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Extended-spectrum beta-lactamase- and carbapenemase-producing Enterobacteriaceae clinical isolates in a Senegalese teaching hospital: A cross sectional study

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Extended spectrum β-lactamase (ESBL) and carbapenemase-producing Enterobacteriaceae (CPE) have been increasingly reported worldwide. The objective of this study is to determine the prevalence of these multidrug-resistant strains in a major university teaching hospital in Dakar, Senegal. A total of 1205 Enterobacteriaceae stains were tested for ESBL and carbapenemase production. Antibiotics susceptibility test was performed with disk diffusion method. ESBL was detected using a double-disk synergy method. Carbapenemase production was detected with ertapenem 10 µg disk charge. The overall prevalence of ESBL- and carbapenemase-producing Enterobacteriaceae was 26.2 (316/1205) and 5.1% (62/1205), respectively. Interestingly, 3.8% of these pathogens were both ESBL-carbapenemase producers. Among the Enterobacteriaceae ESBL positive, *Escherichia coli* (45.2%, 143/316), *Klebsiella pneumoniae* (26.3%, 83/316), *Enterobacter cloacae* (12.7%, 40/316), and *Proteus vulgaris* (9.2%, 29/316) were the most prevalent. These strains were mainly isolated from urine (56.6%) and pus (22.7%) specimen. The most prevalent CPEs were *E. coli* (45.2%, 28/62), *K. pneumoniae* (27.4%, 17/62), and *E. cloacae* (16.1%, 10/62), particularly isolated from urine (58%) and pus (19.3%). The majority of these MDR strains were isolated from patients hospitalized in urology (32.4%), surgery (27.7%), internal medicine (18.5%), and intensive care units (10%). ESBL-producing Enterobacteriaceae remain highly susceptible to fosfomycin (94.1%), amikacin (92.5%) and ertapenem (88.6%), while carbapenemase producers were fully susceptible to amikacin (100%), and to a lesser extent, fosfomycin (66.7%) and colistin (60%). This study revealed increasing prevalence of ESBL- and carbapenemase-producing Enterobacteriaceae with limited therapeutic options, suggesting a need for continuous multi-drug resistant (MDR) surveillance patterns particularly in hospital settings.

Key words: Extended spectrum β-lactamase, carbapenemase, Enterobacteriaceae.

INTRODUCTION

Multi-drug resistant (MDR) Gram-negative bacilli have been increasingly associated with life-threatening infections worldwide (Girish et al., 2012; Poulou et al., 2014). In the last two decades, bacterial resistance to antibiotics, particularly by extended-spectrum β-lactamase (ESBL) production has become a major public health concern, particularly in resource limited settings (Vasoo et al., 2015). ESBLs have the ability to hydrolyze oxyimino-β-lactam antibiotics (e.g., cefotaxime, ceftriaxone, and ceftazidime) and monobactams (aztreonam), but not cephamycins (e.g., cefoxitin and cefotetan) and carbapenems (imipenem, meropenem, doripenem, and ertapenem) (Paterson et al., 2005).

Although ESBLs have been described in a range of Enterobacteriaceae, these enzymes are predominantly found in the bacterial species of *Klebsiella pneumoniae* and *Escherichia coli* (Pitout and Laupland 2008).

ESBL-producing strains of *E. coli* and *K. pneumoniae* are increasingly reported all over the world, and are important pathogens in community- and hospital-onset infections (Paterson and Bonomo, 2005). ESBL-producing Enterobacteriaceae are associated with life-threatening infections, increased morbidity and mortality and healthcare-associated costs (Pitout, 2010).

Extensive use of expanded-spectrum antibiotics including β-lactams is one of the most important risk factors associated with high prevalence of ESBLs (Chopra et al., 2015; Shukla et al., 2004; Oteo et al., 2010).

