*2015) in the Central African Republic showed that 84.8% of the strains tested were resistant to ciprofloxacin. In Côte d'Ivoire, Gadou *et al.,* (2019) obtained a higher resistance rate than our results (86.7%). Ouattara *et al.,* (2014) reported a resistance rate of 93.2% to ciprofloxacin. The resistance rate of *E. coli* strains in our study was 72.22% for ofloxacin. A study carried out in Sudan gave a high resistance rate (80%). In a study carried out by Fissou *et al.,* (2019) in Chad, a resistance rate of 50.09% to ofloxacin was obtained. This resistance is thought to be due to the over-prescription of ofloxacin by healthcare professionals. The high resistance rate could be explained by the fact that fluoroquinolones are the most widely prescribed drugs after β-lactams in Africa (Dosso *et al.,* 2000).

Cotrimoxazole proved ineffective against *E. coli* strains, with a resistance rate of 88.89%. The ineffectiveness of this antibiotic must be taken into account. This rate is confirmed in Chad by Yandai *et al.,* (2014) with a resistance of 100%. Several studies have revealed high levels of *E. coli* resistance to cotrimozaxole: in Nigeria 91% (Iroha *et al.,* 2009); in Sudan 98.6% (Ibrhim *et al.,* 2013) and in France 50 to 80% (Goldstein, 2006). The high level of *E. coli* resistance to cotrimoxazole is thought to be linked in part to over-consumption of the drug. A new therapeutic approach will therefore have to be adopted in the coming years to reduce the ineffectiveness of this antibiotic.

Table.1 Resistance to β-lactam antibiotics

Families	Antibiotic	Disk loading in µg	Critical value	Intermediate resistance n=36(%)	Sensitivity n=36(%)
Aminopenicillin	Amoxicillin/AC (AUG)	30	13-18	31(86,11)	5 (13,89)
Cephalosporins	Amoxicillin (AMX)	10	13-17	30 (83,33)	6 (16,67)
	Cefotaxime (CTX)	30	14-23	31 (86,11)	5 (13,89)
	Ceftriaxone (CRO)	30	13-21	30(83,33)	6 (16,67)
	Cefepime (FEP)	30	14-18	26 (72,22)	10 (27,88)
	Ceftazidime (CAZ)	30	14-18	21 (63,60)	15 (41,4 0)
C4G Carbapenes	Imipenene (IMI)	10	13-16	0 (0,0)	36 (100,0)

Table.2 Aminoglycoside resistance

Family	Antibiotic	Disk loading in µg	Critical value	Intermediate resistance n=36(%)	Sensitivity n=36(%)
Aminoglycosides (Aminoside)	Gentamicin (CN)	10	12-15	28 (77,78)	8 (22,22)
	Tobramycin (TOB)	10	12-15	29 (80,56)	7 (19,44)
	Amikacin (AK)	30	14-17	16 (44,44)	20 (55,56)

Table.3 Resistance to quinolones and fluor quinolones

Family	Antibiotic	Disk loading in µg	Critical value	Intermediate resistance n=36(%)	Sensitivity n=36(%)
Quinolones	Nalidixic acid (AK)	30	13-19	25 (69,44)	11 (30,56)
Fluoroquinolone	Ciprofloxacin (CRP)	5	15-21	29(80,56)	7 (19,44)
	Ofloxacin (OFX)	5	12-16	26 (72,22)	10 (27,78)

Infections caused by *E. coli* resistant to several families of antibiotics pose therapeutic impasses. The search for alternatives to control the phenotypic mechanisms of resistance involves several factors. The strains in question have virtually developed acquired resistance to the antibiotics currently used in clinical practice. This situation is linked to the circulation of multi-resistant strains in community and hospital settings, and represents a serious threat to the treatment of *E. coli* bacterial infections in the future. The use of antibiotics must therefore be based on laboratory results proving not only their efficacy against the isolated pathogen responsible for the infection observed, but also the sensitivity of the pathogen to possible antibiotics.

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Author Contribution

Bertille Dewa: Investigation, formal analysis, writing—original draft. Bessimbaye Nadlaou: Validation, methodology, writing—reviewing. Nadjiroum Ngam-Asra:—Formal analysis, writing—review and editing. Abdoulaye Hissein Ousman: Investigation, writing—reviewing. Pedros Mitan: Resources, investigation

writing—reviewing. Brahim Boy Otchom: Validation, formal analysis, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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