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Resistance of Clinical Isolates of *Escherichia coli* and *Klebsiella pneumoniae* in "Boucle du Mouhoun, Burkina Faso": one year's Experience in Antibiotic Resistance Surveillance

Kafando H.[1], Zangréyanogo H.[1*], Dionou P.[2], Bayala D.[1], Seihon M.[1], Traoré N.[1], Barro M.[1], Dipama S.[1], Nikiema G.[1], Ramdé D.[1], Ouédraogo A-S.[3, 4]

[1]Department of the laboratories of medical biology of the Regional Hospital Center of Dedougou, BP 61 Dedougou, Burkina Faso

[2]Department of surgery of the Regional Hospital Center of Dedougou, Burkina Faso

[3]National Reference Laboratory for the Control of Antimicrobial Resistance (NRL-AMR), Bobo Dioulasso, Burkina Faso

[4]National Institute of Health Sciences, NAZI BONI University, Bobo Dioulasso, Burkina Faso

ABSTRACT

Introduction: *Escherichia coli* and *Klebsiella pneumoniae* account for a large proportion of clinically isolated pathogenic bacteria. However, their resistance to antibiotics is increasingly becoming a global health threat. The aim of this study was to describe the current antibiotic resistance profile of these two species.

Materials and method: This was a retrospective descriptive study at the Dedougou regional hospital. The results of antibiotics susceptibility testing of non-redundant clinical isolates of Enterobacteriaceae were used. Bacteria were isolated and identified using standard bacteriology methods. The antibiogram was performed by the Kirby-Bauer method and the interpretation was made according to the recommendations of the Antibiogram Committee of the French Microbiology Society (CASFM 2017). Data were entered into WHOnet 2018 and analysed using EPI-INFO 7.2.4.0.

Results: A total of 138 non-redundant Enterobacteriaceae strains were isolated, of which almost 90% were *E. coli* (75.4%) and *Klebsiella pneumoniae* (13.8%). The most frequent resistance was observed with amoxicillin + clavulanic acid (81.3%), ceftriaxone (66.7%) and cotrimoxazole (82.9%). *E. coli* showed very high resistance to ampicillin (95.2%). Relatively moderate to high resistance was also observed with ciprofloxacin 69.1% and gentamicin 39%. The most active antibiotics were imipenem and cefoxitin, with resistance frequencies of 2.4% and 5.7% of all strains respectively. ESBL-producing strains were the most frequently encountered phenotypes (59.3%), followed by high-level penicillinases (19.5%). 3GC resistance was associated to ESBL production in almost 90% of cases, and both ciprofloxacin and gentamicin resistance were significantly associated with 3GC resistance ($p<0.001$).

Conclusion: *Escherichia coli* and *Klebsiella pneumoniae* are the main enterobacteria isolated in clinics. The production of extended-spectrum beta-lactamases is their main mechanism of resistance to beta-lactam antibiotics.

KEYWORDS: Enterobacteriaceae, ESBL, antibiotic resistance

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INTRODUCTION

Antibiotic resistance is on the increase worldwide. Long considered to be a hospital phenomenon, antibiotic resistance has become a global public health problem and is found in all ecosystems (environment, human, animal). Enterobacteriaceae account for a large proportion of pathogenic bacteria isolated in clinics (1). Among them, Escherichia coli and Klebsiella pneumoniae are edifying examples of acquired resistance to antibiotics, since they are the bacteria most frequently implicated in infectious pathology. Over 70% of enterobacteria isolated in clinics belong to these two species (2). The laboratory at the Boucle du Mouhoun Regional Hospital has been one of the sentinel

sites for monitoring antimicrobial resistance (AMR) since January 2022.

The aim of this study was to describe the current resistance profile of clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* in the laboratory of the Boucle du Mouhoun regional hospital after one year's experience in AMR surveillance.