Carbapenems are generally stable against ESBLs and still mainly used as treatment of last resort in infections caused by MDR Gram-negative bacilli (Morosini et al., 2006). The emergence of carbapenemase-producing Enterobacteriaceae (CPE) is causing an unprecedented public health threat leaving few treatment options, and consequently leads to high clinical mortality rates (Tzouvelekis et al., 2012).

Carbapenemases are the most versatile family of β-lactamases and are able to hydrolyze carbapenems and other β-lactams (Queenan and Bush 2007). The most important mechanism of carbapenem resistance in Enterobacteriaceae is the production of carbapenemases, although resistance can also result from the synergistic activity between AmpC-type or ESBL combined with decreased outer membrane permeability (Pitout et al., 2015; Ruppé et al., 2015).

The recognition of MDR isolates is a major laboratory challenge and their inappropriate or delayed detection may have negative impacts on patients' management and on the implementation of infection control measures (Nordmann and Poirel, 2014). To our knowledge, the prevalence of MDR Enterobacteriaceae is not well documented in Senegal. Therefore, this study was designed to determine the prevalence of ESBL- and carbapenemase-producing Enterobacteriaceae and its antibiotic susceptibility profiles at Aristide Le Dantec University Teaching Hospital in Dakar, Senegal.

MATERIALS AND METHODS

Bacterial isolates

A total of 1205 non-duplicate clinical isolates were collected from January to December 2016. Clinical specimens were cultured on Eosin Methylene Blue (EMB) agar (Merck, Germany), and incubated at 37°C for 24 h. Clinical isolates were identified using standard biochemical galleria (for *E. coli*, *K. pneumoniae*, *Salmonella* species, *Shigella* species) or Api 20E (for *Citrobacter freundii*, *Serratia marcescens*, *Morganella morganii*, and *Providencia stuartii*) for Enterobacteriaceae.

Antibiotic susceptibility testing

Antimicrobial susceptibility test was performed using the disk diffusion method (Bio-Rad, France) as recommended by the Antibiogram Committee of the French Society for Microbiology (CA-SFM, 2016). Briefly, bacterial suspensions of 10^7 CFU/ml, adjusted with a McFarland densitometer, were inoculated on Mueller-Hinton agar and incubated for 16 to 24 h at 37°C. The following antibiotics were tested: amoxicillin (AMX, 25 µg), amoxicillin-clavulanic acid (AMC, 20/10 µg), cefalotin (CF, 30 µg), cefamandole (MA, 30 µg), cefoxitin (FOX, 30 µg), cefotaxim (CTX, 30 µg), ceftriaxone (CRO, 30 µg), ceftazidime (CAZ, 30 µg), aztreonam (ATM, 30 µg), ertapenem (ERT, 10 µg), gentamicin (GM, 10 µg), amikacin (AN, 30 µg), chloramphenicol (C, 30 µg), tetracycline (TE, 30 µg), sulphamethoxazole-trimethoprim (SXT, 1.25/23.75), ciprofloxacin (CIP, 5 µg), and fosfomycin (FOS, 50 µg). *E. coli* ATCC 25922 was used for quality control.

ESBL production was detected by double-disk synergy test with disks of amoxicillin-clavulanic acid surrounded at a radius of 30 mm by cefotaxime, ceftriaxone, ceftazidime and aztreonam. Bacterial suspensions at a concentration of 10^7 CFU/ml were inoculated on Mueller-Hinton agar.

Carbapenemase producing strains were detected with an inhibition zone diameter of <25 mm with ERT antibiotic disk as in CASFM (2016).

Statistical analysis

Differences in continuous and categorical variables between groups were analyzed with non-parametric Mann-Whitney U and chi-squared tests, respectively. The level of significance for all statistical tests was set at $p < 0.05$. Statistical analyses were performed with SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) software.

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Table 1. Demographics and specimen type characteristics from patients infected with ESBL-and carbapenemase-producer and non-producer isolates.

