**Prevalence of beta-lactam-resistant*, Escherichia coli* strains isolated in healthcare settings in the commune of Lomé, Togo**

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ABSTRACT

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| **Aims:** This study focuses on beta lactam resistance in, *Escherichia coli* (*E. coli*) strains isolated from healthcare facilities in the commune of Lomé  **Study design:** This descriptive study was conducted over a six-month period (February - July 2024) on all beta-lactam-resistant*, E. coli* strains isolated from all types of bacteriological samples received at the CHUs Sylvanus Olympio (SO) and Campus and at Bè Hospital.  **Methodology:** The Kirby-Bauer disc diffusion method was used for antibiotic susceptibility testing and the results were interpreted according to the guidelines of the Antibiogram Committee of the French Society of Microbiology and The European Committee on Antimicrobial Susceptibility Testing (CA-SFM / EUCAST, 2023). ESBLs was carried out using the classic method based on the detection of synergy between an amoxicillin-clavulanic acid disk and two third-generation cephalosporin disks (cefotaxime and ceftazidime) and a fourth-generation cephalosporin disk (cefepime).  **Results:** Of the 173 ß-lactam-resistant*, E. coli* strains collected, 65.90% (114/173) came from CHU SO, 21.38% (37/173) from Chu Campus and 12.72% (22/173) from Hospital de Bè. The overall significantly prevalence rate of extended-spectrum beta-lactamase-producing E. coli isolates were 93,64% (108/173)) and rate of carbapenem-resistant*, E. coli* isolates were 6,35% (11/173). Prevalence significantly rates of extended-spectrum beta-lactamase-producing*, E. coli* isolates were 94,64% (108/114) from CHU SO, followed by 94,59% (35/37) from Chu Campus and 86,36% (19/22) from Hospital de Bè. Prevalence of carbapenem-resistant*, E. coli* isolates were 5,26% (6/114), 5,41% (2/37), and 6,36% (3/22) at CHU SO, Chu Campus, and Hospital de Bè, respectively.  **Conclusion:** This study highlights the emergence of *E coli* BLSE and Carba strains in the commune of Lomé, and therefore probably multi-resistant strains everywhere. Determining the prevalence of multi-resistant bacteria remains a means of monitoring resistance and the emergence of antibiotics. |

*Keywords: Prevalence, beta-lactam-resistant, healthcare, Escherichia coli*

1. INTRODUCTION

Infections caused by antimicrobial resistance (AMR) are responsible for the deaths of around 700,000 people throughout the world every year, and this number is anticipated to climb to 10 million by 2050 (Gharavi *et al.,* 2021). Geographical differences may have an impact on the prevalence rates of ESBL seen across various investigations (Mohammed *et al*., 2016). The emergence and spread of bacteria that have acquired resistance to antibiotics is a huge public health problem (Rastogi *et al*., 2012). The direct consequence of antibiotic resistance is the increase in morbidity and mortality due to infectious diseases (Hailaji *et al.,* 2016). Beta lactam antibiotics are of critical importance to human health.

Since the early 2000, the number of human infections due to C3G-resistant Enterobacteriaceae, and in particular producers of Extended-Spectrum-Lactamases (ESBL), has increased (Madec *et al.,* 2015). From 2002 to 2017, the proportion of, *E. coli* species among ESBL-producing Enterobacteriaceae rose from 19% to 54% (Santé publique France, 2019).

A first approach in the fight against antibiotic resistance involves prevalence studies of multi-resistant strains multi-resistant strains circulating in hospitals as well as their sensitivity to antibiotics. In Cameroon, research revealed 14.3% of Extended-Spectrum Beta-Lactamase Beta-Lactamases (Gangoué-Piéboji *et al.,* 2005). At Benin, in the department of Zou et Collines, 22% of ESBL *E. coli* strains were isolated were isolated (Ahoyo *et al*., 2007). Togo, like these countries, is undoubtedly not spared spared from the problem, as evidenced by the results reported by several authors (Dagnra *et al*., 2000; Karou *et al*., 2012).