MATERIALS AND METHOD

Type and period of study: This was a retrospective descriptive study covering a 12-month period from 01 January to 31 December 2022 in the bacteriology unit of the multipurpose medical biology laboratory of the Dedougou regional hospital, Boucle du Mouhoun region, Burkina Faso. It concerned all the results of antibiotic susceptibility tests on patients with enterobacterial infections.

Inclusion criteria: antibiograms of clinical strains of Enterobacteriaceae were included in this study. Clearly identified duplicates were excluded (isolation of the same species with the same sensitivity profile in the same patient).

Isolation and identification of bacteria: bacteria were isolated on selective or enriched agar media depending on the samples. Identification was carried out using conventional methods based essentially on morphology, cultural and biochemical characteristics using API20E identification strips (BioMerieux).

Antibiotic susceptibility testing: all antibiograms were performed using the diffusion method on Mueller Hinton medium (Kirby-Bauer method). The following antibiotics were tested (Thermo Fisher Diagnostics, Oxoid, France): ampicillin (AM) 10µg, amoxicillin-clavulanic acid (AMC) 20-10µg, ceftriaxone (CRO) 30µg, ceftazidime (CAZ) 10µg, cefepime (FEP) 30µg, cefoxitin (FOX) 30µg, imipenem (IMP) 10µg, gentamicin (GN) 10µg, ciprofloxacin (CIP) 5µg, trimetoprim-sulfamethoxazole (SXT) 1.25-23.75µg. The screening for ESBL secretion was established by a synergy test involving a central disc of amoxicillin-clavulanic acid 30mm away from a disc of 3rd generation cephalosporin (C3G) or 4th generation cephalosporin (4GC). The antibiogram was interpreted according to the recommendations of the French Microbiology Society Antibiogram Committee 2017 (CA-SFM) (3).

Statistical analyses: the data from this work were entered into WHOnet 2018 and analysed in EPI-INFO 7.2.4.0. A Chi2 test with $p < 5\%$ as the statistical significance threshold was used to compare the different proportions. Tables and graphs were produced using Excel 2013.

Ethical considerations: Personal data collected on patients was not disclosed.

RESULTS AND DISCUSSION

A total of 138 strains of Enterobacteriaceae were isolated in 2022, almost 90% (123/138) of which were *E. coli* (104; 75.4%) and *Klebsiella pneumoniae* (19; 13.8%) (Figure 1). The strong involvement of *Klebsiella pneumoniae* and *E. coli* in infectious pathologies no longer needs to be demonstrated, even if the frequencies vary greatly from one study to another. Sawadogo *et al* reported that *E. coli* accounted for more than 33% of the bacteria responsible for bacteraemia (1). In urinary tract infections, this tendency is practically the rule, with *E. coli* frequencies varying between 50 and 80 % (4), (5), (6) and *Klebsiella pneumoniae* around 10% (6).

