Title: Molecular characterization of extended-spectrum beta-lactamase-producing extra-intestinal pathogenic *Escherichia coli* isolated in a university teaching hospital Dakar-Senegal

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Abstract

Extra-intestinal pathogenic *Escherichia coli* (ExPEC), a predominant Gram-negative bacterial pathogen, express a wide range of virulence factors and is responsible of several diseases including urinary tract infections (UTI), nosocomial pneumonia, bacteremia, and neonatal meningitis. ExPEC isolates are often multidrug resistant (MDR) and clones producing extended-spectrum beta-lactamases (ESBL) are increasingly reported all over the world.

Seventy-eight clinical ExPEC strains were selected for this study. The majority was from UTIs (n=51), while the rest (n=27) was from pus, sputum, bronchial fluid and vaginal samples (non-uropathogenic ExPEC). Interestingly, 49 out of the 78 ExPEC isolates where considered as community-acquired (CA) and 29 hospital-acquired (HA) bacteria. Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method. Standard polymerase chain reaction (PCR) was used to screen major ESBL genes ($bla_{\text{CTX-M}}$, $bla_{\text{OXA-1}}$, bla_{TEM}, bla_{SHV}) and $_{\text{bla}}$CTX-M variants ($bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-9}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-25}}$).

All the ExPEC strains were resistant to ampicillin, ticarcillin, amoxicillin/clavulanic acid combination, cefalotin, cefotaxime, ceftazidime, cefepime and aztreonam, but showed a high susceptibity to fosfomycin (98.7%, n = 77), ertapenem (96.2%, n = 75), and imipenem (100%). Moreover, isolates harbored at least one ESBL gene, including $bla_{\text{CTX-M}}$ (98.7%), $bla_{\text{OXA-1}}$ (78.2%), bla_{TEM} (44.9%) and bla_{SHV} (3.8%). The CTX-M variants were also found with the predominance of $bla_{\text{CTX-M-1}}$ (90.9%) and $bla_{\text{CTX-M-15}}$ (90.9%) followed by $bla_{\text{CTX-M-9}}$ (11.7%), while $bla_{\text{CTX-M-25}}$ was not detected.

Despite the resistance to most of the tested antibiotics, ExPEC isolates showed fortunately a good susceptibility to fosfomycin and carbapenems. $bla_{\text{CTX-M}}$ ($bla_{\text{CTX-M1}}$, $bla_{\text{CTX-M15}}$) and $bla_{\text{OXA-1}}$ seem

to be *E. coli* major ESBL genes circulating in Senegal. No significant difference was noted when comparing prevalence of ESBL genes detected from CA and HA strains, and from UPEC and non-uropathogenic ExPEC. The high level of resistance to antimicrobials observed stresses the need of establishing an epidemiological surveillance of antimicrobial resistance in both community and hospital settings.

***Key words**: Escherichia coli*, Extended Spectrum β-lactamase, hospital, Dakar-Senegal

Words count: 299

Introduction

Escherichia coli (*E. coli*), a common bacteria found in various parts of the human body, is also the predominant bacterial species responsible for community-acquired (CA) and hospital-acquired (HA) infections at all ages in human [1]. Human pathogenic *E. coli* strains are classified into two large groups, strains responsible for intestinal infections and those causing extra-intestinal diseases (ExPEC) [2, 3].

ExPECs are among the most common Gram-negative bacterial pathogens affecting Human with diverse infections, including urinary tract infections (UTI), bacteremia, meningitis, nosocomial respiratory infections, peritonitis, prostatitis, skin and soft tissue infections [4–6].

In addition, multidrug resistant (MDR) ExPECs are now common both in community-acquired and hospital-acquired infections, including resistance to beta-lactams (β-lactams), which are the commonly used antibiotics in human and animal health. The β-lactams resistance is mediated by production of extended-spectrum beta-lactamases [7–9]. These enzymes hydrolyze

penicillins, cephalosporins (first, second, third, and fourth generation), and monobactams, but were generally inactive against cephamycins and carbapenems. ESBLs are generally inhibited by beta-lactamase inhibitors (BLI) [10, 11]. A worrying fact is that mobile genetic elements that harbor ESBL genes also carry others genes conferring resistance to quinolones, aminoglycosides and even carbapenems [12–15].

ESBL-producing ExPECs infections are responsible of extended hospital stays, accompanying high cost and mortality and morbidity [2]. Hence, the importance to establish innovative diagnostic toolkits and performant surveillance system for early detection and monitoring of ExPECs cases, especially in developing countries. In this study, we investigated the antibiotic resistance profile and the ESBL genes carried by ESBL-producting ExPEC isolated at the laboratory of bacteriology laboratory, Aristide le Dantec University Teaching Hospital (HALD) in Dakar, Senegal. Additionally, we compared community-acquired (CA) to hospital-acquired (HA), and uropathogenic *E. coli* (UPEC) to no-uropathogenic ExPEC isolates (No-UPEC).

Materials and methods

Bacterial isolates

This is a retrospective study and all ExPEC isolates analyzed in this study were collected between January 1st, 2018 and December 31th, 2020 at the Hospital Laboratory of HALD during routine activities and stored at -80°C. Seventy-eight no-duplicate strains were randomly selected from the Laboratory. Strains were isolated from urine (UPEC, n = 51), pus, sputum, bronchial fluid and vaginal samples (no-uropathogenic ExPEC, n = 27). Of the 78 strains, 49 and 29 were CA

and HA respectively. Culture and Isolation were done based on gold standard microbiological tests and identification by using Api 20E for *Enterobacteriaceae* (bioMérieux France).

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method and results were interpreted according to the committee of the French society of microbiology (CA-SFM, 2020) recommendations. Briefly, bacterial suspensions were prepared at 0.5 Mc Farland and inoculated onto Mueller-Hinton agar for an overnight incubation at 37 °C. These following antibiotic disks were tested: ampicillin (AMP, 10 µg), ticarcillin (TIC, 75 µg), amoxicillin-clavulanic acid (AMC, 20/10 µg), cefalotin (CEF, 30 µg), cefoxitin (FOX, 30 µg), cefotaxime (CTA, 30 µg), ceftazidime (CAZ, 30 µg), cefepime (CEP, 30 µg), aztreonam (AZT, 30 µg), imipenem (IMP, 10 µg), ertapenem (ERT, 10µg), Nalidixic acid (NAL, 30 µg), ciprofloxacin (CIP, 5 µg), gentamicin (GEN, 10 µg), amikacin (AMI, 30 µg), fosfomycin (FOS, 50 µg), tetracycline (TET, 30 µg) and sulfamethoxazole-trimethoprim (TMS, 1.25 µg / 23.75 µg). The *E. coli* ATCC 25922 was used for quality control. ESBL production was appreciated by double-disk synergy test with disks of amoxicillin-clavulanic acid surrounded at a radius of 30 mm by cefepime, ceftriaxone, ceftazidime and aztreonam.

