

(RESEARCH ARTICLE)



Antimicrobial resistant in Gram-negative bacilli: *Enterobacteriaceae* and non-fermenting bacilli isolated at Sominé DOLO Hospital of Mopti, Mali

Moussa Diawara ^{1,*}, Modibo Coulibaly ², Dramane Samaké ³, Soumaila Touré ², Dramane Cissé ⁴, Abdoulaye Traoré ⁵, Djibril Traoré ⁴, Pierre Coulibaly ⁶, Bréhima Traoré ⁴, Oumar Guindo ⁷, Bakary Maiga ⁸ and Amagana Dolo ⁹

¹ Department of Biomedical laboratory, Military hospital infirmary of Kati, Mali.

² Department of Biomedical laboratory, Sominé DOLO Hospital of Mopti, Mali.

³ Department of Medicine, Sominé DOLO Hospital of Mopti, Mali.

⁴ Department of General surgery, Sominé DOLO Hospital of Mopti, Mali.

⁵ Department of Anesthesia and resuscitation, Sominé DOLO Hospital of Mopti, Mali.

⁶ Department of Gynecology and obstetrics, Sominé DOLO Hospital of Mopti, Mali.

⁷ Department of Public Health, Sominé DOLO Hospital of Mopti, Mali.

⁸ Faculty of Medicine and Odonto-Stomatology, University of Technical and Technological Sciences, Mali.

⁹ Faculty of Pharmacy, University of Technical and Technological Sciences, Mali.

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Abstract

Background: β -lactams and carbapenems are the major antibiotics used to treat gram-negative bacteria and non-fermenting bacilli. However, the increasing production of β -lactamase and carbapenemase limits the therapeutic options. Our study aims to determine the resistant phenotypes of these bacteria while describing their epidemiological aspect.

Material and Methods: This was a cross-sectional study by consecutive enrollment from January 2018 to December 2019 at Sominé DOLO Hospital of Mopti, Mali. We performed manual method for bacteria culture, identification and antibiotics sensitivity testing. The antibiotics sensitivity testing was accessed by the diffusion method according to CA-SFM/EUCAST ("Comité de l'Antibiogramme de la Société Française de Microbiologie" / European Committee on Antimicrobial Susceptibility Testing) recommendations V1.0 february 2018 and V2.0 may 2019 V.2.0. May recommendation released in 2019. Data were analyzed by software R 4.0.3 GUI 1.73 Catalina build (7892).

Results: At all 904 samples were included in this study. Out of the 904 cultures, 297 sample (32.85%) were positive. The rates of enzymes production were as follow: Extended-spectrum β -lactamase (ESBL) 56.42% (101/179), cephalosporinase hyperproduction (HCASE) 15.64% (28/179), cephalosporinase production (CASE) 6.14% (11/179), penicillinase hyperproduction (HP) 5.58% (10/179), carbapenemase production (CP) 6.14% (11/179) and savage strains 10.05% (18/179).

Conclusion: Our data showed a high prevalence of resistance to β -lactamins and carbapenemes in gram-negative bacteria and non-fermenting bacilli. The A high level of β -lactamase and carbapenemase production by gram negative bacilli were also reported by others authors calls for the rational use of antibiotic in hospital setting.

Keywords: Gram-negative bacilli; Antimicrobial resistant; Rational use of antibiotics.

* Corresponding author: Dr. Moussa Diawara, Pharm.D; Email: moussadiaw13@gmail.com
Department of Biomedical laboratory, Military hospital infirmary of Kati, Mali.

1. Background

Gram-negative bacilli, most notably *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, are among the most important causes of serious hospital-acquired and community-onset bacterial infections in humans [1]. Resistant to antibiotics of these bacteria is increasing worldwide in healthcare settings and in the community [2]. Gram-negative bacteria because of their high resistant rate are among a global priority list of antibiotic-resistant bacteria to guide research, discovery and development of the new antibiotic [3]. The β -Lactam antibiotics are a major drug class used to treat serious community-onset or hospital-acquired infections caused by gram negative bacilli, resistance to these agents will continue to challenge clinical therapeutic choices [4]. Carbapenemase and β -lactamases are bacterial enzymes that inactivate β -lactam antibiotics by hydrolysis, which results in ineffective compounds. In Gram-negative bacilli, these enzymes production remains the most important contributing factor to β -lactam resistance and these dissemination is alarming [2]. Low and middle income countries (LMICs) are and will be particularly affected [5]. A few data concern antibiotic-drug-resistance is available in Mali, that is also a middle and income country and that not have a real antimicrobial resistance policy. The objective of this study was to determine the resistant phenotypes of gram negative bacteria while describing their epidemiological aspect at Sominé DOLO hospital of Mopti and the prevalence of these bacilli.