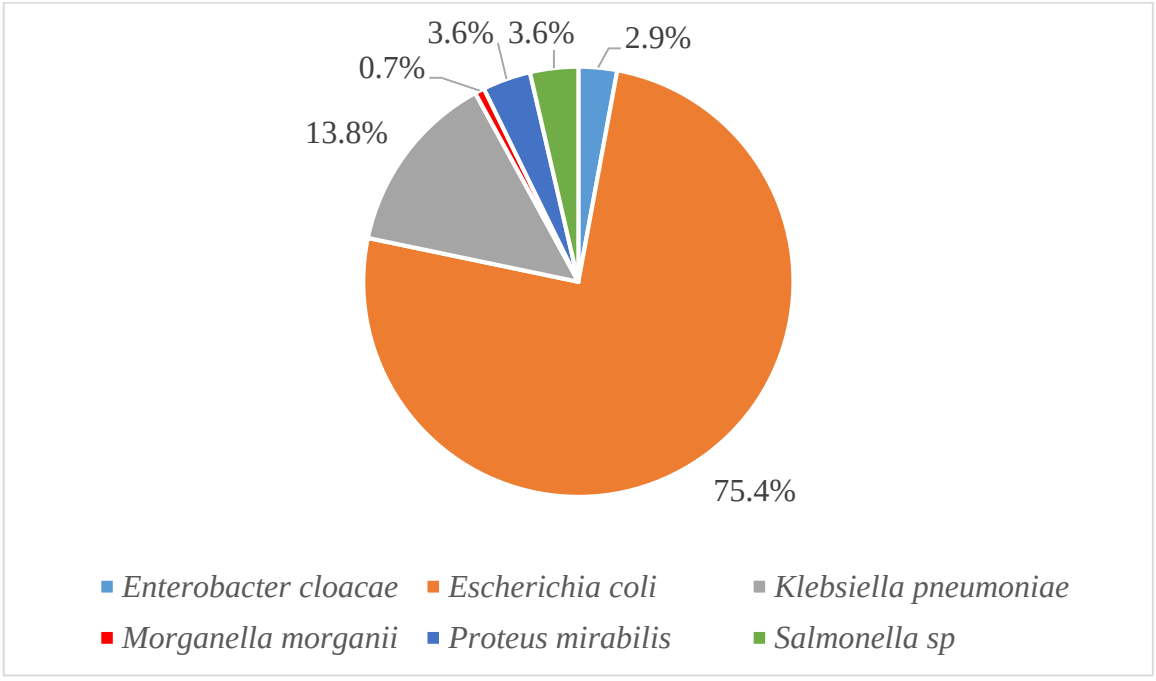

Figure 1: Distribution of enterobacteria species isolated

In this study, isolates came mainly from urine 71.5% (88/123), pus 17.9% (22/123), blood cultures 4.9% (6/123), genital samples 4% (5/123) and faeces 1.6% (2/123). Urinary tract infection is one of the most common bacterial infections,

making urine cytobacteriological examination one of the most frequently prescribed tests. Similar results were reported by Diawara *et al*, who found that 82.68% of isolates in their study came from urine, followed by pus (5.59%) (7).

In terms of acquired resistance to the antibiotics tested, *Escherichia coli* strains showed more resistance than *Klebsiella pneumoniae* strains overall. Resistance to aminopenicillins, penicillins + beta-lactamase inhibitors, 3rd generation cephalosporins, sulphonamides and fluoroquinolones was by far the most frequent, with 81.3% (100/123) to amoxicillin-clavulanic acid (81.7% vs. 79%); 66.7% (82/123) to ceftriaxone, i.e. 67.3% versus 63.2%; 82.9% (92/111) to cotrimoxazole, i.e. 86% versus 66.7% respectively in *E. coli* and *Klebsiella pneumoniae*. *E. coli* showed very high resistance to ampicillin 95.2% (99/104) (figure 2). Relatively moderate to high resistance was also observed with ciprofloxacin 69.1% (85/123) or 75% versus 36.8% and gentamicin 39% (48/123) or 36.5% versus 52.6%. The most active antibiotics were imipenem and cefoxitin, with resistance frequencies of 2.4% (3/123) and 5.7% (7/123) respectively (Figure 2).