DNA extraction

Bacterial DNA extraction was performed mechanically by thermal choc. Briefly, a well-separated bacterial colony was dispersed in a tube contained 1ml of sterile distilled water, vortexed, boiled for 15 minutes at 100°C and centrifuged at 13,200 rpm for 10 min. The supernatant was carefully

recovered, aliquoted and stored at -20°C until used. To confirm results, extraction was done by Qiagen kit (DNeasy Blood & Tissue Kit (50) Cat. No. / ID: 69504).

ESBL genes amplification

A simplex end-point PCR was performed (on Thermocycler 2720, Applied Biosystems, Lincoln Centre Drive, Foster City, California 94404, USA) to detect ESBL genes. Specific primer pairs (Table 1) were used to amplify ESBL genes (*bla*$_{\text{CTX-M}}$, *bla*$_{\text{CTX-M-1}}$, *bla*$_{\text{CTX-M-9}}$, *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{CTX-M-25}}$, *bla*$_{\text{OXA-1}}$, *bla*$_{\text{TEM}}$, *bla*$_{\text{SHV}}$). Each reaction included positive and negative controls. PCRs were carried out in 20 µl reaction volume (2.5 µl DNA + 17.5 Master Mix FIREPol®). The amplification program consisted of an initial denaturation at 95°C for 3 min., 35 PCR cycles (denaturation: 94° C, 30 sec., 72°C, 60 sec.) and a final elongation at 72°C for 7 min. Ten microliters of each amplicon were separated on 2% agarose gel in 1X TAE buffer for 35 min at 135 volts and the amplified fragment detected using a GelDoc imager (BioRad).

Table 1. Oligonucleotide primers sequence used for PCR to detect ESBL genes.

Target genes	Sequences genes	Sizes (bp)	Annealing Temp (°C)	References
bla$_{\text{CTX-M}}$	F: 5' - ATGTGCAGYACCAGTAARGTKATGGC - 3' R: 5' - TGGGTRAARTARGTSACCAGAAYSAGCGG - 3'	592	55	[16]
bla$_{\text{CTX-M-1}}$	F: 5' - GGTTAAAAAATCACTGCGTC - 3' R: 5' - TTACAAACCGTYGGTGACGA - 3'	873	50	[16]
bla$_{\text{CTX-M-9}}$	F: 5' - GTGACAAAGAGAGTGCAACGG - 3' R: 5' - ATGATTCTCGCCGCTGAAGCC - 3'	856	55	[16]

$bla_{\text{CTX-M-15}}$	F: 5' - CACACGTGGAATTTAGGGACT - 3' R: 5' - GCCGTCTAAGGCGATAAACA - 3'	995	50	[16]
$bla_{\text{CTX-M-25}}$	F: 5' - GCACGATGACATTCGGG - 3' R: 5' - AACCCACGATGTGGGTAGC - 3'	327	52	[16]
$bla_{\text{OXA-1}}$	F: 5' - ATGAAAAACACAATACATATC - 3' R: 5' - AATTTAGTGTGTTTAGAATGG - 3'	830	56	[17]
bla_{TEM}	F: 5' - TTGGGTGCACGAGTGGGTTA - 3' R: 5' - TAATTGTTGCCGGGAAGCTA - 3'	506	55	[16]
bla_{SHV}	F: 5' - TCGGGCCGCGTAGGCATGAT - 3' R: 5' - AGCAGGGCGACAATCCCGCG - 3'	628	52	[16]

Statistical analysis

Statistical analysis and multiple correspondence analysis and data analysis methods were performed with R software. The statistic test used is the Chi-square at 5% risk threshold. p-values are obtained from the proportion comparison test and the level of significance for all statistical tests was set at $p < 0.05$.

Results

Antibiotic susceptibility testing

All the 78 ExPEC isolates were MDR (resistance to at least one drug from at least three classes of antibiotics), and were resistant to ampicillin, ticarcillin, amoxicillin/clavulanic acid combination, cefalotin, cefotaxime, ceftazidime, cefepime and aztreonam (Table 2). Besides,

resistance to ciprofloxacin (93.6%, *n* = 73), tetracycline (91%, *n* = 71) and sulfamethoxazole-trimethoprim (91%, *n* = 71) was high, while less frequent for aminoglycosides (gentamicin, 60.3%, *n* = 47; amikacin, 42.3%, *n* = 33). In contrast, only 3.8% (*n* = 3) and 1.3% (*n* = 1) of the isolates were resistant to ertapenem and fosfomycin respectively, while all were sensitive to imipenem (Table 2). Comparison of resistance profiles between CA and HA, and between UPEC and no-UPEC strain did not show any significant difference, except for ciprofloxacin (Table 2).

Table 2. Antibiotics resistance rate of total strains, CA and HA strains, UPEC and no-UPEC strains.

Antibiotics		**Total strains**	**Pathogenicity**			**Origin**		
Class	**Drug**	N (%)	**UPEC** N (%)	**No-UPEC** N (%)	**p**	**CA** N (%)	**HA** N (%)	**p**
Beta-lactams	AMP	78 (100)	51 (100)	27 (100)	1	49 (100)	29 (100)	1
	TIC	78 (100)	51 (100)	27 (100)	1	49 (100)	29 (100)	1
	AMC	78 (100)	51 (100)	27 (100)	1	49 (100)	29 (100)	1
	CEF	78 (100)	51 (100)	27 (100)	1	49 (100)	29 (100)	1
	FOX	5 (6.4)	3 (5.9)	2 (7.4)	0.06	4 (8.2)	1 (3.5)	0.06
	CTA	78 (100)	51 (100)	27 (100)	1	49 (100)	29 (100)	1
	CAZ	78 (100)	51 (100)	27 (100)	1	49 (100)	29 (100)	1
	CEP	78 (100)	51 (100)	27 (100)	1	49 (100)	29 (100)	1
	AZT	70 (89.7)	48 (91.4)	22 (81.5)	0.08	46 (93.9)	24 (82.8)	0.12
	IMP	0	0	0	-	0	0	-
	ERT	3 (3.8)	3 (5.9)	0	0.01*	2 (4.1)	1 (3.5)	0.04*
Quinolones and Fluoroquinolones	NAL	76 (97.4)	51 (100)	25 (92.6)	0.97	49 (100)	27 (93.1)	0.97
	CIP	73 (93.6)	50 (98)	23 (85.2)	0.03*	48 (98)	25 (86.2)	0.04*

Aminoglycosides	GEN	47 (60.3)	33 (64.7)	14 (51.9)	0.27	29 (59.2)	16 (62.1)	0.72
	AMI	33 (42.3)	24 (47.1)	9 (33.3)	0.24	19 (38.8)	14 (48.3)	0.41
Phosphonic acid	FOS	1 (1.3)	1 (2)	0	0.01*	0	1 (3.6)	0.01*
Cyclines	TET	71 (91)	45 (88.2)	26 (96.3)	0.24	44 (89.8)	27 (93.1)	0.62
Antifolates	TMS	71 (91)	45 (88.2)	26 (96.3)	0.24	45 (91.8)	26 (89.7)	0.74

UPEC, Uropathogenic *E. coli*; CA, Community-acquired; HA, Hospital-acquired; AMP, ampicillin; TIC, ticarcillin; AMC, Amoxicillin-clavulanic acid; CEF, cefalotin; FOX, cefoxitin; CTA, cefotaxim; CAZ, ceftazidime; CEP, cefepime; AZT, aztreonam; IMP, imipenem; ERT, Ertapenem; NAL, nalidixic acid; CIP, ciprofloxacin; GEN, gentamicin; AMI, amikacin; FOS, fosfomycin; TET, tetracycline; TMS, sulphamethoxazole-trimethoprim; *, significant p-value ($p < 0.05$).

