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Prevalence of difficult-to-treat resistance in ESKAPE pathogens in a third level hospital in Mexico

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SUMMARY

Background: Antimicrobial resistance and difficult-to-treat resistance (DTR) in ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) is a threat to human health. The aim of this study was to determine the prevalence of antimicrobial resistance and DTR rates in ESKAPE pathogens over six years in a third-level hospital from Monterrey, Mexico.

Methods: Antimicrobial susceptibility testing was determined by either disk diffusion or broth microdilution in strains from 2018 to 2023. Isolates were screened for carbapenemase genes. Multidrug resistance (MDR), extensively drug resistance (XDR), carbapenem resistance (CR), extended-spectrum cephalosporin-resistance (ESCR), fluoroquinolone resistance (FQR), and DTR were determined.

Results: From 3,239 strains, 48.5% were from respiratory infections, resistance was 87.5% to meticillin in *Staphylococcus* spp. and 39.8% in *S. aureus*, and 13.9% to vancomycin in *Enterococcus* spp. MDR, FQR and ESCR rates were between 54−90% in *A. baumannii*, 20−60% in Enterobacterales and 17−25% in *P. aeruginosa*. CR was 85.7% in *A. baumannii*, 33.3% in *P. aeruginosa* and <5% in Enterobacterales. Most frequent CR genes were OXA-24/40-like in *A. baumannii* and NDM and OXA-48 in carbapenem-resistant Enterobacterales. DTR rates were 59.7% in *A. baumannii* (49.2% in 2018 vs 62.9% in 2023), 8.9% in *P. aeruginosa* and <3% in Enterobacterales. XDR in *A. baumannii* was 14.4%.

Conclusions: Antimicrobial resistance rates were high in Gram-negative pathogens. CR and DTR rates were higher in *A. baumannii* than *P. aeruginosa* and Enterobacterales. DTR surveillance in healthcare providers should be continuous updating local and regional DTR trends among Gram-negative bacteria.

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Introduction

Antimicrobial resistance (AMR) is a global threat to human health. According to the Centers for Disease Control and Prevention (CDC), over 2.8 million infections exhibiting AMR occur each year in the United States, causing over 35,000 deaths [1]. In Mexico, previous studies reported high frequency of drug-resistant pathogens, including 75.3% of carbapenem-resistant *Acinetobacter baumannii* and 62% of meticillin-resistant *Staphylococcus aureus* (MRSA), particularly in the intensive care unit [2]. Similarly, a ten-year report from more than 22 states of Mexico showed that resistance to some antibiotics such as carbapenems was increasing in several bacterial species, especially in *Enterobacter* spp., *Klebsiella* spp., and *A. baumannii* [3]. AMR is especially significant for pathogens related to healthcare-associated infections (HAI) [4], and in individuals who are colonised by multidrug-resistant (MDR) organisms [5]. The emergence of these MDR pathogens often occurs by transmission of resistant clones or genes, or by selective pressure after antimicrobial treatment, or due to lack of prevention and infection control measures [6,7].

The Infectious Diseases Society of America (IDSA) highlighted the clinical importance of specific HAI-causing pathogens harbouring resistance to critical antimicrobials needed for their treatment [8]. The ESKAPE group includes vancomycin-resistant *Enterococcus* (VRE), MRSA, carbapenem-resistant *Klebsiella pneumoniae*, MDR *A. baumannii*, MDR *Pseudomonas aeruginosa*, and carbapenem-resistant *Enterobacter cloacae* [4]. Carbapenem-resistant Enterobacterales are also among the most frequent antimicrobial-resistant bacteria causing HAI [1].