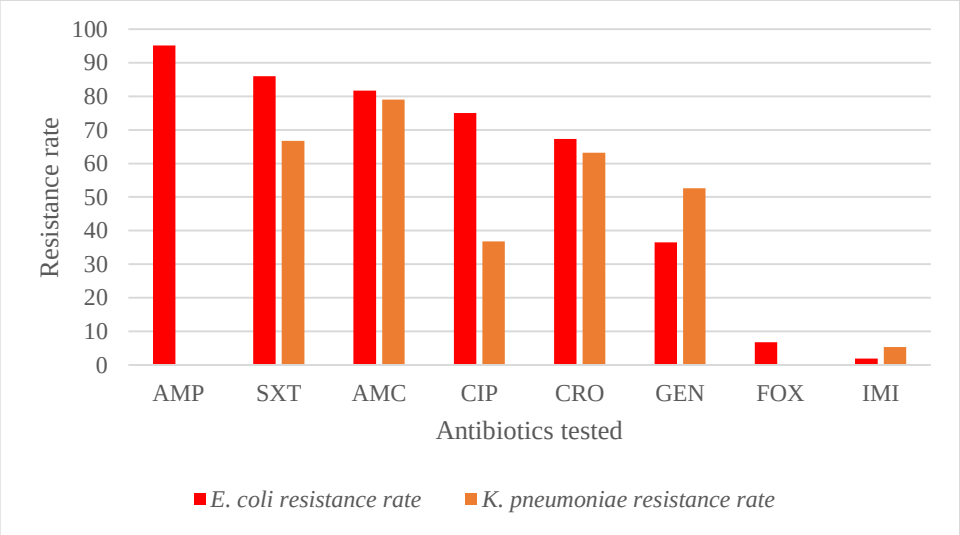

Figure 2: Antibiotic resistance profile of the strains studied

A study of the antibiotic susceptibility of the strains shows that there is no antibiotic or family of antibiotics spared from the phenomenon of bacterial resistance. These results are similar to those described in several studies(6), (7), (8),(9). Betalactam antibiotics are a family of antibiotics that are highly homogeneous structurally, pharmacologically and therapeutically, and are now essential antibiotics in the antibiotic therapy chain(10). The high levels of resistance to these antibiotics are thought to be the result of selection pressure due to their excessive use. Imipenem and cefoxitin are among the antibiotics still active on the vast majority of strains studied. These results are superimposed on those reported in other studies(5), (8), (11).

Resistance to 3GCs was associated to ESBL production in almost 90% of cases, and resistance to ciprofloxacin and gentamicin were significantly associated with resistance to 3GCs (p<0.001) (table 1).

Table 1: Co-resistance of isolated strains to ciprofloxacin and gentamicin

Ciprofloxacin	**CRO-R**	**CRO-S**	**Total**	
Resistant n (%)	70 (85,4)	15 (36,6)	85 (69,1)	
Sensitive n (%)	12 (14,6)	26 (63,4)	38 (30,9)	P< 0,001
Total n (%)	82 (100)	41(100)	123 (100)	
Gentamicin	**CRO-R**	**CRO-S**	**Total**	
Resistant n (%)	46 (56,1)	2 (4,9)	48 (39)	
Sensitive n (%)	36 (43,9)	39 (95,1)	75 (61)	P< 0,001
Total n (%)	82 (100)	41 (100)	123 (100)	

Co-resistance of ESBLs to fluoroquinolones and aminoglycosides is commonly described in the literature (5), (7). Co-resistance of ESBLs to quinolones is most often linked to the presence of mobile resistance genes qnr (A, B and S alleles) and especially the aac(6')-Ib-cr gene conferring dual aminoglycoside-piperazinyl-amine-quinolone resistance, which are frequently found in CTX-M plasmid carriers (12). CTX-Ms are also often associated with AAC(3) and/or AAC(6') type aminoglycoside acetylases, conferring variable resistance patterns for gentamicin and amikacine (13).

As for beta lactam resistance phenotypes, ESBL-producing strains were the most frequently encountered phenotypes (59.3%), i.e. 59.6% in *E. coli* versus 57.9% in *Klebsiella pneumoniae*, followed by high-level penicillinases (19.5%). Low-level penicillinases and wild-type strains were the least common, with frequencies of 7.3% and 6.5% respectively (Figure 3).

