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Prevalence of Multidrug-Resistant Enterobacterales Isolated from 2019 to 2020 in a Tertiary Hospital in Dakar-Senegal

Farma Thiam [a], Abdou Diop [b], Babacar Ndiaye [b], Ndeye Maguette Bèye [a], Issa Ndiaye [c], Ousmane Sow [c], Magatte Ndiaye [a], Thierno Abdoulaye Diallo [b] and Abdoulaye Seck [b,d*]

[a] Medical Biology Laboratory, Hospital of Pikine, Senegal.
[b] Medical Biology Laboratory, Pasteur Institute of Dakar, Senegal.
[c] Microbiology Center, Pasteur Institute of Dakar, Senegal.
[d] Bactériology-virology Laboratory, Faculty of Medecine, Pharmacy and d'Odonto-stomatology, Dakar, Senegal.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Multi-resistant bacteria have emerged as a global threat to human health. In Africa, there are few data on AMR. The objective of this study was to determine antibiotic resistance profile of enterobacteria strains and prevalence of multidrug-resistant bacteria isolated in a university hospital in the suburbs of Dakar.

**Corresponding author: E-mail: ablayseck@gmail.com;*

Methods: Enterobacterales were isolated from a wide range of clinical specimens (urine, pus, blood, catheter tip and bronchoalveolar fluid) from inpatients and outpatients at Medical Biology Laboratory of National University Hospital Center of Pikine from November 2019 to October 2020. Enterobacterales were identified using API 20E. Antimicrobial susceptibility testing was performed with ATB G-EU (08) (bio Mérieuxs®) on all enterobacterales in accordance with CA-SFM/EUCAST 2020 guidelines.
Results: Of the 3422 different clinical specimens tested, 623 (17.1%) were culture positive. Enterobacteriaceae accounted for 57.6% (n=359) of the strains; *Escherichia coli* and *Klebsiella pneumoniae* were predominant isolates with 53.5% and 20.6% respectively. Seventy-four(%) strains of enterobacterales were ESBL. Antibiotic resistance patterns showed a prevalence of multidrug resistant strains of 32.6%. The most active antibiotics on isolates were imipenem (25%), followed by amikacin (15%), fosfomycin (12%) and piperacillin-tazobactam (10%).
Conclusion: High rates of ESBL and multidrug-resistant strains were found in both outpatients and inpatients. These results indicate need for an active surveillance system for antimicrobial resistance. Also, the application of good hospital hygiene practices and antibiotic therapy adapted to local data must be adopted.

Keywords: Prevalence; ESBL; multidrug-resistant; enterobacterales.

1. INTRODUCTION

Antimicrobial resistance (AMR) is a public health problem. The inappropriate use of antibiotics in community and hospital settings has led to appearance of multi-resistant bacteria (MRB). In recent years, the prevalence of MRB has increased worldwide [1]. These MRB cause infections that can lead to increased morbidity, mortality, and healthcare costs. Western sub-Saharan Africa had the highest burden, with 27.3 deaths per 100.000 (20.9–35.3) attributable to AMR and 114,8 deaths per 100 000 (90.4–145.3) associated with AMR [2]. These strains may be of community or healthcare-associated origin. Healthcare-associated infections are infections that first appear 48 hours or more after hospitalization or within 30 days after having received health care [3].

The increase in bacterial antimicrobial resistance (AMR) is considered as one of the major threats to human health worldwide [4].

Antimicrobials are commonly used in modern medicine and 50% of prescriptions are considered inappropriate. This misuse of antimicrobials is a major cause of increased AMR [5].

To reduce spread of MRBs, one of the preventive measures is based on the fight against cross-transmission by lack of hygiene in the hospital environment at the level of the nursing staff, but also on the screening of asymptomatic carriers who constitute a potential reservoir from which these bacteria can spread [4].

To our knowledge, few data are available on the prevalence of MRB in Senegal, particularly in Dakar and especially in the suburbs.

The objectives of this study were to determine the antibiotic resistance profile of enterobacteria and the prevalence of multi-resistant enterobacteria.

2. METHODOLOGY

This prospective study was conducted at Medical Biology Laboratory of National University Hospital Center of Pikine from November 2019 to October 2020. All inpatients and outpatients with a bacteriological diagnostic sample taken in suspicion of a bacterial infection were included in the study regardless of age and sex. Results of a second bacteriological sample with isolation of same bacteria as previously from a patient already included were not considered. Various specimens (urine, blood, pus, catheter tip, and bronchoalveolar fluid) were plated on appropriate culture media (Müeller-Hinton, Chocolate agar, Chapman, thioglycolate broth). These media were incubated in an appropriate atmosphere according to morphological aspect of type of germ observed on microscopic examination after Gram staining.

