

Prevalence of Extended-Spectrum Beta-Lactamase-Producing Strains Isolated at Zinder National Hospital (ZNH) in 2021 and Their Antibiotic Susceptibility Profile

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How to cite this paper: Saïdou, M.S.F., Ousmane, A., Inoussa, A., Bachir, A.M., Boureima, H., Gora, L., Daouda, A.M., Mahamadou, D., Laouali, B.S.M., Boukar, S.M.B. and Saidou, M. (2023) Prevalence of Extended-Spectrum Beta-Lactamase-Producing Strains Isolated at Zinder National Hospital (ZNH) in 2021 and Their Antibiotic Susceptibility Profile. *Open Journal of Medical Microbiology*, **13**, 43-51. https://doi.org/10.4236/ojmm.2023.131004

Received: September 8, 2022 Accepted: February 20, 2023 Published: February 23, 2023

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Abstract

Purpose: Bacterial resistance to antibiotics has become a global public health problem. Enterobacteriaceae ESBL is among the most incriminated in this emergence which reduces the therapeutic possibilities. Thus, the objective of this study is to determine the prevalence of the extended-spectrum betalactamase (ESBL) producing Enterobacteriaceae at ZNH and their antibiotic susceptibility profile. Materials and Methods: This is a prospective study carried out over 5 months in all hospitalized and non-hospitalized patients in whom a culture was taken for the diagnosis of an infection. The search for ESBL is done by the double disc diffusion method. Results: In total, 21 out of 45 of our strains are ESBL-producing, *i.e.* a frequency of 46.7%. The mean age is 41.62 (\pm 22.90) with extremes of 2.6 - 78 years. The distribution of ESBL producing species showed a predominance of E. coli with 66.7% followed by K. pneumoniae and K. oxytoca each 9.5%. All ESBL strains were resistant to Amoxicillin, Cefalotin, Pipiracillin, Piperacillin + tazobactam, Ticarcillin, Ticarcillin + clavulanic acid. Resistance to C3G and Aztreonam was each 95.5%, to Amoxicillin/clavulanic acid 9.1%. All strains were sensitive to imipenem. E. coli strains showed resistance: 85.7% to ciprofloxacin, 50% to Amikacin, 57.1% to Gentamicin. For K. pneumoniae, it

is 66.7% for Gentamicin and Ciprofloxacin. **Conclusion:** Our study reports a high prevalence of ESBL at the HNZ. This must be taken into account in order to monitor this phenomenon which constitutes a public health problem. The study also reports sensitivity to Amoxicillin/clavulanic acid and Aminoside which can be an alternative.

Keywords

Bacterial Resistance, ESBL, Enterobacteriaceae, Zinder, Niger

1. Introduction

Bacterial resistance is a phenomenon that was discovered soon after the first antibiotic molecules were used [1]. According to a global report, more than 1.2 million people died worldwide in 2019 from infections caused by antibiotic-resistant bacteria [2]. Extended-spectrum beta-lactamase (ESBL)-secreting Enterobacteriaceae are a major contributor to the emergence of resistance. These are bacteria that have the ability to hydrolyze the beta-lactam ring of all penicillin making it inactive, with the exception of Cephamycin and Carbapenem. This reduces the therapeutic arsenal, creates a deadlock situation and has a considerable economic impact [3] [4] [5]. This situation constitutes a real threat to global public health, which is why the WHO has made it a priority [6].

In Africa the prevalence of ESBL varies between 30% - 50% during infectious processes. In Ghana, half of the Enterobacteriaceae (49.4%) isolated from various infections diagnosed at Korle-Bu Hospital were ESBL producers [7] [8]. Although ESBLs have been described in some African countries, there is no information on ESBLs here. In view of these results, an effective control plan is needed. This plan will start with a prevalence study to confirm and know the level of progress of this obstacle. The objective of this study is to determine the prevalence of ESBL and their sensitivities to the different families of antibiotics in the National Hospital of Zinder (NHZ) to better enable us to deal with the problem and guide the choice of prescriptions.

2. Materials and Methods

We conducted a prospective descriptive study at the National Hospital of Zinder (HNZ) over a period of 6 months from June to December 2021 to determine the prevalence of ESBL and their susceptibility to different families of antibiotics. Our study population consisted of inpatients and outpatients in whom a bacteriological examination was requested for the diagnosis of any infection with as inclusion criteria, patients from whom an Enterobacteriaceae was isolated.

The sample size was not calculated. We contacted each other to collect the strains depending on the time.

Excluded from our study were any individuals from whom a

non-Enterobacteriaceae was isolated, a sample or a bulletin that did not comply

with the criteria established by the laboratory. Ethical aspects such as research authorization by the NHZ and preservation of anonymity were respected.