Parameter	Value	% of patients					
		ESBL producer (n=316)	Non ESBL producer (n=889)	p	CPE (n=62)	Non CPE (n=1143)	p
Sex	M/F	191/125	569/320	0.367	38/24	722/421	0.905
Age, year, median (IQR)	-	46 (29-65)	50 (29-64)	0.774	51.5 (32-65)	48 (29-65)	0.753
Hospital unit, n (%)	Internal medicine	50 (18.6)	141 (17.5)	0.634	7 (13)	184 (18)	0.476
	Pediatrics	9 (3.3)	34 (4.2)		4 (7.4)	39 (3.8)	
	ICU	27 (10)	64 (7.9)		7 (13)	84 (8.2)	
	Surgery	74 (27.5)	210 (26)		16 (29.6)	268 (26.2)	
	Urology	92 (32.3)	370 (37.7)		17 (31.5)	369 (36.1)	
	Other	21 (7.8)	59 (7.3)		3 (5.5)	77 (7.5)	
Specimen type, n (%)	Wound swab	76 (22.7)	281 (26)	0.482	12 (19.3)	322 (29.8)	0.711
	Urine	189 (56.6)	583 (54)		36 (58)	711 (65,8)	
	Liquid of effusion	5 (1.5)	17 (1.6)		1 (1.6)	20 (1.8)	
	Genital	9 (2.7)	50 (4.6)		3 (4.8)	50 (4.6)	
	Blood	12 (3.6)	28 (2.6)		3 (4.8)	36 (3.3)	
	Catheter	7 (2.1)	27 (2.5)		0	30 (2.8)	
	Respiratory	32 (9.6)	87 (8)		7 (11.3)	102 (9.4)	
	Fecal	4 (1.2)	7 (0.6)		0	10 (0.9)	

ESBL: Extended-spectrum beta-lactamase; CPE: carbapenemase producing *Enterobacteriaceae*; M: male; F: female; IQR: inter quartile range; ICU: intensive care unit.

RESULTS

Prevalence of ESBL- and carbapenemase-producing Enterobacteriaceae (CPE)

During the study period, a total of 1205 Enterobacteriaceae stains were tested for ESBL and carbapenemase production. The overall prevalence of ESBL- and carbapenemase-producing Enterobacteriaceae was 26.2 and 5.1%, respectively. 3.8% of these pathogens were, however, both ESBL and carbapenemase producers.

The demographic characteristics of the study population are shown in Table 1. Male patients predominated either in ESBL-producer and CPE or in non ESBL-producer and non-CPE isolates. There was no significant difference regarding age between patients infected by these two groups of pathogens.

The majority of these MDR (ESBL and CPE) strains were isolated from patients hospitalized in urology (32.4%), surgery (27.7%), internal medicine (18.5%), and ICUs units (10%).

The prevalence of strains is depicted in Table 2. Among the Enterobacteriaceae ESBL positive, *E. coli* (45.2%), *K. pneumoniae* (26.2%), *Enterobacter cloacae* (12.6%), and *Proteus vulgaris* (9.2%) were the most prevalent. These strains were mainly isolated from urine (56.6%) and pus (22.7%) specimens. The most prevalent CPEs were *E. coli* (46.8%), *K. pneumoniae* (27.4%), and *E. cloacae* (16.1%) particularly isolated from urine (58%) and pus (19.3%).

Susceptibility of ESBL- and carbapenemase-producing Enterobacteriaceae to antimicrobial agents

Table 3 shows the results of the susceptibility test of 316 ESBL- and 62 carbapenemase-producing clinical isolates against several antibiotics. All ESBL producing isolates were resistant to amoxicillin, cefalotin, cefamandole, and cefotaxim or ceftriaxone. The majority of these ESBL strains had associated high resistance rates to non-β-lactam antibiotics, including chloramphenicol (76.4%), sulfamethoxazole/trimethoprim complex (76.2%), tetracycline (61.3%), and ciprofloxacin (50.2%). Interestingly, fosfomycin, amikacin, and imipenem, remain very effective against the majority of the ESBL strains, with 94.1, 92.5 and 88.6% activities, respectively, while colistin activity is declining (66.7%).