Presence of ESBL genes

All 78 strains carried at least one ESBL gene. bla_{CTX-M} group was the most prevalent (77/78; 98.7%), followed by bla_{OXA-1} (61/78; 78.2%), bla_{TEM} (35/78; 44.9%) and bla_{SHV} (3/78; 3.8%) (Table 3) and (Fig 1). 51/51 (100%) of UPEC strains and 29/29 (100%) of hospital - acquired strains carried the bla_{CTX-M} gene and none of " no-uropathogenic ExPEC " strains carried a bla_{SHV} gene (Table 3) and (Fig 2). (9/78; 11.5%) carried only bla_{CTX-M} or bla_{OXA-1} and (69/78; 88.5%) carried several types of ESBL gene. Indeed, (2/78; 2.6%) carried bla_{CTX-M} + bla_{OXA-1} + bla_{TEM} + bla_{SHV}; (23/78; 29.4%) carried bla_{CTX-M} + bla_{OXA-1} + bla_{TEM}; (1/78; 1.3%) carried bla_{CTXM} + bla_{OXA-1} + bla_{SHV}; (32/78; 41%) carried bla_{CTX-M} + bla_{OXA-1} and (11/78; 14.1%) of strains carried "bla_{CTX-M} + bla_{TEM}" (Table 3). None of strains carried bla_{TEM} or bla_{SHV} gene alone (Table 4).

Table 3. Prevalence of ESBL genes in total strains, CA and HA strains, UPEC and non-UPEC strains.

ESBL		**Total strains**	**Pathogenicity**			**Origin**		
Family	**Genes**	N (%)	**UPEC** N (%)	**No-UPEC** N (%)	**p**	**CA** N (%)	**HA** N (%)	**p**
Cefotaximase-Munich	*bla*$_{CTX-M}$	77 (98.7)	51 (100)	26 (96.3)	0.98	48 (98)	29 (100)	0.99
	bla$_{CTX-M-1}$	70 (89.7)	48 (94.1)	22 (81.5)	0.08	44 (89.8)	26 (89.7)	0.98
	bla$_{CTX-M-9}$	9 (11.5)	5 (9.8)	4 (14.8)	0.25	5 (10.2)	4 (13.8)	0.45
	bla$_{CTX-M-15}$	70 (89.7)	48 (94.1)	22 (81.5)	0.08	44 (89.8)	26 (89.7)	0.98
	bla$_{CTX-M-25}$	0	0	0	-	0	0	-
Oxacillinase	*bla*$_{OXA-1}$	61 (78.2)	42 (82.4)	19 (70.4)	0.22	38 (77.6)	23 (79.3)	0.86
Temoneira	*bla*$_{TEM}$	35 (44.9)	23 (45.1)	12 (44.4)	0.95	19 (38.8)	16 (55.2)	0.16
Sulfhydryl variable	*bla*$_{SHV}$	3 (3.8)	3 (5.9)	0	0.01*	2 (4.1)	1 (3.5)	0.04*

UPEC, Uropathogenic *E. coli*; CA, community-acquired; HA, hospital-acquired; %, percentage; N, number of isolates, *, significant p-value (< 0.05).

Fig. 1. Prevalence of ESBL genes in total strains.

Fig. 2. Prevalence of ESBL genes in UPEC and no-uropathogenic ExPEC.

Table 4. Prevalence of ESBL genes combinations in total strains, CA and HA strains, UPEC and non-UPEC strains.

Combination of ESBL genes	Total strains	Pathogenicity			Origin		
	N (%)	UPEC N (%)	No-UPEC N (%)	p	CA N (%)	HA N (%)	p
$bla_{CTX-M-1} + bla_{CTX-M-15} + bla_{CTX-M-9} + bla_{OXA-1} + bla_{TEM}$	2 (2.6)	2 (3.9)	0	0.01*	1 (2)	1 (3.4)	0.1
$bla_{CTX-M-1} + bla_{CTX-M-15} + bla_{OXA-1} + bla_{TEM} + bla_{SHV}$	2 (2.6)	2 (3.9)	0	0.01*	1 (2)	1 (3.4)	0.1
$bla_{CTX-M-1} + bla_{CTX-M-15} + bla_{OXA-1} + bla_{TEM}$	21 (26.9)	14 (27.4)	7 (33.3)	0.68	10 (20.4)	11 (37.9)	0.12
$bla_{CTX-M-1} + bla_{CTX-M-15} + bla_{OXA-1} + bla_{SHV}$	1 (1.3)	1 (2)	0	0.01*	1 (2)	0	0.01*
$bla_{CTX-M-1} + bla_{CTX-M-15} + bla_{OXA-1}$	32 (41)	21 (41.2)	11 (40.7)	0.98	23 (46.9)	9 (31)	0.15
$bla_{CTX-M-1} + bla_{OXA-1} + bla_{TEM}$	1 (1.3)	1 (2)	0	0.01*	1 (2)	0	0.01*
$bla_{CTX-M-1} + bla_{CTX-M-15} + bla_{TEM}$	7 (9)	4 (7.8)	3 (11.1)	0.21	5 (10.2)	2 (6.9)	0.23
$bla_{CTX-M-1} + bla_{CTX-M-15}$	3 (3.8)	2 (3.9)	1 (3.7)	0.97	2 (4.1)	1 (3.4)	0.04
$bla_{CTX-M-15} + bla_{CTX-M-9}$	2 (2.6)	2 (3.9)	0	0.01*	1 (2)	1 (3.4)	0.1
$bla_{CTX-M-1} + bla_{OXA-1}$	1 (1.3)	1 (2)	0	0.01*	0	1 (3.4)	0.01*
$bla_{CTX-M-9} + bla_{TEM}$	2 (2.6)	0	2 (7.4)	0.01*	1 (2)	1 (3.4)	0.1

UPEC, Uropathogenic *E. coli*; CA, community-acquired; HA, hospital-acquired %, percentage; N, number of isolates; *, significant p-value (< 0.05).