The strains of Enterobacteriaceae in our study were isolated from various pathological products which were analyzed in the NHZ Laboratory Bacteriology Unit using conventional bacteriological techniques and procedures [9].

Macroscopic, cytobacteriological examination was performed on all samples. Urine was inoculated on CLED medium. For stools, MacConkey medium with sorbitol, Hektoen were used. Colonies suspected on Hektoen were plated in Kligler-Hajna. Plus, in addition to Mac Conkey, CLED, we used fresh blood agar under CO_2 . The reading is done after 24 hours and an examination after staining is done on a colony confirming the purity (Gram control). We used the API 20E gallery (Bio Mérieux, France) for strain identification.

We used the agar diffusion method to interpret the critical diameter of the inhibition zones also according to the table established by CA-SFM/ EUCAST2020 [10].

Penicillin such as Amoxicillin, Amoxicillin/clavulanic acid, Ticarcillin, Ticarcillin + clavulanate, Piperacillin, Piperacillin + tazobactam, Cefalotin, Cefoxitin, Ceftriaxone, Cefotaxime, Cefepime, Ceftazidime, Aztreonam and Imipenem were tested. Some aminosides were also tested including Gentamicin, Tobramicyn and Amikacin. Only Ciprofloxacin of the quinolone family was tested. The sensitivity of colistin was also studied.

We used the phenotypic method. Double disc synergy technique, between discs of third generation cephalosporins (Cefotaxime, Ceftazidime, Ceftriaxone etc) and a disc containing the combination Amoxicillin/clavulanic acid (a β -lactamase inhibitor). This synergy results in the formation of an image known as a champagne cork, which indicates the restoration of the activity of C3Gs opposite the Amoxicillin/clavulanic acid combination [11].

The data were processed and analyzed using Epi info 3.5.4 and Excel 2010. Variables such as socio-demographic characteristics, nature of the specimen and service their behavior towards antibiotics was effectively investigated.

3. Results

In our study, fifty (50) strains of Enterobacteriaceae were isolated from the different specimens. Blood culture was not performed in our study. **Table 1** shows the distribution of Enterobacteriaceae species. Ten (10) different species of Enterobacteriaceae were isolated including *Citobacter koseri, Escherichia coli, Klebsiella ornithinolytica, Klebsiella oxytoca, Klebsiella pneumoniae, Morganella morganii, Proteus mirabilis, Providencia stuartii, Salmonella Spp, Serratia odifera.* Among them *Escherichia coli* was the most isolated species (62%) followed by *Klebsiella pneumoniae* (6 12%). Of these fifty (50) strains, twenty-one (21) were beta-lactamase producing, *i.e.* a prevalence of 42.0%. A clear male predominance with 71.4% was observed. Female represented only 28.6%. Urine is the product from which most ESBL strains have been isolated. *E. coli* was the most isolated species in both specimens.

Table 2 shows the distribution of strains according to services and environment. Outpatients are in the majority with a frequency of 61.9% (n = 13). In the inpatient population, the nephrology department reported more EBLSE strains (23.8%), P = 0.207.

Figure 1 shows the resistance of the beta-lactamase producing strains to the beta-lactams. All strains n = 21 (100%) were resistant to: Amoxicillin, Ticarcillin, Ticarcillin + ac clavulanic, Piperacillin, Piperacillin-tazobactam, first and third generation Cephalosporins (C1 and C3) and Aztreonam. However, all n = 21 (100%) strains were susceptible to Imipenem and 90.5% were susceptible to Amoxicillin/clavulanic acid. A 45% resistance to Cefoxitin was found.

Resistance to aminoglycosides including Gentamicin, Tobramicyn and Amikacin was 66.7%, 61.9% and 47.6% respectively. Strains were resistant to ciprofloxacin at a frequency of 85.7% (Figure 2).

Species	Number	Percentage
Citobacter koseri	1	2.0%
Escherichia coli	31	62.0%
Klebsiella ornithinolytica	1	2.0%
Klebsiella oxytoca	2	4.0%
Klebsiella pneumoniae	6	12.0%
Morganella morganii	1	2.0%
Proteus mirabilis	3	6.0%
Providencia stuartii	1	2.0%
Salmonella Spp	2	4.0%
Serratia odifera	2	4.0%
Total	50	100.0%

Table 1. Distribution of Enterobacteriaceae strains and their frequencies.