Regarding CPE, all isolates were resistant to β-lactam antibiotics, except aztreonam (17.8%). High rates of

Table 2. Distribution of *Enterobacteriaceae* strains ESBL-and CARB-producer and non-producer isolates.

Parameter	Value	% of patients					
		ESBL producer (n=316)	Non ESBL producer (n=889)	p	CPE (n=62)	Non CPE (n=1143)	p
Isolated micro-organisms, n (%)	*Escherichia coli*	143 (45.2)	408 (45.9)	0.773	28 (45.2)	523 (45.7)	0.876
	Klebsiella pneumoniae	83 (26.3)	233 (26.2)		17 (27.4)	299 (26.2)	
	Enterobacter cloacae	40 (12.6)	104 (11.7)		10 (16.1)	134 (11.7)	
	Citrobacter freundii	13 (4.1)	45 (5)		1 (1.6)	57 (5)	
	Proteus vulgaris	29 (9.2)	71 (8)		4 (6.5)	96 (8.4)	
	Salmonella spp.	1 (0.3)	5 (0.6)		-	6 (0.5)	
	Shigella spp.	-	1 (0.1)		-	1 (0.1)	
	Serratia marcescens	-	2 (0.2)		-	2 (0.2)	
	Morganella morganii	7 (1.4)	1 (0.1)		2 (3.2)	18 (1.6)	
	Providencia stuarti	-	7 (0.8)		-	7 (0.6)	

ESBL: Extended-spectrum beta-lactamase; CPE: carbapenemase producing Enterobacteriaceae.

Table 3. Susceptibility rate for ESBL- and carbapenemase-producing isolates to different antibiotics.

Anti-biotics	MDR Enterobacteriaceae	
	% ESBL producer	CPE (%)
Amoxicillin	0	0
Amoxicillin-clavulanic acid	44.2	40.7
Cefalotin	0	0
Cefamandole	0	0
Cefotaxim	0	0
Ceftriaxone	0	0
Aztreonam	33.3	17.8
Ertapenem	88.6	0
Gentamicin	61.5	57.4
Amikacin	92.5	100
Chloramphenicol	23.6	17.7
Tetracycline	38.7	9.7
Sulphamethoxazole-trimethoprim	23.8	14.3
Ciprofloxacin	49.8	28
Fosfomycin	94.1	66.7
Colistin	66.7	60

ESBL: Extended-spectrum beta-lactamase; CPE: carbapenemase producing Enterobacteriaceae.

resistance patterns were detected with tetracycline (90.3%), sulfamethoxazole/trimethoprim complex (85.7%), chloramphenicol (82.3%), and ciprofloxacin (72%). Interestingly, amikacin remained fully active to all isolates.

DISCUSSION

ESBL- and carbapenemase-producing Enterobacteriaceae (CPE) strains are increasingly reported worldwide pathogens in community- and hospital-onset infections (Paterson and Bonomo 2005; Tzouvelekis et al., 2012), suggesting the need for continuous surveillance of antimicrobial resistance (AMR) patterns. In this study, the prevalence of ESBL- and CPE and its antibiotic susceptibility patterns in Aristide Le Dantec Teaching Hospital in Dakar, the major university hospital of Senegal was investigated. The rate of ESBL- and carbapenemase-producing strains was found to be 23.6 and 5.1%, respectively.

High rate of Enterobacteriaceae producing ESBLs have also been reported across Africa, namely Ghana (49.3%) (Obeng-Nkrumah et al., 2013), Gabon (45%) (Schaumburg

et al., 2013), Egypt (38.5%) (Bouchillon et al., 2004), and Tanzania (21%) (Blomberg et al., 2005).