Identification of enterobacterial strains was performed using the API 20E gallery (bio Mérieux®). Antibiotic susceptibility profiling was performed with the ATB G- EU (08) gallery (bio Mérieux) according to recommendations of CA-SFM/EUCAST version 2020. Several antibiotics were tested: ampicillin, amoxicillin-clavulanic

acid, piperacillin, piperacillin + tazobactam, cefalotin, cefuroxime, cefixime, cefotaxime, cefoxitin, ceftazidime, cefepime, ertapenem, meropenem, imipenem, nalidixic acid, ofloxacin, levofloxacin, ciprofloxacin, gentamicin, amikacin, tobramycin, tetracycline, fosfomycin and cotrimoxazole. Control strain *E. coli* ATCC 25922 was used for internal quality control.

Search for ESBL was performed with the synergy test by placing a third-generation cephalosporin (ceftazidime or cefotaxime) disc opposite an amoxicillin-clavulanic acid disc. Presence of ESBL was shown by a "champagne cork" image. Enterobacteria strains resistant to three families of antibiotics were considered Multi- resistant Bacteria (MRB) [4] [Magiorakos and al, 2012]. Data were collected and analyzed with Microsoft Excel software 2022 version.

3. RESULTS

In this study, 3422 specimens including urine, blood, pus, catheter tip, and bronchoalveolar fluid (BAL) samples were analyzed. The overall isolation rate was 18.2% (Table 1).

A total of 623 bacterial strains were isolated ; 57.6% of isolates were enterobacteria (n=359), 18.3% were non-fermenting Gram-negative bacilli (n=113), 23.8% were Gram-positive cocci (n=148) and 0.48% were anaerobic germs (n=3).

Enterobacteria were mainly *E. coli* (n=192; 53.5%), followed by *K. pneumoniae* (n=74; 20.6%) and *E. cloacae* (n=39; 10.9%). Healthcare-associated strains accounted for 39.6%, compared with 60.4% for community-acquired strains (Table 2).

Antibiotic susceptibility profile of *E. coli* isolates had shown high rates of resistance from 53% to 89.1% to ampicillin, ticarcillin and piperacillin.

Resistance rates greater than 50% were noted for *E. cloacae and E. aerogenes* strains with ticarcillin and piperacillin. The combination of amoxicillin and clavulanic acid was more active on *E. coli* than on *K. pneumoniae* (53.1% versus 61.7% resistance).

Table 1. Distribution of biological samples and culture isolation rates

Specimens	Number	Isolation rates (%)
Urine	2795	13.8
Blood	362	16.9
Pus	231	63.2
Catheter tip	18	88.9
BAL	16	93.8

Table 2. Distribution of enterobacterales according to species and hospital department of origin

Bacteria	Origin								Total
	Community	Healthcare-associated infections							
	EC	IM	SUR	AR	PED	ME	GO	NEU	
E. coli	117	27	25	03	06	11	01	02	192
K. pneumoniae	41	12	03	08	03	05	01	01	74
K. oxytoca	05	02	0	0	0	0	0	0	07
E. cloacae	21	02	02	06	07	01	0	0	39
E. aerogenes	02	0	0	0	0	0	0	0	02
P. mirabilis	09	01	0	02	01	0	0	0	13
P. vulgaris	02	0	0	0	0	0	0	0	02
C. freundii	05	1	0	0	0	0	0	0	06
S. enterica	05	03	0	0	01	01	0	0	10
M. morganii	06	0	0	0	0	0	0	0	06
S. marcescens	03	0	01	0	01	0	0	0	05
S. liquefaciens	0	0	0	0	02	0	0	0	02
R. ornithinolytica	1	0	0	0	0	0	0	0	01
Total	217	48	31	19	21	18	02	03	359

EC : External Consultation, IM : Internal Medicine, SUR : Surgery, AR : Anaesthesia Resuscitation, PED : Pediatrics, ME: Medical Emergency, GO : Gynecology Obstetrics, NEU : Neurology.

The piperacillin-tazobactam combination was more active on *Klebsiella* strains (17.3% resistance) than on *E. coli* (20.8% resistance) and *Enterobacter* (34.1% resistance).

Cefoxitin had a better efficacy on *E. coli* and *K. pneumoniae* strains (77.1% and 63%), than third and fourth generation cephalosporins with sensitivity rates of 39.5% to 76%.

Cephalotin was less active on *E. coli* (47.9%) and *K. pneumoniae* (34.6%) strains. ESBL production was noted in 20.6% of the enterobacterial strains (n = 74).

These enterobacterales kept a good sensitivity to carbapenems, amikacin, gentamicin and fosfomycin with resistance rates ranging from 2.1% to 36.6% contrary to tetracycline and cotrimoxazole with resistance rates ranging from 51.2% to 69.8% (Table 3).