The World Health Organization designated ESBL-producing *Enterobacteriaceae* as the highest public health risk in its 2021 report. The present study aimed to determine the prevalence of beta-lactam-resistant *E coli* isolates in three health facilities in Lomé.

2. material and methods

This descriptive study was conducted over six months from February to July 2024 on all beta-lactam-resistant*, E. coli* strains isolated from all types of bacteriological samples at CHU Sylvanus Olympio, Campus, and Bè Hospital.

**2.1 Isolation and identification**

Urine, vaginal swab, pus, effusion, sperm, and blood culture samples received at SO and Campus CHUs, then Bè Hospital, were plated on blood agar, Uriselect, CLED and EMB media and incubated for 24 hours at 37°C. Any cultures observed at the end of this incubation period were identified using the API 20E gallery when they were GRAM-negative bacilli after GRAM staining.

**2.2 Susceptibility testing and detection of ESBLs**

Antibiotic susceptibility testing was carried out using the disk diffusion method on agar (Müller Hinton medium) and interpreted after measurement of inhibition diameters according to the recommendations of the EUCAST / CA-SFM (Antibiogram Committee of the French Society of Microbiology and The European Committee on Antimicrobial Susceptibility Testing), version 2023. Twenty antibiotics such us Ampicillin (AMP; 10µg), Amoxicillin + clavulanic acid (AMC; 20-10µg), Ticarcillin (TIC; 30µg), Imipenem (IPM; 10µg), cefalexin (CFL; 30µg), Cefoxitin ((FOX; 30 μg), Ceftazidime (CAZ; 30µg), Amikacin (AK; 30µg), Ceftriaxone (CRO; 30µg), Cefotaxime (CTX; 30µg), Gentamicin (CN; 10µg), Chloramphenicol (C; 30µg), Colistin (CT; 10µg), Ciprofloxacin (CIP; 05µg), Pefloxacin (PEF; 05µg), Tetracycline (TET; 30µg), Ofloxacin (OFX; 05µg), Levofloxacin (LEV; 05µg), Doxycycline (DO; 30µg), Trimethoprim + sulfamethoxazole (SXT; 1,25-23,75) were tested.

The search for ESBLs was carried out using the classic method based on the detection of synergy between an amoxicillin-clavulanic acid disk and two third-generation cephalosporin disks (cefotaxime and ceftazidime) and a fourth-generation cephalosporin disk (cefepime). Although all the above-mentioned discs were tested, our study focused on Ertapenem, Imipenem, Aztreonam, and Temocillin in addition to ESBL detection discs.

**2.3 Statistical evaluation**

Data processing was performed using STATA® statistical processing software version 15. The variables were described by their absolute (n) and relative (%) frequencies. Microsoft Excel® 2019 software was used to make the graphs.

3. results and discussion

**3.1 Distribution of beta-lactam resistant*, E. coli* isolates according to type of specimen**

A total of 3246 samples were collected from all sites. The isolation frequency for *E. coli* strains was 25.29% (821/3246) and for beta-lactam-resistant *E. coli* 21.07% (173/821). Beta-lactam-resistant *E. coli* isolates were obtained from urine cultures and vaginal swabs at CHU SO and Campus and Bè Hospital. Others were obtained from blood cultures at both CHUs and pus at CHU SO. The beta-lactam-resistant *E. coli* isolates were predominantly from urine, i.e. 73.41% (127/173). A few isolates were obtained from vaginal swabs, pus, and blood cultures. However, no isolates were obtained from pleural fluid, semen, or cerebrospinal fluid. Figure 1 below illustrates these data.

**Figure 1: Distribution by sample type and location of beta-lactam-resistant, *E. coli* isolates**

These results agree with those of Toudji *et al.,* 2017, who obtained a frequency of isolation of *E coli* of 22,44% and a predominance of *E coli* in the urine of 54,94%. Our result is even closer to that of Alghamdi *et al*., 2023, who obtained a predominance of 63% of *E coli* in urine.