In this study, ESBL production was mainly detected towards *E. coli* (45.2%) and *K. pneumoniae* (26.3%). Similar results have been reported from USA (Ajao et al., 2013) and India (Taneja et al., 2010), showing ESBL prevalence rates from 60 to 71% for *K. pneumoniae* and 35.0 to 42% for *E. coli*. The majority of ESBL producers were recovered from urine specimens (56.6%) and pus (22.7%). This is consistent with data reported in other studies (Obeng-Nkrumah et al., 2013; Severin et al., 2010). Our findings confirm the reports of Pitout et al. (Pitout and Laupland 2008) showing higher frequencies of ESBL-producing Enterobacteriaceae among patients with severe infections including UTIs, suppurative infections, bacteremia, and intra-abdominal. Indeed, ESBL-producing Enterobacteriaceae have been associated with serious nosocomial infection outbreaks that lead to prolonged hospital stay, increased morbidity and mortality, and consequently increased healthcare associated costs with limited therapeutic options (Pitout, 2010). Increasing rate of community-acquired infections caused by ESBL-producing Enterobacteriaceae has, however, been recently reported (Lonchel et al., 2012), representing a potential reservoir for ESBL producers.

ESBL-producing strains were more dominant among patients admitted in urology, surgery, internal medicine, and intensive care units, as reported elsewhere (Obeng-Nkrumah et al., 2013; Shu et al., 2010).

In this study, all strains were resistant to cefotaxim or ceftriaxone (99.9%). These ESBLs are plasmid mediated β-lactamases resistance and are associated with co-resistance to other classes of antibiotics (Paterson and Bonomo 2005). This would explain the high rates of resistance to non-β-lactam antibiotics observed in our study, including tetracycline, sulfamethoxazole/ trimethoprim complex, chloramphenicol, and ciprofloxacin, which are comparable to rates found in other studies (Lin et al., 2012; Simner et al., 2011). Low rates of amikacin and fosfomycin resistance were detected, which is in agreement with findings from Korea (Lee et al., 2012), Taiwan (Liu et al., 2015), and Japan (Wachino et al., 2010). Carbapenems are generally stable against ESBLs and still mainly used as treatment of last resort in infections caused by multi-drug resistant bacteria. As reported elsewhere (Liu et al., 2015), the result of the present study data showed that imipenem remains very active against ESBL-producing *E. coli* and *K. pneumoniae* clinical isolates. However, ESBL-producing Enterobacteriaceae carbapenem resistant are emerging worldwide. This might be due to acquisition of cabapenem-hydrolyzing β-lactamases (Nordmann et al., 2011) or a combination of plasmid-mediated AmpC β-lactamase and loss of an outer membrane protein (Dahmen et al., 2012), limiting thus the drug treatment choices. The most prevalent CPEs detected in this study were *E. coli* (45.2%), *K. pneumonia* (27.4%), and *E. cloacae* (16.1%), particularly isolated from urines (58%), and pus (19.3%). All these isolates were resistant to β-lactam antibiotics. In addition, high resistance rates were observed with tetracycline (90.3%), sulfamethoxazole/trimethoprim complex (85.7%), chloramphenicol (82.3%), and ciprofloxacin (72%). Interestingly, amikacin remained fully active, while fosfomycin and colistin were respectively effective only in 66.7 and 60% of these MDR strains. In fact, only few remaining antibiotics are currently in use to treat infections caused by carbapenemase-producing Gram-negative bacilli including, colistin, tigecycline, amikacin, fosfomycin, and temocillin. Appropriate combination therapy with 2 or more drugs is superior to monotherapy and associated with better survival rate (Tumbarello et al., 2015; Daikos et al., 2014; Zarkotou et al., 2011).

Conclusion

ESBL- and carbapenemase-producing Enterobacteriaceae strains are important pathogens in community- and hospital-onset infections. Emergence of carbapenem-resistant clinical isolates underscores the need for continuous surveillance of antimicrobial resistance patterns. This study shows high prevalence rates of ESBL-producing isolates and emerging prevalence of CPE with limited therapeutic options, suggesting a need for continuous MDR surveillance patterns and antibiotic combination recommendation, particularly in hospital settings.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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