Analysis of resistance patterns showed that 32.6% (n=117/359) of strains were multidrug-resistant enterobacteria (MRE), including 60 community strains and 57 hospital strains. MRE consisted of *E. coli* (n=55), *K. pneumoniae* (n=34), *K. oxytoca* (n=2), *E. cloacae* (n=17), *E. aerogenes* (n=2), *M. morganii* (n=2), *C. freundii* (n=2), *S. marcescens* (n= 1), *S. liquefaciens* (n= 1), and *R. ornithinolytica* (n=1) (Table 4).

ESBL production was found in 45.3% of MRE (n = 53) including *E. coli* (n=24), *K. pneumoniae* (n=18) and *E. cloacae* (n=6).

Majority of MRE (75.2%, n=88) were resistant to both β-lactams, quinolones and aminoglycosides. Some MRE were still sensitive to some antibiotics. Piperacillin-tazobactam combination was active on 70.6% of *K. pneumoniae* strains. Carbapenems, amikacin and fosfomycin were more active against *E. coli* and *K. pneumoniae* than *E. cloacae*. Gentamicin is more active on *K. pneumoniae* (70,5%) than *E. coli* (54,5%) and *E. cloacae* (52,9%) (Table 5).

4. DISCUSSION

The main objective of this study was to determine the prevalence of multi-resistant enterobacteria isolated from 2019 to 2020 in medical biology laboratory of university hospital of Pikine, in the periphery of Dakar.

Table 3. Antibiotic resistance rate of most isolated enterobacterales

Antibiotics	% Resistance		
	E. coli (n=192)	***K. pneumoniae-K. oxytoca*** **(n=81)**	***E. cloacae-E. aerogenes*** **(n=41)**
Ampicillin	89,1	-	-
Amox-ac clav	53,1	61,7	-
Ticarcillin	89,1	-	56,1
Piperacillin	89,1	-	53,7
Pip - tazo	20,8	17,3	34,1
Cephalotin	52,1	65,4	-
Cefoxitin	22,9	37	-
Ceftazidim	28,1	60,5	51,2
Cefepim	24	46,9	41,5
Ertapenem	2,6	3,7	19,5
Meropenem	2,6	3,7	19,5
Imipenem	2,1	3,7	19,5
Nalidixic acide	58,3	49,4	51,2
Ciprofloxacin	41,7	45,7	53,7
Tobramycin	27,1	48,1	19,5
Gentamicin	18,8	18,5	17,1
Amikacin	3,6	9,9	22
Tetracyclin	52,6	56,8	51,2
Fosfomycin	12,7	16	36,6
Cotrimoxazol	69,8	59,3	51,2

Table 4. Distribution of MRE according to their community and hospital origins

Bacteria	MRE origin						Total
	Community	Healthcare-associated infections					
	EC	IM	SUR	AR	PED	ME	
E. coli	31	10	9	2	1	2	55
K. pneumoniae	16	5	2	6	2	3	34
K. oxytoca	1	1	0	0	0	0	2
E. cloacae	8	2	1	2	3	1	17
E. aerogenes	1	0	0	0	1	0	2
C. freundii	1	1	0	0	0	0	2
M. morganii	2	0	0	0	0	0	2
S. marcescens	0	0	0	0	1	0	1
S. liquefaciens	0	0	0	0	1	0	1
R. ornithinolytica	0	0	0	1	0	0	1
Total	60	19	12	11	9	6	117

Table 5. Antibiotic susceptibility profile of the 3 main MRE

Antibiotics	% Resistance EMR		
	E. coli **(n=55)**	***K. pneumoniae*** **(n=34)**	***E. cloacae*** **(n=17)**
Piperacillin-tazobactam	38,2	70,6	41,2
Ertapenem	90,9	91,2	52,9
Meropenem	90,9	91,2	52,9
Imipénème	92,7	91,2	52,9
Amikacine	89,1	79,4	58,8
Gentamicine	54,5	70,5	52,9
Fosfomycine	87,3	82,4	41,2

Six hundred and twenty-three bacterial strains were isolated from different pathological products with an isolation rate of 18.2%. Enterobacterales accounted for 57.6% of isolates (n=359). *E. coli* and *K. pneumoniae* were the most frequently isolated bacteria with 53.5% and 20.6% respectively. Predominance of these two bacterial pathogens has been reported in other studies [6], this could be explained by predominance of uropathogenic isolates which represented 59.8% of which these two strains were the majority. Preponderance of uropathogenic enterobacteria is often reported in microbiology laboratories where urine is by far most analyzed specimen [7,8,9]. Majority of enterobacterales strains were of community origin (58.9%), which could be explained by the greater number of outpatient samples than inpatients.