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Table 2	Distribution	OT ESBL	strains	nv de	nartment	and	environn	nent
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Species	Surgery	External	Medicine/women	Nephrology
Escherichia coli	1 (4.76%)	9 (42.85%)	1 (4.76%)	3 (14.28%)
Klebsiella ornithinolytica	1 (4.76%)	0 (0%)	0 (0%)	0 (0%)
Klebsiella oxytoca	0 (0%)	1 (4.76%)	0 (0%)	1 (4.76%)
Klebsiella pneumoniae	0 (0%)	2 (9.52%)	0 (0%)	0 (0%)
Morganella morganii	0 (0%)	1 (4.76%)	0 (0%)	0 (0%)
Serratia odifera	0 (0%)	0 (0%)	0 (0%)	1 (4.76%)
Total	2 (9.52%)	13 (61.90%)	1 (4.76%)	5 (23.80%)



Figure 1. Sensitivity of EBLSE to beta-lactams.



Figure 2. The susceptibility profile of EBLS to the aminoglycoside and quinolone family.

4. Discussions

At the end of our study, 42.0% of the isolated strains were extended spectrum

beta-lactamase spectrum beta-lactamase. This prevalence is alarmingly high in many African countries [8]. In Togo, Toudji and al report a prevalence of 22.44% [12]. A much lower prevalence (12.5%) was reported in Mali and 12.2% in Morocco in urinary tract infections [13] [14]. This is probably due to the lack of adequate surveillance and antimicrobial use in the region. It has been shown that the lack of resources (human and financial) in our countries where hygiene is poor and antibiotics are poorly used, the absence of an antimicrobial surveillance program, increases the risk of the emergence and spread of multidrug resistant bacteria [8]. In contrast, a much higher value was found in Kano (Nigeria) at 66.7% [15]. Here, as in other African countries, antibiotics can be bought over the counter without a prescription. The sale of a few tablets is possible when the patient does not have enough money. Still many patients interrupt the treatment as soon as they feel better before the end of the treatment, keep the remaining tablets in bad condition, for another time or give them to another person with similar complaints without even medical advice [16].

The anatomical characteristics of the female urethra are short, wide, straight and close to the perianal area [17]. Despite these factors favoring infection, male sex was dominant with a sex ratio of 1.38 in favor of males. Our results disagree with those reported by Mohamed Sbiti and coll. in Morocco [14]. This can be explained by the socio-cultural aspect in our women (modesty, embarrassment to consult for urinary/vaginal infections). They are generally treated with probabilistic antibiotics.

In our study, strains from the outside environment were the most frequently isolated with a frequency of 61.9%. Long limited to the hospital setting, the epidemiology of EBLSE has changed considerably since the 2000s. Since then, EBLSE have been spreading in the community [18]. *Escherichia coli* were the most isolated species with a frequency of 66.7%. Similar results were reported by Ouédraogo in Burkina Faso (67.5%) [16]. This result can be explained by the promiscuity of the anal and urinary orifice, self-infection is possible. On the one hand *Escherichia Coli* has factors such as adhesion through which it clings to the epithelial cells of the urinary tract. This mechanism makes it the most incriminated in urinary tract infections [19]. However, Anastay and Coll. in France and Belmonte *et al.* in Reunion Island report *E. cloacae* as the most isolated species [20] [21]. The genera Salmonella, Shigella and Yersinia have not been found due to their rare isolation in the samples analyzed [12].

The resistance phenotypes of ESBL strains to different antibiotic families showed 100% resistance to all beta-lactams except Imipenem and Cefoxitin. Both molecules have a methoxyl-0-CH3 radical on the beta-lactam nucleus which protects them from enzyme action. The low resistance observed is probably due to the overexpression of chromosomal cephalosporinase [12] [22] [23]. Resistance to the Amoxicillin/clavulanate combination was 9.5%. Higher results were reported by Ahoyo in Benin and Mahamat in France [24] [25].

Resistance to aminoglycosides was lower than that observed with beta-lactams. These antibiotics are difficult to access compared to beta-lactams. Their toxicity is sometimes more remarkable and there are only injectable forms, which limits self-medication [12]. On the other hand, Ciprofloxacin, a widely used antibiotic, was 85.7% resistant. The same finding was made by Mutasim and coll. in Sudan [26]. This level of resistance to Aminosides and Ciprofloxacin may be due to the fact that the plasmid carrying the beta-lactam resistance gene may also carry other resistance genes such as QnrA (quinolone resistance gene) [27] [28].

Finally, the limitations of this study remain the non-availability of certain materials and reagents to continue the analysis of the isolated bacterial strains.

5. Conclusion

Our study revealed a high prevalence of EBLSE of 42% *Escherichia coli* was the most isolated species (62%) followed by Klebsiella pneumoniae (12%). Cross-resistance to other families of antibiotics including aminoside and quinolon was observed. This must be taken into account in order to curb this scourge which constitutes a real public health problem. Studies on the circulating gene will allow us to know more about these beta-lactamases.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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