**3.2 *E. coli* isolates phenotypic profile**

Of the 173 ß-lactam-resistant *E. coli* strains collected, 65.90% (114/173) were from CHU SO, 21.38% (37/173) from Chu Campus and 12.72% (22/173) from Bè hospital. The overall prevalence rate of extended-spectrum beta-lactamase-producing *E. coli* isolates was 93.64% (108/173) and the carbapenem-resistant *E. coli* isolates rate was 6.35% (11/173). By site, the prevalence rate of extended-spectrum beta-lactamase-producing *E. coli* isolates was 94.64% (108/114) from CHU SO, followed by 94.59% (35/37) from Chu Campus and 86.36% (19/22) from Bè hospital. Also, by site, the prevalence of carbapenem-resistant *E. coli* isolates was 5.26% (6/114), 5.41% (2/37), and 6.36% (3/22) in CHU SO, Chu Campus, and Bè hospital, respectively. Figure 2 below illustrates these results.

**Figure 2: ESBL and carbapenem resistant *E. coli* prevalence rates**

The prevalence rate of ESBL *E coli* in our study varies around 90% in all health centers. This prevalence is slightly higher than that reported by Malande *et al.,*2019 who obtained a prevalence of 63%. Other authors have also recently reported impressive results. In their studies, the highest prevalence (52.9%) was recorded in Ivory Coast in 2018 (Yao *et al*., 2018), while the lowest prevalence (16.1%) was reported in Tunisia in 2019 (Sghaier *et al*., 2019). In Asia, ESBL *E. coli* prevalence reached 66.4% in 2022 in countries such as China and Pakistan (Liu *et al.,* 2022; Shafiq *et al*., 2022), with the lowest value of 3.7% in 2020 in Malaysia and South Korea (Song *et al*., 2020; Kamaruzzaman *et al.,* 2020). In the Americas, a higher prevalence of 48.3% was observed in the northern region, such as the United States (Carey *et al.,* 2022), in contrast to the southern region, where a prevalence of 3.0% was reported in countries such as Chile (Benavides *et al.,* 2021). Spain, France, the United Kingdom, the Netherlands, and Italy reported ESBL *E. coli* in cattle, with the highest value (24.5%) reported in 2023 (Kerluku *et al*., 2023). Although these differences may be due to differences in the geographical location of the studies, they are evidence of the emergence of ESBLs on a global scale.

Our study reported very low rates of resistance to carbapenems (7%), in contrast to Nasser *et al*., 2020 who reported a significant 64.4%. This difference could be explained by the fact that their study was conducted in Arabia and lasted 20 years, whereas ours was conducted in six months and in Togo.

**3.3** **Antibiotic resistance profile of *E. coli* isolates**

Although our study focused on beta-lactam resistance, we also tested other antibiotic families such as aminoglycosides and fluoroquinolones. We found that all strains were resistant to the other antibiotics tested. Overall, all isolates were 100% resistant to cefotaxime, ceftriaxone, and aztreonam (n=173). High levels of resistance, around 90%, were observed for cefepime and fluoroquinolones (ciprofloxacin, levofloxacin). Resistance to amoxicillin + clavulanic acid and gentamicin is around 70%. However, low levels of resistance are observed for carbapenems (5-10%). Table 1 below shows the results of susceptibility testing for the different antibiotics in the strains collected.

**Table 1: Resistance rate of the collected *E. coli* isolates to the tested antibiotics**

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| Antibiotics | CHU SO  (n=114) | CHU Campus  (n=37) | Bè hospital  (n=22) |
| Ampicillin | 100% | 100% | 100% |
| Amoxicillin + Clavulanic Acid | 82,45% | 72,98% | 77,28% |
| Cefotaxime | 100% | 100% | 100% |
| Ceftriaxone | 100% | 100% | 100% |
| Ceftazidime | 82,86% | 81,98% | 82,51% |
| Cefepime | 95,61% | 94,6% | 95,46% |
| Cefoxitin | 77,35% | 72,97% | 72,98% |
| Aztreonam | 100% | 100% | 100% |
| Temocillin | 86,84% | 86,48% | 63,63% |
| Ertapenem | 8,77% | 10,81% | 22,72% |
| Imipenem | 7,89% | 5,4% | 13,64% |
| Ciprofloxacin | 92,10% | 91,89% | 86,36% |
| Levofloxacin | 90,35% | 86,48% | 86,36% |
| Gentamicin | 88,59% | 75,67% | 54,54% |
| Amikacin | 52,63% | 40,54% | 27,27% |