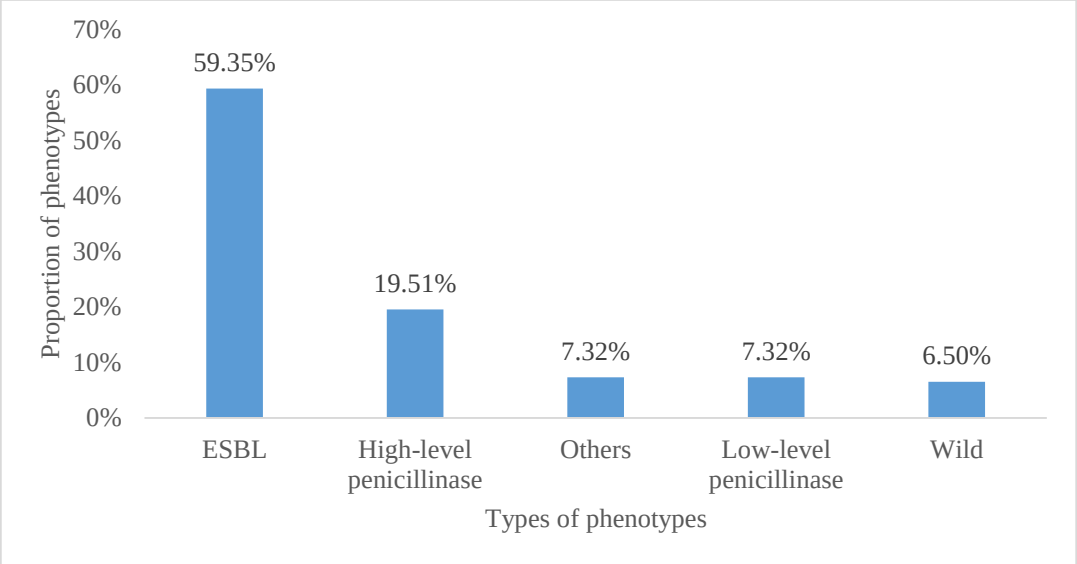

Figure 3: Distribution of beta-lactam resistance phenotypes of the strains studied

The predominance of ESBL-producing strains within the *E. coli* and *K. pneumoniae* species was reported by Diawara *et al.* (7). The current spread of ESBLs among enterobacteria, described as a pandemic, is thought to be due to CTXM-type ESBLs, which are carried by mobile genetic elements, in this case plasmids (14). The advent of these types of ESBLs has completely changed epidemiological trends, with E. coli in the lead, unlike the traditional ESBLs derived from TEM or SHV penicillinases, which mainly spread among *Klebsiella* (13). Traditional ESBLs retained the property of being inhibited by beta-lactamase inhibitors such as clavulanic acid, tazobactam and sulbactam (13).

In addition, the high rates of ESBLs reflect a number of factors, some of which are specific to West African countries, which have an impact on the emergence and spread of AMR, led by the misuse of antibiotics:

- Public health policies relating to access to medicines:

Policies on access to medicines, with the introduction of the Bamako initiative, have focused much more on the accessibility of medicines, and antibiotics in particular, without any fundamental concern for the circuit. In the WHO African region, pharmacist coverage is low and unevenly distributed. It is less than 2.4 pharmacists per 10,000 inhabitants (15). This has led to a decentralisation of the public pharmaceutical distribution circuit at all levels of the health pyramid (1st level, 2nd level, 3rd level), with a poor distribution of pharmaceutical professionals such as pharmacists, who are most often found at central level (3rd level), to the detriment of peripheral and rural areas(16). This dichotomy has led to an increase in the consumption of antibiotics. In sub-Saharan Africa, antibiotics are often bought directly at the point of sale, without prescription or advice from qualified personnel. Added to this are antibiotics sold on the parallel market, which is highly developed in West Africa, and whose therapeutic indications are often vague and broad (16). Alongside the formal distribution circuit, a parallel circuit has developed, particularly in French-speaking Africa. Initially encouraged by the malfunctioning of the formal circuit (poor geographical distribution, stock shortages), the illicit market is now sustained by the globalisation of production and the circulation of industrial pharmaceutical medicines, as well as by the disengagement of governments from pharmaceutical distribution (16).