2. Material and methods

We enrolled a total of 904 samples for bacteriological analysis in a prospective study during 2 years January 2018 to December 2019 at Sominé DOLO hospital of Mopti (SDH-M)/Mali. SDH-M is the unique secondary health care setting in Mopti area, it's also in transition to third reference health care. In this study we included all bacteriological specimens sent to our laboratory for bacteria identification and antibiotics susceptibility (urine, stool, sperm, ascistes fluid, pleural fluid, prostatic fluid, throat swab, pus and others specimens). All patients with positive culture containing an *Enterobacteriaceae* or non-fermentative bacilli were therefore included in antibiotic susceptibility analysis. The manual method was used for bacteria culture, identification, and sensitivity testing. Uriselect® medium (a chromogen medium) served as a medium for culture and identification for bacteria isolated in urine combined with others biochemistry tests (oxydase, catalase, urease, indole) after microscopic observation to identified bacteria morphology. Bacteria isolated from others samples had grown on different mediums (Ordinary medium, Drigalski medium, Salmonella/Shigella mediums, and Hektoen medium). Analytical Profile Index 20- *Enterobacteriaceae* (API 20-E®) and Analytical Profile Index 20- Non- *Enterobacteriaceae* (API-20 NE®) systems (BioMérieux, Marcy l'Etoile, France) were used to identified bacteria isolated on specimens others that urines. Antibiotic susceptibility testing was performed by the diffusion method by agar-agar according to CA-SFM/ EUCAST ("Comité de l'Antibiogramme de la Société Française de Microbiologie" / European Committee on Antimicrobial Susceptibility Testing) recommendations V1.0 february 2018 and V2.0 may 2019 2019 V.2.0. May. Extended-spectrum β lactamase (ESBL) production and Carbapenemase production by gram negative bacilli bacteria was detected by phenotypic method. This method is based on the principle that most ESBLs hydrolyze third generation of cephalosporins although they are inhibited by clavulanate. For ESBLs phenotypic detection, we used synergy method between two antibiotic discs on standard antibiogram, one disc of third or fourth generation of cephalosporin (cefotaxime, ceftriaxone, ceftazidime, cefepime) or aztreonam in combination with one disc of clavulanic acid at a distance of 30 mm. ESBL presence is expressed by the appearance of a synergy << champagne cork >>. For a clinical isolated producers ESBL who had others β -lactam resistant mechanisms as cephalosporinase production, the synergy image was detected by the rapprochement of cephalosporin disc of the clavulanate acid disc or by performing a standard antibiogram on Mueller- Hinton agar supplemented with 250 mg/L of cloxacillin (cephalosporinase inhibitor). For carbapenemase detection we measured only carbapenem disc diameter. Data were captured in Excel Microsoft and analyzed by the statistical software R 4.0.3 GUI 1.73 Catalina build (7892).

3. Results

During the study period, we received 904 samples for bacteriological analysis. Out of the 904 cultures, 297 sample (32.85%) were positive. At these positives cultures, 60.27% (179/297) were gram negative bacilli: Enterobacteria and non-fermentative bacilli, account for 53.20% (158/297) and 7.07% (21/297), respectively. Among the patients what we isolate gram negative bacilli, 63.13% (113/179) were female and 36.87% (66/179) were males. The mean age was 37 years with extremes to 1 to 90 years, and the age group of 31 to 45 years was predominant 27.93% (Figure 1). Urine culture was the most performed analysis 82.68% (148/179), followed by pus culture 5.59% (10/179) and sperm culture 5.03% (9/179) (Figure 2). Out of the 179 gram-negative bacilli, the most clinical bacteria isolates were *Escherichia coli* 61.45% (110/179) , *Klebsiella pneumonia* 13.97% (25/179) and *Pseudomonas aeruginosa* 7.26% (8/179) (Table 1). Antibiotic susceptibility test reveals that the main antibiotics resistance rates were as follow:

amoxicillin 99.44% (178/179), amoxicillin + clavulanic acid 98.88% (177/179), third generation cephalosporine 79.89% (143/179) (Figure 3). The rates of enzymes production were as follow: Extended-spectrum β -lactamase (ESBL) 56.42% (101/179), cephalosporinase hyperproduction (HCASE) 15.64% (28/179), cephalosporinase production (CASE) 6.14% (11/179), penicillinase hyperproduction (HP) 5.58% (10/179), carbapenemase production (CP) 6.14% (11/179) and savage strains 10.05% (18/179) Figure 4.