All isolates were resistant to ampicillin, cefotaxime, ceftriaxone, and aztreonam. These results are consistent with those of Saleem *et al*., 2017 in Pakistan, who showed that *E. coli*-producing extended-spectrum beta-lactamases (ESBLs) are resistant to several antimicrobials, particularly penicillins, and cephalosporins. Our results are also consistent with those of Abalkhail *et al.*, 2022. Indeed, these authors have shown that the highest antibiotic resistance among ESBL *E. coli* concerns the penicillin subclass (100% for ampicillin and 89.41% for amoxicillin-clavulanate), the cephalosporin subclass (99. 80% for cephalothin, 99.61% for cefotaxime and ceftriaxone individually, 99.41% for cefuroxime, 98.43% for ceftazidime and 97.45% for cefepime), the monobactam subclass (99.22% for aztreonam). Our study also shows a high level of resistance to fluoroquinolones and gentamicin. These results are much higher than those of Godonou *et al*., 2020 who showed average resistance to ciprofloxacin (58.4%), levofloxacin (39.53%), and gentamicin (9.30%).

Our study showed low resistance to carbapenems and moderate resistance to amikacin. These results contradict those of Godonou *et al*., 2020 and Alghamdi *et al.*, 2023 who demonstrated the absence of resistance to these antibiotics.

4. Conclusion

This study allowed us to determine the level of resistance of ESBL and carbapenem-resistant *E. coli* in the hospital environment of the commune of Lomé. A rate of 90% of ESBL and 7% of carbapenem-resistant bacteria demonstrates the urgency of the fight against the emergence of antibiotic resistance. however, other complementary studies on the characterization of these bacteria will allow us to determine the level of virulence to better orientate the guidelines for this fight in our region.