167

In 4 *bla*$_{\text{CTX-M}}$ group, *bla*$_{\text{CTX-M-1}}$ (70/77; 90.9%) with *bla*$_{\text{CTX-M-15}}$ (70/77; 90.9%) was the most prevalent followed by *bla*$_{\text{CTX-M-9}}$ (9/77; 11.7%). *bla*$_{\text{CTX-M-25}}$ was not detected in any of the 77 strains (Table 4). Among strains which carried the *bla*$_{\text{CTX-M}}$ type, 89.6% carried 2 variants of *bla*$_{\text{CTX-M}}$ while 7.8% carried only one variant and 2.6% carried 3 *bla*$_{\text{CTX-M}}$ variants (Table 4). No significant difference was found by comparing the prevalence of ESBL genes in hospital-acquired and community-acquired strains on the one hand and UPEC and no-uropathogenic ExPEC strains on the other hand (Figs 2-5).

Fig. 3. Prevalence of ESBL genes in community-acquired and hospital-acquired strains.

Fig. 4. Prevalence of bla$_{CTX-M}$ variants in community and hospital-acquired strains.

Fig. 5. Prevalence of bla$_{CTX-M}$ variants genes in UPEC and no-uropathogenic ExPEC.

Discussion

Potentially pathogenic *Escherichia coli* which produce extended spectrum betalactamases (ESBL) are frequently isolated from urinary tract infections [6, 18] can be resistant to many molecules of this class. The most worrying thing is that these strains, which spread rapidly both in hospitals and in the community, are often resistant to many other antibiotics such as those of the aminoglycosides and quinolones classes, thus making the treatment failure of these infections.

The rate of resistance to multiple antibiotics among ESBL- producing isolates is usually common due to carrying multi- resistant genes and plasmids [12–14].

Nowadays, ESBL type CTX-M are the most widespread in the world, unlike ESBL TEM and SHV which are becoming less prevalent [19, 20]. Our study also confirmed this trend with

98.7% of strains positive for *bla*$_{CTX-M}$, followed by *bla*$_{OXA-1}$ (78.2%), *bla*$_{TEM}$ (44.9%) and *bla*$_{SHV}$ (3.8%). Several studies carried out in Togo [21], in Saudi Arabia [22], and in Mozambique [23] reported high prevalence rates of *bla*$_{CTX-M}$ in ESBL ExPEC strains 100%; 93,94% and 77%, respectively. Moreover, other authors [24, 25] had already pointed out that currently, *bla*$_{OXA-1}$ was the second most prevalent ESBL gene type in the world behind *bla*$_{CTX-M}$. In disagreement to these studies, [23] rather mentioned 52% of prevalence for *bla*$_{SHV}$ and 1% for *bla*$_{TEM}$ in 2021 in Mozambique. [26] mentioned 3.12% of prevalence rate for *bla*$_{SHV}$ in 2019 in Senegal. ESBLs SHV-gene type therefore seem to be rare in *E. coli* strains circulating in Senegal.

Interestingly, 55.1% of the strains harbored 2 ESBL gene types while 30.8% of the strains carried 3 and 2.6% carried all the 4 ESBL genotypes. While 33.33% and 12.12% of strains carrying 2 and 3 ESBL gene types, respectively were reported from Riyadh, Saudi Arabia [22]. The very high proportion of strains (88.5%) combining several ESBL gene types seems to be one of the major causes of the 100% resistance to ampicillin, ticarcillin, (clavulanic acid + amoxicillin), cefalotin, cefotaxime, ceftazidime, cefepime and aztreonam. None of the strains carried only *bla*$_{TEM}$ or *bla*$_{SHV}$ gene.

Globally, *bla*$_{CTX-M-15}$ had long been cited as the most prevalent variant of *bla*$_{CTX-M}$ in *E. coli* [27–29]. The high prevalence rate of *bla*$_{CTX-M-15}$ (90.9%) observed among *bla*$_{CTX-M}$ positive strains in our study corroborates these earlier studies. An interesting fact in our study was that *bla*$_{CTX-M-1}$ was as prevalent as *bla*$_{CTX-M-15}$ with respectively 90.9% and 85.7% rates of the strains concomitantly carried *bla*$_{CTX-M-15}$ and *bla*$_{CTX-M-1}$. These data suggest that *bla*$_{CTX-M-15}$ is not the only major variant of *bla*$_{CTX-M}$ circulating in Senegal. The low prevalence rates of *bla*$_{CTX-M-9}$ (11.7%) and *bla*$_{CTX-M-25}$ (0%) follow trends observed in other parts of the world [27, 30].

No significant difference was noted when comparing the prevalence of ESBL genes from community-acquired and hospital-acquired strains. This seems to imply either a port of ESBL

ExPEC in community or that the community strains are the same ones encountered in a hospital environment. We did not notice any significant difference in the prevalence of ESBL genes between UPEC strains and non-uropathogenic ExPEC strains. It seems that in Senegal, non-uropathogenic ExPEC are as resistant as UPEC strains. Future studies could confirm this.

The high prevalence of $bla_{\text{CTX-M}}$ genes suggests the involvement of mobile genetic elements (plasmids, integrons and transposons) [20, 31] in the spread of antibiotic resistance in Dakar, as reported in many studies leading increasing resistance to fluoroquinolones, aminoglycosides and even carbapenems antibiotics [12–14]. This suggests the importance to study and monitor the mobile genetic elements from strains isolated in healthy carriers, environment, and hospital settings in order to initiate others actions that can help fighting against antibiotic resistance.

Conclusion

All the 78 ExPEC strains tested in this study were MDR patterns, and resistant to almost all antibiotics families, except fosfomycin and carbapenems. Based on our results, we recommend avoiding monotherapy and prohibiting fluoroquinolones, C3G and C4G as empiric treatment of UTIs in Senegal. $bla_{\text{CTX-M}}$ ($bla_{\text{CTX-M1}}$, $bla_{\text{CTX-M15}}$) and $bla_{\text{OXA-1}}$ seem to be the major ESBL genes circulating in Senegal. No significant difference was noted when comparing the prevalence of ESBL genes between hospital-acquired and community-acquired strains; As well as by comparing UPEC and ExPEC strains isolated from other types of samples. The high resistance to antimicrobials observed, underscore the relevance to implement an epidemiological antimicrobial resistance (AMR) surveillance system to improve the management of treatment protocols in patients infected with MDR bacteria.

Acknowledgements

The authors thank Abdoul Aziz Wane, Amadou Mactar Gueye, Ousmane Sow and El Hadji Aly Niang for their technical assistance.

References

1. Savage DC. Microbial biota of the human intestine: a tribute to some pioneering scientists. Curr Issues Intest Microbiol. 2001;2: 1–15.