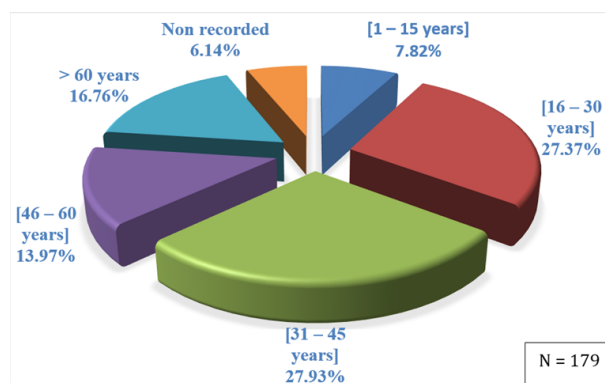


Figure 1 Age group proportions of study subjects

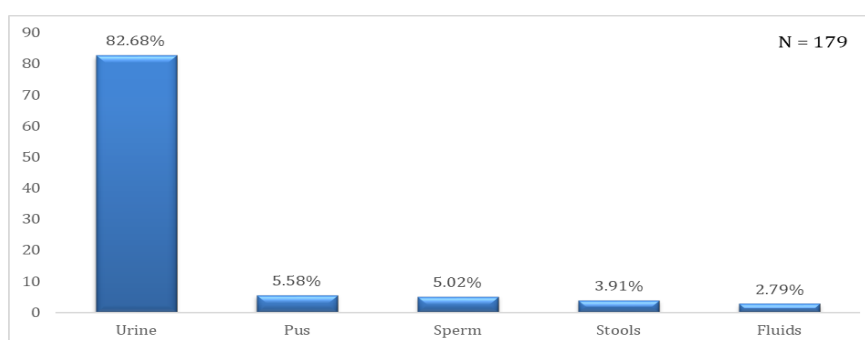


Figure 2 Proportions of received biological samples

Table 1 Distribution of clinical isolated bacteria

Isolated spp	N	%
<i>Escherichia coli</i>	110	61.45
<i>Klebsiella pneumoniae</i>	25	13.97
<i>Pseudomonas aeruginosa</i>	13	7.26
<i>Acinetobacter baumannii</i>	8	4.47
<i>Enterobacter cloacae</i>	6	3.35
<i>Salmonella spp</i>	5	2.79
<i>Serratia marcescens</i>	3	1.68
<i>Pseudomonas vulgaris</i>	1	0.56
<i>Pseudomonas multocisa</i>	1	0.56
<i>Pseudomonas luteola</i>	1	0.56
<i>Pseudomonas damsele</i>	1	0.56
<i>Citrobacter koresi</i>	1	0.56
<i>Citrobacter braaki</i>	1	0.56
Total	179	100

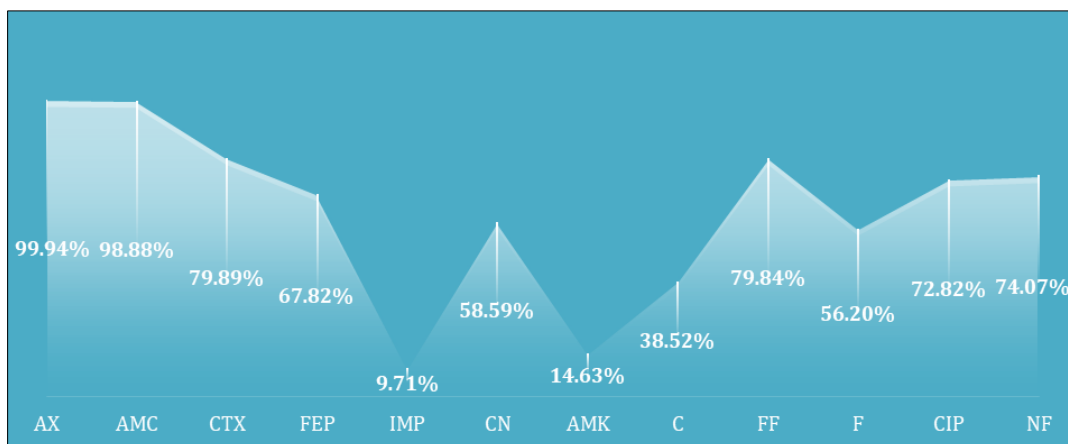


Figure 3 AX = amoxicillin, AMC = amoxicillin + clavulanic acid, CTX = cefotaxime, FEP = cefepime, IMP = imipenem, CN = gentamycin, AMK = amikacin, C = chloramphenicol, FF = Fosfomycin, F = nitrofurantoin, CIP = ciprofloxacin, NF = norfloxacin

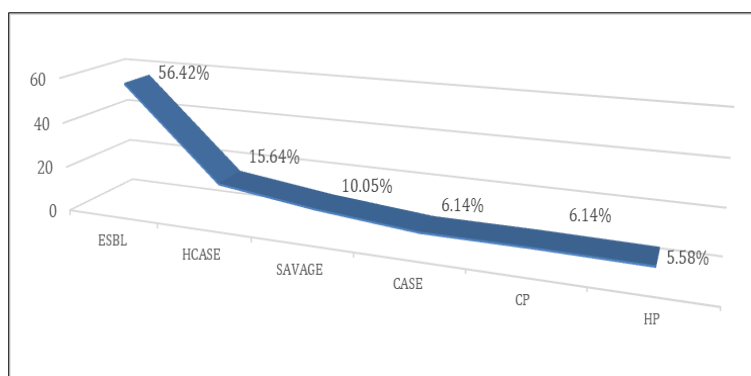


Figure 4 Distribution of resistant phenotypes. ESBL = extended-spectrum β -lactamase, HCASE = high cephalosporinase, CASE = cephalosporinase, CP = carbapenemase, HP = penicillinase hyperproduction