References

1. Abalkhail, A., Alyami, A. S., Alrashedi, S. F., Almushayqih, K. M., Alslamah, T., Alsalamah, Y. A., Elbehiry, A. (2022). The Prevalence of Multidrug-Resistant *E. coli* Producing ESBL among Male and Female Patients with Urinary Tract Infections in Riyadh Region, Saudi Arabia healthare, *10*, 1778. <https://doi.org/10.3390/healthcare10091778>
2. Ahoyo, A. T., Baba-Moussa, L., Anago A. E., Avogbe, P., Missihoun T. D., Loko F., Prévost G., Sanni A., Dramane, K. (2007). Incidence d’infections liées à *E. coli* producteur de bêta lactamases à spectre élargi au Centre hospitalier départemental du Zou et Collines au Bénin. Médecines et Maladies Infectieuses, 37(11): 746-752.
3. Alghamdi, S. A. A., Mir, S. S., Alghamdi, F. S., Al Banghali, M. A. M. M. A., Almalki, S. S. R. (2023). Evaluation of Extended-Spectrum Beta-Lactamase Resistance in Uropathogenic, *E. coli* Isolates from Urinary Tract Infection Patients in Al-Baha, Saudi Arabia. Microorganisms, *11*, 2820. <https://doi.org/10.3390/microorganisms11122820>
4. Godonou, A. M., Gbeasor-Komlanvi, F. A., Tsogbale, N., Lack, F., Dossim, S., Batawui, K. B., Ameyapoh, Y. A., Ekouevi, K., Dagnra, A. Y., and Salou, M., (2020). High Carrying Rate of Extended-Spectrum Beta-Lactamase (ESBL) Producing Enterobacteriaceae by Slaughterhouse Workers in Lomé, Togo in 2019. Microbiology Research Journal International, 30(11): 30-41. DOI: 10.9734/MRJI/2020/v30i1130281.
5. Benavides, J. A., Salgado-Caxito, M., Opazo-Capurro, A., González, M., P., Piñeiro, A., Otto M. M., Rivas, L., Munita, J., Millán, J. (2021). ESBL-Producing*, E. coli* Carrying CTX-M Genes Circulating among Livestock, Dogs, and Wild Mammals in Small-Scale Farms of Central Chile. Antibiotics, *10*, 510.
6. Carey, A. M., Capik, S. F., Giebel, S., Nickodem, C., Piñeiro, J. M., Scott, H. M., Vinasco, J., Norman, K. N. (2022). Prevalence and Profiles of Antibiotic Resistance Genes mph(A) and qnrB in Extended-Spectrum Beta-Lactamase (ESBL)-Producing*, E. coli* Isolated from Dairy Calf Feces. Microorganisms, *10*, 411
7. CASFM. (2023). French Society of Microbiology. https://www.sfm-microbiologie.org/wp-content/uploads/2023/06/CASFM2023\_V1.0.pd
8. Dagnra, A. Y., Tigossou, S., Prince-David, M. (2000). Prevalence and antimicrobial susceptibility of bacterial meningitis. Medecine and Infectious Diseases, 30: 291-294.
9. Gangoué-Piéboji, J., Bedenic, B., Koulla-Shiro, S., Randegger, C., Adiogo, D., Ngassam, P., Peter Ndumbe, P., Hächler, H. (2005). Extended-spectrum-β-lactamase producing Enterobacteriaceae in Yaounde, Cameroon. Journal of Clinical Microbiology, 43(7): 3273-3277.
10. Gharavi, M. J., Zarei, J., Roshani-Asl, P., Yazdanyar, Z., Sharif, M., Rashidi, N. (2021). Comprehensive study of antimicrobial susceptibility pattern and extended spectrum beta-lactamase (ESBL) prevalence in bacteria isolated from urine samples. Scientifics Reports, *11*, 578
11. Hailaji, N. S. M., Ould Salem, M. L., Ghaber, S. M. (2016). Antibiotic susceptibility of uropathogenic uropathogenic bacteria in the city of Nouakchott-Mauritania. Progrès urology, 26 : at 346-352.
12. Kamaruzzaman, E. A., Abdul Aziz, S., Bitrus, A. A., Zakaria, Z., Hassan, L. (2020). Occurrence and Characteristics of Extended-Spectrum β-Lactamase-Producing*, E. coli* from Dairy Cattle, Milk, and Farm Environments in Peninsular Malaysia. Pathogens, *9*, 1007
13. Karou, D. S., Balaka, A., Bamoké, M., Tchelougou, D., Assih, M., Anani, K., Agbonoko, K., Simpore, J., de Souza, C. (2012). Epidemiology and antibiotic resistance of bacterial meningitis in Dapaong, northern Togo. Asian Pacific Journal of Tropical Medecine. : 848-852.
14. Kerluku, M., Jankuloski, D., Manovska, M., Prodanov, M., Dimzoska, B., Dodovski, A., Blagoevska. (2023). K. β-Lactamase genes (blaCTX-M, blaSHV, blaTEM, blaOXA1 AND blaOXA2) and phylogenetic groups in ESBL producing commensal *E. coli* isolated from faecal samples from dairy farm in the Municipality of Debar. Macedonian Veterinary Review, *46*, 89–97.