2. Kaper JB, Nataro JP, Mobley HLT. Pathogenic Escherichia coli. Nat Rev Microbiol. 2004;2: 123–140. https://doi.org/10.1038/nrmicro818

3. Braz VS, Melchior K, Moreira CG. Escherichia coli as a Multifaceted Pathogenic and Versatile Bacterium. Front Cell Infect Microbiol. 2020;10: 548492. https://doi.org/10.3389/fcimb.2020.548492

4. Russo TA, Johnson JR. Medical and economic impact of extraintestinal infections due to Escherichia coli: focus on an increasingly important endemic problem. Microbes Infect. 2003;5: 449–456. https://doi.org/10.1016/s1286-4579(03)00049-2

5. Johnson JR, Russo TA. Extraintestinal pathogenic Escherichia coli : "The other bad E coli ." J Lab Clin Med. 2002;139: 155–162. https://doi.org/10.1067/mlc.2002.121550

6. Pitout J. Extraintestinal Pathogenic Escherichia coli: A Combination of Virulence with Antibiotic Resistance. Front Microbiol. 2012;3. https://doi.org/10.3389/fmicb.2012.00009

7. Camara M, Mane MT, Ba-Diallo A, Dieng A, Diop-Ndiaye H, Karam F, et al. Extended-spectrum beta-lactamase- and carbapenemase-producing Enterobacteriaceae clinical isolates in a Senegalese teaching hospital: A cross sectional study. Afr J Microbiol Res. 2017;11: 1600–1605. https://doi.org/10.5897/AJMR2017.8716

8. Toudji AG, Djeri B, Karou SD, Tigossou S, Ameyapoh Y, Souza C de. Prévalence des souches d'entérobactéries productrices de bêta-lactamases à spectre élargi isolées au Togo et de leur sensibilité aux antibiotiques. Int J Biol Chem Sci. 2017;11: 1165–1177. https://doi.org/10.4314/ijbcs.v11i3.19

9. Ouedraogo A-S, Sanou M, Kissou A, Sanou S, Solaré H, Kaboré F, et al. High prevalence of extended-spectrum ß-lactamase producing enterobacteriaceae among clinical isolates in Burkina Faso. BMC Infect Dis. 2016;16: 326. https://doi.org/10.1186/s12879-016-1655-3

10. Ghafourian S, Sadeghifard N, Soheili S, Sekawi Z. Extended Spectrum Beta-lactamases: Definition, Classification and Epidemiology. Curr Issues Mol Biol. 2015;17: 11–22. https://doi.org/10.21775/cimb.017.011

11. Bradford PA. Extended-Spectrum β-Lactamases in the 21st Century: Characterization, Epidemiology, and Detection of This Important Resistance Threat. Clin Microbiol Rev. 2001;14: 933–951. https://doi.org/10.1128/CMR.14.4.933-951.2001

12. Gibreel TM, Dodgson AR, Cheesbrough J, Fox AJ, Bolton FJ, Upton M. Population structure, virulence potential and antibiotic susceptibility of uropathogenic Escherichia coli from Northwest England. J Antimicrob Chemother. 2012;67: 346–356. https://doi.org/10.1093/jac/dkr451

13. Doumith M, Day M, Ciesielczuk H, Hope R, Underwood A, Reynolds R, et al. Rapid Identification of Major Escherichia coli Sequence Types Causing Urinary Tract and Bloodstream Infections. J Clin Microbiol. 2015;53: 160–166. https://doi.org/10.1128/JCM.02562-14

282 **14.** Dale AP, Woodford N. Extra-intestinal pathogenic Escherichia coli (ExPEC): Disease, carriage and
283 clones. J Infect. 2015;71: 615–626. https://doi.org/10.1016/j.jinf.2015.09.009

284 **15.** Poirel L, Dortet L, Bernabeu S, Nordmann P. Genetic Features of blaNDM-1-Positive
285 Enterobacteriaceae. Antimicrob Agents Chemother. 2011;55: 5403–5407.
286 https://doi.org/10.1128/AAC.00585-11

287 **16.** Gundran RS, Cardenio PA, Villanueva MA, Sison FB, Benigno CC, Kreausukon K, et al. Prevalence
288 and distribution of blaCTX-M, blaSHV, blaTEM genes in extended- spectrum β- lactamase-
289 producing E. coli isolates from broiler farms in the Philippines. BMC Vet Res. 2019;15: 227.
290 https://doi.org/10.1186/s12917-019-1975-9

291 **17.** Weill F-X, Guesnier F, Guibert V, Timinouni M, Demartin M, Polomack L, et al. Multidrug
292 resistance in Salmonella enterica serotype Typhimurium from humans in France (1993 to 2003). J
293 Clin Microbiol. 2006;44: 700–708. https://doi.org/10.1128/JCM.44.3.700-708.2006

294 **18.** Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol
295 Rev. 2005;18: 657–686. https://doi.org/10.1128/CMR.18.4.657-686.2005

296 **19.** Bevan ER, Jones AM, Hawkey PM. Global epidemiology of CTX-M β-lactamases: temporal and
297 geographical shifts in genotype. J Antimicrob Chemother. 2017;72: 2145–2155.
298 https://doi.org/10.1093/jac/dkx146

299 **20.** Canton R, Gonzalez-Alba JM, Galán JC. CTX-M Enzymes: Origin and Diffusion. Front Microbiol.
300 2012;3. https://www.frontiersin.org/article/10.3389/fmicb.2012.00110

301 **21.** Dossim S, Salou M, Ihou-Wateba M, Bidjada B, Godonou AM, Aoussi E, et al. Molecular
302 Characterization Of Extended Spectrum Betalactamases Genes (Blactxm And Blashv) In

303 Enterobacteria Isolates In Medical Specimens In Lomé (Togo). https://researcher-
304 app.com/paper/3400456

305 **22.** Alqasim A, Abu Jaffal A, Alyousef AA. Prevalence of Multidrug Resistance and Extended-
306 Spectrum β-Lactamase Carriage of Clinical Uropathogenic Escherichia coli Isolates in Riyadh, Saudi
307 Arabia. Int J Microbiol. 2018;2018: e3026851. https://doi.org/10.1155/2018/3026851

308 **23.** Estaleva CEL, Zimba TF, Sekyere JO, Govinden U, Chenia HY, Simonsen GS, et al. High
309 prevalence of multidrug resistant ESBL- and plasmid mediated AmpC-producing clinical isolates of
310 Escherichia coli at Maputo Central Hospital, Mozambique. BMC Infect Dis. 2021;21: 16.
311 https://doi.org/10.1186/s12879-020-05696-y

312 **24.** Faezi Ghasemi M, Dibadji SN. Prevalence of blaoxa-1 and blashv Genes in E. coli Isolates from
313 Hospitalized Patients in Rasht. Med Lab J. 2016;10: 65–70.
314 https://doi.org/10.18869/acadpub.mlj.10.5.65