- Organisation of the public health system:

Since the advent of the Bamako initiative, the desire to improve access to care has led to the structuring of the public health system into 3 levels (the peripheral level or 1st level, the intermediate level or 2nd level and the central level or 3rd level) depending on the technical facilities and care on offer. In most cases, bacteriological diagnosis is only possible at the central level, and to a lesser extent at the intermediate level. This inaccessibility to microbiological diagnosis and assessment of antibiotic resistance, due to a lack of infrastructure and qualified staff, has fundamentally contributed to the over-consumption of antibiotics(17). The way the healthcare system is organised is such that by the time patients reach the 3rd level, they have received several treatment by antibiotics from the 1st level and the 2nd level. This cascade of antibiotic treatments is undoubtedly responsible for the unprecedented pressure at the root of ESBL selection, with patients arriving at the central level having used almost all the antibiotics available on the market with multi-resistant bacteria, so that even after a well-

established microbiological diagnosis, the therapeutic options are very limited, with only carbapenems as the last alternative. The ultimate consequence is an increase in the prescription of carbapenems and a selection of carbapenem-resistant bacteria that very often lead to therapeutic impasses.
- Treatment based on syndromic algorithms:
The endemicity of infectious diseases in the West African region no longer needs to be demonstrated, and combined with the difficulty of access to biological diagnosis, the ministries in charge of health in the various countries have developed and made available to practitioners at the first level of the health pyramid, diagnostic and treatment guides (DTG) to enable the standardisation of diagnostic and therapeutic attitudes. The development of DTGs is one of the measures taken to preserve essential generic medicines (18). The DTG consists of algorithms or flowcharts and treatment protocols for priority diseases. The main aim of these guidelines was to improve the quality of care by standardising the preventive, diagnostic and therapeutic attitudes of healthcare staff (19). Unfortunately, the misuse of these antibiotic therapy guidelines instead of microbiological documentation has led to overuse of antibiotics. As evidence of this, 3rd generation cephalosporins such as ceftriaxone and cefotaxime, whose indication in the DTG is limited to the treatment of acute bacterial meningitis, are used in almost all febrile episodes in the periphery. In addition, the DTG's shortcomings are also to be deplored, while learned societies and even the national guide to good antibiotic prescribing advise against the systematic use of antibiotics for rhinopharyngitis, bronchiolitis and other viral infections such as measles (20), (21), the GDT recommends the administration of amoxicillin or cotrimoxazole in addition to symptomatic treatment in children under 5 years of age (19).
In this study, high-level penicillinases were found in almost one-fifth (19.5%) of the strains studied, second only to ESBL phenotypes. These enzymes hydrolyse penicillins and penicillins + beta-lactamase inhibitors. The high expression of high-level penicillinases within the E. coli species has been reported in Cameroon and Madagascar (8), (22). This resistance could be explained by a reduction in the activity of the beta-lactamase inhibitor (clavulanic acid), as a result of hyperproduction of penicillinase, or by inactivation of the inhibitor itself (8). In addition, the high prescription of the amoxicillin-clavulanic acid combination is thought to be the source of selection pressure. Low-level penicillinases and wild strains were the least frequently encountered, with frequencies of 7.3% and 6.5% respectively. Gonsu Kanga *and al* (22) reported 13.6% low-level penicillinases compared with 6.8% wild-type phenotypes in E. coli strains involved in urinary tract infections in the community.

CONCLUSION

Escherichia coli and *Klebsiella pneumoniae* are the main *Enterobacteriaceae* isolated in clinics. The production of extended-spectrum beta-lactamases is the main mechanism of beta-lactam resistance in the strains studied. These strains have associated resistance to fluoroquinolones and aminoglycosides. In the light of these data, it is vital to step up AMR surveillance in order to gain a better understanding of the local epidemiology of resistance in relation to infections, and to readapt probabilistic treatments.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest in connection with this article.

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