15. Liu, Z., Wang, K., Zhang, Y., Xia, L., Zhao, L., Guo, C., Liu, X., Qin, L., Hao, Z. (2022). High Prevalence and Diversity Characteristics of blaNDM, mcr, and blaESBLs Harboring Multidrug-Resistant*, E. coli* From Chicken, Pig, and Cattle in China. Frontiers in Cellullar and Infection Microbiology, *11*, 755545.
16. Madec, J. Y., Haenni, M., Métayer, V., Saras, E., Nicolas-Chanoine, M. H. (2015). "High Prevalence of the Animal-Associated blaCTX-M-1 IncI1/ST3 Plasmid in Human, *E. coli* Isolates." Antimicrobial Agents and Chemotherapy. 59, 5860.
17. Malande, O. O., Nuttall, J., Pillay, V., Bamford, C., Eley, B. (2019) A ten-year review of ESBL and non-ESBL*, E. coli* bloodstream infections among children at a tertiary referral hospital in South Africa. PLOS ONE 14(9): e0222675. <https://doi.org/10.1371/journal.pone.0222675>
18. Mohammed, M. A., Alnour, T. M., Shakurfo, O. M., Aburass, M. M. (2016). Prevalence and antimicrobial resistance pattern of bacterial strains isolated from patients with urinary tract infection in Messalata Central Hospital, Libya. Asian Pacific Journal of Tropical Medecine, *9*, 771–776.
19. Nasser, M., Palwe, S., Bhargava, R. N., Feuilloley, M. G. J., Kharat, A. S. (2020). Retrospective Analysis on Antimicrobial Resistance Trends and Prevalence of β-lactamases in*, E. coli* and ESKAPE Pathogens Isolated from Arabian Patients during 2000–2020. Microorganisms, *8*, 1626. <https://doi.org/10.3390/microorganisms8101626>
20. Rastogi, V., Mishra, P. K., Bhatia, S. (2012). Emerging antimicrobial resistance in hospital a threat to public health. Indian Journal of Community Health, 24(4) : 260-263.
21. Saleem, R., Ejaz, H., Zafar, A., Younas, S., Rathore, A. W. (2017). Phenotypic characterization of extended-spectrum-beta—lactamase producing *E. coli* from healthy individuals, patients, sewage sludge, cattle, chickens and raw meat. Pakistan Jounal of Medical Sciences, 33(4):886-890.
22. Santé Publique France, (2019). "Surveillance des bactéries multirésistantes dans les établissements de santé. Réseau BMR-Raisin, France. Résultats 2017." Santé Publique France, 1-94.
23. Sghaier, S., Abbassi, M. S., Pascual, A., Serrano, L., Díaz-De-Alba, P., Said, M. B., Hassen, B., Ibrahim, C., Hassen, A., López-Cerero, L. (2019). Extended-spectrum β-lactamase-producing Enterobacteriaceae from animal origin and wastewater in Tunisia: First detection of O25b-B23-CTX-M-27-ST131 *,,E. coli* and CTX-M-15/OXA-204-producing *Citrobacter freundii* from wastewater. Journal of Global Antimicrobial Resistance 17, 189–194.
24. Shafiq, M., Rahman, S. U., Bilal, H., Ullah, A., Noman, S. M., Zeng, M., Yuan, Y., Xie, Q., Li, X., Jiao, X. (2022). Incidence and molecular characterization of ESBL-producing and colistin-resistant *E. coli* isolates recovered from healthy food-producing animals in Pakistan. Journal of Applied Microbiology, *133*, 1169–1182
25. Shah, C., Baral, R., Bartaula, B., Shrestha, L. B. (2019). Virulence factors of uropathogenic*, E. coli* (UPEC) and correlation with antimicrobial resistance. BMC Microbiology, *19*, 204.
26. Song, J., Oh, S. S., Kim, J., Park, S., Shin, J. (2020). Clinically Relevant Extended-Spectrum β-Lactamase-Producing *E. coli* Isolates From Food Animals in South Korea. Frontiers in Microbiology, *11*, 604.
27. Toudji, A. G., Djeri, B., Karou, S. D., Tigossou, S., Ameyapoh, Y., De Souza, C. (2017). Prevalence of extended-spectrum beta-lactamase-producing Enterobacteriaceae strains strains isolated in Togo and their sensitivity to antibiotics. International Journal of Biological and Chemical Sciences, 11(3): 1165-1177.
28. Yao, R. K., Coulibaly, J. K., Tiekoura, B. K., Yapi, F. H., Djaman, J. A. (2018). Molecular Characterisation of Extended-Spectrum Beta-lactamase Producing *,,E. coli* Isolated from Cattle Faeces in Abidjan District, Ivory Coast. Microbiological Research Journal International, 25, 1–10.