315 **25.** Abrar S, Ain NU, Liaqat H, Hussain S, Rasheed F, Riaz S. Distribution of blaCTX − M, blaTEM,
316 blaSHV and blaOXA genes in Extended-spectrum-β-lactamase-producing Clinical isolates: A three-
317 year multi-center study from Lahore, Pakistan. Antimicrob Resist Infect Control. 2019;8: 80.
318 https://doi.org/10.1186/s13756-019-0536-0

319 **26.** Diagne R. Recherche de gènes BLSE de type TEM, SHV, et OXA-1 sur des souches de E. coli
320 isolées au laboratoire de Bactériologie de Fann, Sénégal. Rev Afr Malgache Rech Sci Santé. 2019;1.
321 http://publication.lecames.org/index.php/sante/article/view/1469

322 **27.** Lahlaoui H, Ben Haj Khalifa A, Ben Moussa M. Epidemiology of Enterobacteriaceae producing
323 CTX-M type extended spectrum β-lactamase (ESBL). Med Mal Infect. 2014;44: 400–404.
324 https://doi.org/10.1016/j.medmal.2014.03.010

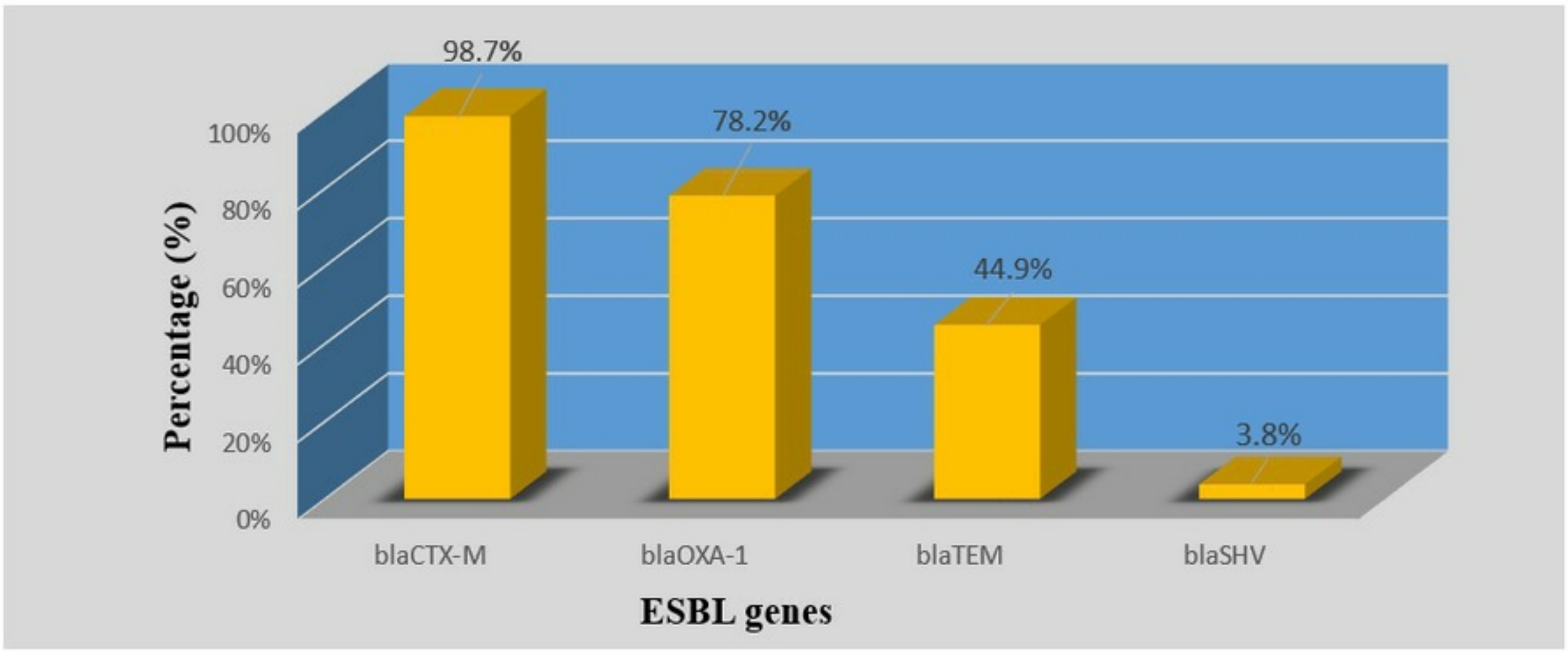
325 **28.** Moghaddam MN, Beidokhti MH, Jamehdar SA, Ghahraman M. Genetic properties of blaCTX-M
326 and blaPER β-lactamase genes in clinical isolates of Enterobacteriaceae by polymerase chain
327 reaction. Iran J Basic Med Sci. 2014;17: 378–383.
328 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4069837/

329 **29.** Zong Z, Partridge SR, Thomas L, Iredell JR. Dominance of blaCTX-M within an Australian
330 Extended-Spectrum β-Lactamase Gene Pool. Antimicrob Agents Chemother. 2008;52: 4198–4202.
331 https://doi.org/10.1128/AAC.00107-08

332 **30.** Pavez M, Troncoso C, Osses I, Salazar R, Illesca V, Reydet P, et al. High prevalence of CTX-M-1
333 group in ESBL-producing *enterobacteriaceae* infection in intensive care units in southern Chile.
334 Braz J Infect Dis. 2019;23: 102–110. https://doi.org/10.1016/j.bjid.2019.03.002

335 **31.** Carattoli A. Plasmids in Gram negatives: molecular typing of resistance plasmids. Int J Med
336 Microbiol IJMM. 2011;301: 654–658. https://doi.org/10.1016/j.ijmm.2011.09.003