Antibiotic susceptibility testing showed that 90% of *E. coli* strains were resistant to ampicillin, ticarcillin and piperacillin compared to 55% of *E. cloacae* isolates for ticarcillin and piperacillin. *E. coli*, *K. pneumoniae* and *E. cloacae* strains had shown piperacillin-tazobactam resistance rates of 20.8%, 17.3% and 34.1% respectively. A study carried out in Dakar showed a resistance of 69.3% to piperacillin-tazobactam in E. cloacae [10].

The resistance rates of *E. coli* and *K. pneumoniae* with amoxicillin-clavulanic acid combination were 53.1% and 61.7% respectively. A meta-analysis of ten studies conducted in Cameroon had reported *E. coli* resistance rates to amoxicillin ranging from 59.7% to 89.7% and to amoxicillin-clavulanic acid ranging from 48.9% to 75.9%; while *K. pneumoniae* resistance rates to amoxicillin ranged from 84.9% to 98.3% [11]. A hospital study conducted in 2019 in northern India of uropathogenic *E. coli* isolates (n=145) had reported amoxicillin and amoxicillin-clavulanic acid resistance rates of 81.37% and 75.86%, respectively [8].

Third generation cephalosporins were ineffective on half of the strains while cefoxitin showed better activity on *E. coli* and *K. pneumoniae* isolates by 77,1% and 63% respectively. This trend was described on strains of enterobacteria isolated from different pathological products collected in two university hospitals in Khartoum, Sudan, with resistance rates to *E. coli* and *K.*

pneumoniae with cefoxitin of 7.2% and 16.7%, respectively, and with ceftazidime of 39.1% and 47.2%, respectively [12]. Enterobacterales isolated during this study were predominantly susceptible to carbapenems. *E. coli* and *K. pneumoniae* isolates showed 90% susceptibility to imipenem and meropenem while *E. cloacae* isolates were 80% susceptible.

A study carried out in Senegal in 2016 showed 45.2% in E. coli and 27.4% in K. pneumonia isolated mainly in urine (58%) and pus (19.3%) [13].

However, overuse of carbapenems in therapy could lead to the emergence of carbapenem-resistant Enterobacteriaceae either by acquisition of carbapenem hydrolyzing betalactamases by the strains or by a combination of a plasmid mediated beta-lactamase AmpC and mutation of an outer membrane protein [14,15], thus posing a real threat to antibiotic therapy.

ESBL production was demonstrated in 20.6% of strains (n=74). Variable prevalence rates have been reported in West Africa. In Mauritania, prevalence rate of ESBL found was 12.8% among 522 uropathogenic enterobacteria isolated from January to June 2014 [16]. In Ivory Coast, a rate of 58.8% of ESBL had been found in 153 enterobacteria isolated from various pathological products [17] while in Burkina Faso 58% of the 308 enterobacteria analyzed were ESBL producers [18]. These ESBL have been described throughout the world. Thus, in France a surveillance of antibiotic resistance carried out in 2019 in 19 at 327 hospital laboratories had reported an ESBL rate of 6.% [19]. In Nepal, a hospital study in Kathmandu of 268 strains of *E. coli* and *K. pneumoniae* had reported 34.5% rate of ESBL-producing strains [20].

The strains isolated in our study had shown resistance rates of 47%, 53% and 60% respectively to ciprofloxacin, tetracycline and cotrimoxazole. They were however sensitive to amikacin (89%), gentamicin (82%) and fosfomycin (78.3%).

The analysis of susceptibility profiles showed that 117 strains (32.6%) were multi-resistant (resistant to at least three families of antibiotics) of which 53 strains (71.6%) were ESBL producers. This multiresistance phenomenon in enterobacteria is due to the acquisition of genes hosted by integrons, transposons, or plasmids [21].

In spite of fact that MRE were resistant to several families of antibiotics, some molecules were still effective on these strains: imipenem (78.9%), amikacin (75.8%), fosfomycin (70.3%) and piperacillin-tazobactam (50%). A retrospective study conducted at LeDantec University Hospital from January to December 2011 on 44 β-lactamase-secreting *K. pneumoniae* strains had shown a 41% resistance rate to amikacin and 2.2% to imipenem while no resistance was observed with fosfomycin [22]. Similarly, rates of 0.2% and 19% for imipenem and fosfomycin, respectively, were reported with the study conducted at Fann university hospital on 89 uropathogenic ESBL isolated from January to June 2017 [9].

A study conducted in Asia Pacific from 2008 to 2014 on 2728 ESBL strains reported an imipenem resistance rate of 92.1% [23].

5. CONCLUSION

High rate of ESBL and MRE was found from outpatient and inpatient. This highlights a need for active surveillance systems, good antibiotic practices and good hospital hygiene. Based on our findings presented in this paper, it will be interesting and important to study the ESBL and MRE in a molecular level. This will highlight the genes responsible for antibiotic resistance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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