4. Discussion

Globally the prevalence of urinary tract infection (UTI) caused by GNB was 12.16%. This high UTI could probably be due to the high proportion of female gender and to the low demand of other bacteriological samples addressed to the laboratory. In our study, the proportion of female was high 63.13% and the age group of 31 to 45 years was the most exposed. The gram negative bacilli (GNB) was isolated mainly in urinary culture (82.68%), followed by pus culture (5.59%) and sperm culture (5.03%). *Escherichia coli* and 61.54% and *Klebsiella pneumoniae* 13.97% were, respectively the most *Enterobacteriaceae* isolated in the urine culture. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* respectively 7.26% and 4.47% were also the most non fermentative bacilli isolated also in the urine culture. Our data are in line with the that of Fissou Henry YANDAI and al (2019) [6] in Tchad where they found a rate of 18.5% of urinary tract infection (UTI). Fissou Henry YANDAI and al identified *E. coli* and *K. pneumoniae* as the two major bacteria involved in UTI 64.5% and 27.7%, respectively. In Cameroun, Ebongue et al (2015) found the same result [7].

Our study reveals a high antibiotic resistance rate, globally 89.95%. Strains with savage phenotype were isolated only in 10.05% of the culture samples. The analyses of the antibiotic susceptibility to β -lactam showed also a high resistance levels of *Enterobacteriaceae* and to non-fermentative bacilli: resistant to amoxicillin 99.44%, resistant to amoxicillin + clavulanate acid 98.88%, resistant to third generation cephalosporine 79.89%, resistant to fourth generation cephalosporine 67.82% and resistant to imipenem 9.71%. This result is in correlation with the study conducted by Samba Adama Sangare and al, 2017 in Mali where they found also a high antibiotic resistance rate to *Enterobacteriaceae* [8]. In our study, imipenem resistance to gram negative bacilli rate was high particularly *P. aeruginosa* 15.38%, *A. baumannii* 12.50% and *E. coli* in 10.91%. This high resistant level to imipenem could be due to the more and more increasing use of imipenem caused by the poor sensibility of hospital strains to third and fourth cephalosporine generation. The different phenotypes resistance rate to gram negative bacilli were as follow: ESBL

(56.24%), HCASE (15.64%), CP (6.14%), CASE (6.14%), and HP (5.58%). Among clinical isolates in Mopti hospital, the prevalence of ESBL producing gram negative bacilli (ESBL-PGNB) was very high (56.24%). ESBL-PGNB prevalence varies widely between geographic areas. In Sub-Saharan Africa, and particularly in West Africa, limited data are available on ESBL-PGNB. The prevalence found in our study (56.74%) was similar to the one reported for other African countries, such as Burkina Faso 58% [9], Mali 61.8% [10] and Ghana 49.4% [11]. In others African countries, prevalence of ESBL-PGNB was relatively low such as Nigeria [12] and Central African Republic [13]. In our study, ESBL and CP affected antibiotics drug susceptibility such as aminosid and fluroquinolone. Samba Adama Sangare and *al* in Mali, 2017 [8], Oumar Ouchar Mahamat and *al* in Tchad [14] reported the same result. This study shed light on the different pathogenic bacteria isolated at Sominé DOLO hospital of Mopti and their antibiotic resistance phenotypes. However, we did not perform molecular testing to confirm the genotype of clinical isolates. The use of control strains would improve this job.

5. Conclusion

Escherichia coli 61,45% (110/179) and *Klebsiella pneumoniae* 13,97% (25/179) were the two majors clinical isolates isolated in clinical specimens particularly in urine. ESBL production by gram-negative bacilli was very high 56,42% and affect antibiotics which are not usually hydrolyzed by ESBLs such as carbapenem 6,14% (11/179). This high level of carbapenemase reported by others authors calls for the rational use of antibiotic in hospital setting.

Compliance with ethical standards

Acknowledgments

We are grateful to all study subjects for participating in this study who gave their informed consent. We also thank all the staff of Mopti hospital for their frank and closed collaboration.

Disclosure of conflict of interest

Authors certify that there is no actual or potential conflict of interest in relation to this article.

Statement of informed consent

Each participant gave fully informed written consent prior to enrollment. The protocol was reviewed and approved by Hospital committee.

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