337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78.2%
44.9%
3.8%
100%
80%
60%
40%
20%
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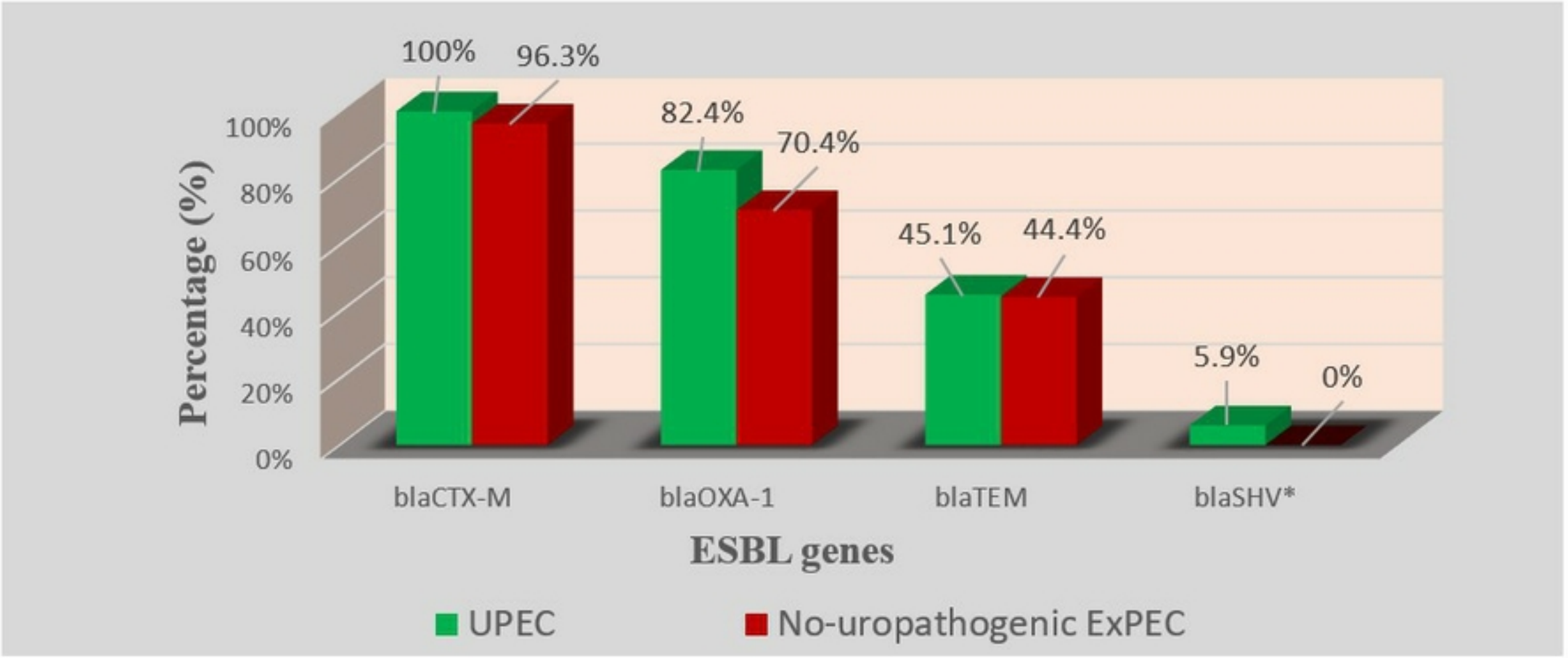
Percentage (%)
blaCTX-M
blaOXA-1
blaTEM
blaSHV
ESBL genes

Fig. 1



* Significant p ($p = 0.01$)

Fig. 2

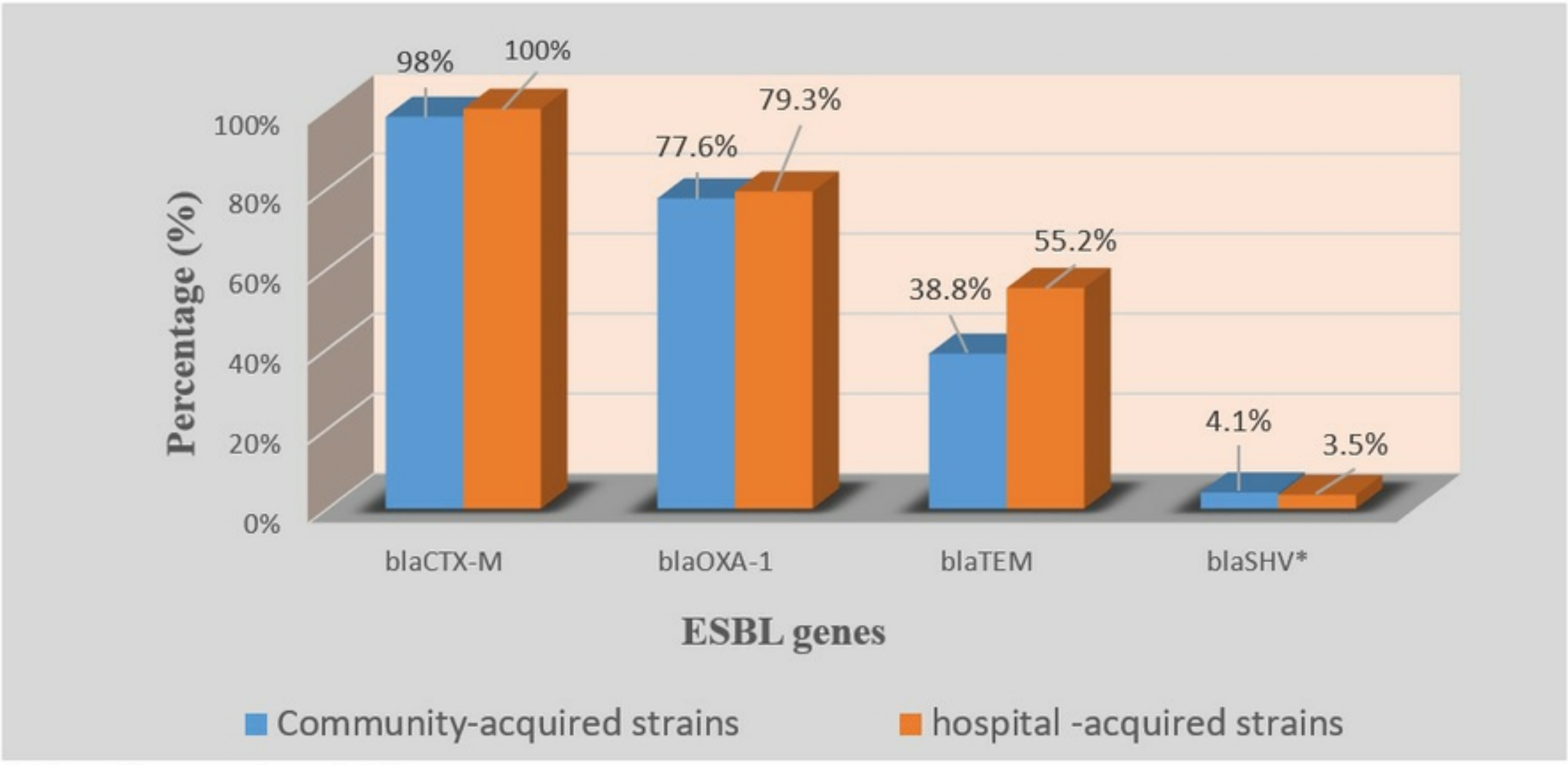


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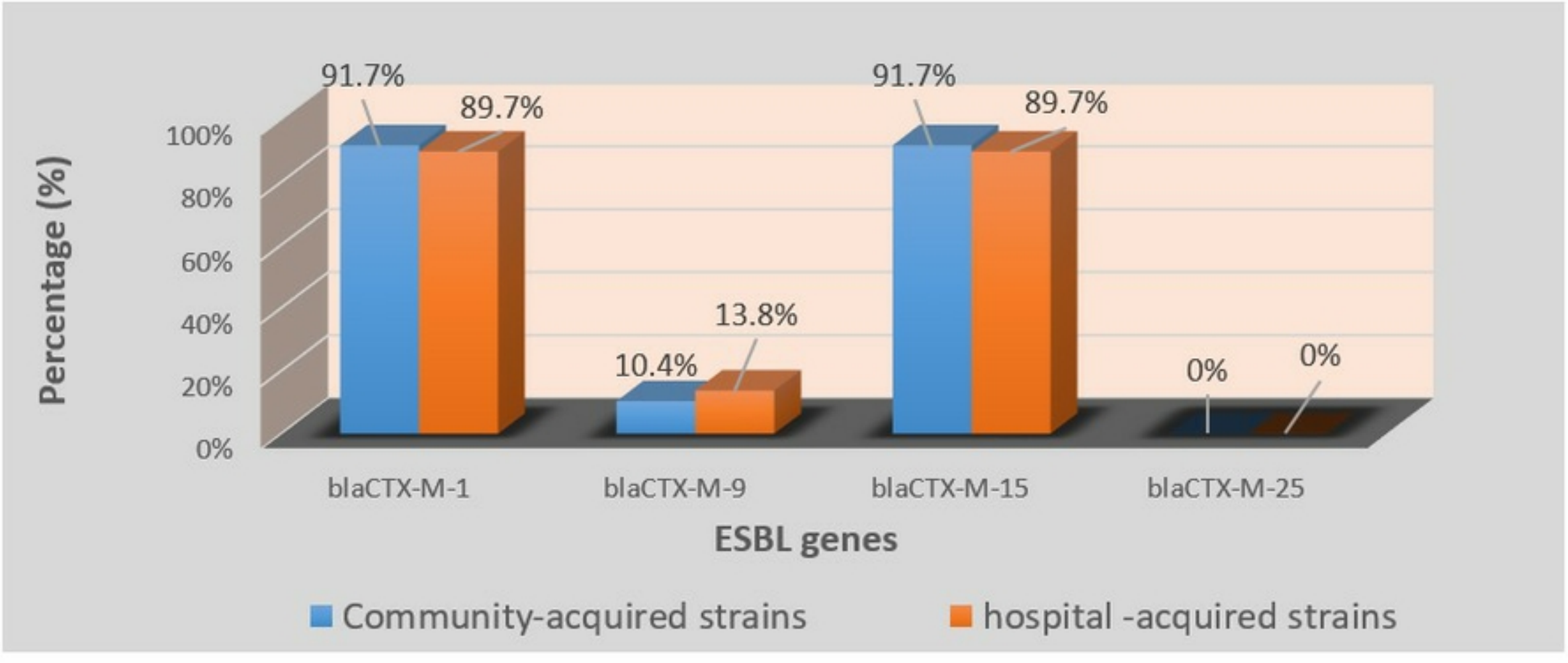
Fig. 3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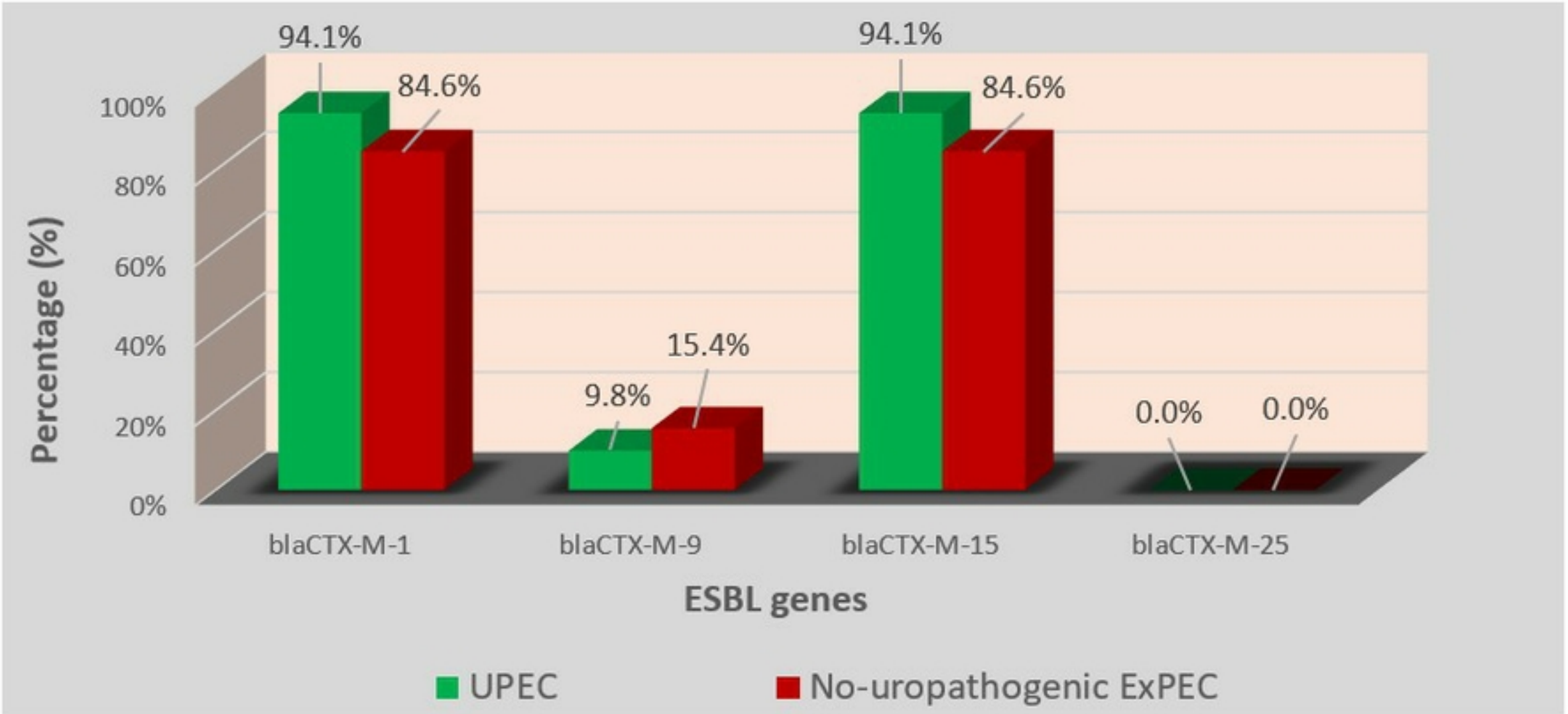
ESBL genes
Community-acquired strains
hospital -acquired strains

Fig. 4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blaCTX-M-1
blaCTX-M-9
blaCTX-M-15
blaCTX-M-25
ESBL genes
UPEC
No-uropathogenic ExPEC

Fig. 5