Difficult-to-treat resistance (DTR) is a term coined by Kadri *et al.* in 2018 which refers to non-susceptibility to all first-line antimicrobial agents [9]. In Gram-negative pathogens such as Enterobacterales, *P. aeruginosa* and *A. baumannii*, the DTR phenotype can be the source of severe infections [10], such as bloodstream infections (BSI) and lower respiratory tract infections (LRTI) [11]. Therapeutic failure and the limited therapeutic options for DTR infections results in higher mortality rates [4,12]. Non-fermenting Gram-negative pathogens such as *Acinetobacter* and *Pseudomonas* can show higher DTR rates [9,13–18] compared to lactose-fermenting Gram-negative bacteria such as Enterobacterales [19]. However, most studies regarding the prevalence of DTR focus on BSI rather than HAI. Furthermore, there has been no study that has assessed DTR rates in Mexico. The aim of this study was to analyse the prevalence of AMR and DTR rates in ESKAPE pathogens during six years in a third-level hospital in Monterrey, Mexico.

Methods

Study design

This retrospective descriptive study aimed to assess the prevalence of AMR and DTR per year in ESKAPE pathogens in a third-level hospital from Monterrey, Mexico.

Site of study

The University Hospital "Dr José Eleuterio Gonzalez" is a third-level teaching hospital located in Monterrey, the third largest city in Northeastern Mexico. It is a multi-building hospital and medical school complex that also includes a COVID-19 facility of 85 beds, and it serves a population that includes the Monterrey metropolitan area, including 51 municipalities (approximately 5.5 million habitants) and surroundings states. It has an average of 25,000 admissions and around 200,000 emergency room visits per year.

Ethics approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee of the University Hospital at which the studies were conducted (Approval number IF23-00003) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Clinical isolates

The strains were collected from January 2018 to December 2023. The isolates were identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS ®, Microflex LT system, Bruker Daltonics, Bremen, Germany) according to the manufacturer's recommendations. The isolates were classified according to the manufacturer's recommended score identification criteria, in which a score $\geq$ 2.00 indicates reliable species identification.

Inclusion criteria were isolates only from clinically relevant specimens. For each microorganism, only one isolate per patient was included, unless the antimicrobial susceptibility profile was different in the same species. Exclusion criteria were when isolates had susceptibility tests performed for less than four antibiotics.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) was determined by two methods. From January 2018 to September 2020, AST was assessed by disk diffusion. A 0.5 McFarland bacterial inoculum was inoculated into Mueller-Hinton plates. Then, antimicrobial susceptibility test discs (Becton Dickinson, Rutherford, NJ, United States) were deposited on the Mueller-Hinton plates, incubated for 18–24 h at 37°C, and the inhibition zone diameter was measured. *S. aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as quality control. Isolates were classified as susceptible, intermediate, and resistant based on Clinical and Laboratory Standards Institute (CLSI) breakpoints criteria for each corresponding year [20–22].

From October 2020 to December 2023, AST was evaluated predominantly by broth microdilution method using the VITEK 2® Compact automatized system (Biomérieux, Craponne, France) according to the manufacturer's instructions. Bacteria were cultured overnight at 35°C on plates containing tryptic soy broth agar with 5% sheep blood. Then, a 0.5 McFarland bacterial suspension in 0.45% sodium chloride was introduced into the equipment, and was used to inoculate an AST-GP75 VITEK card for Gram-positive microorganisms or AST-N271 and AST-N272 VITEK cards for Gram-negative microorganisms. The cards were incubated at 35°C until the test was completed. According to the resulting minimum inhibitory concentration (MIC), isolates were classified as

susceptible, intermediate, and resistant based on CLSI breakpoints criteria from each corresponding year [22–25]. The quality control for the card for Gram-positive and Gram-negative susceptibility were made according to the manufacturer's recommendations using the *S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853.

During 2018, colistin susceptibility testing was conducted using broth microdilution in accordance with the CLSI guidance applicable during that period [20]. Subsequently, following the updated guideline in 2019, screening was performed utilising colistin broth disk elution, with confirmation assessed through broth microdilution [21–25].

For tigecycline, *A. baumannii* isolates were classified based on the British Society for Antimicrobial Chemotherapy (BSAC) breakpoints criteria for the disk diffusion methodology (in which inhibition diameters $\geq$ 24 mm were classified as susceptible and inhibition diameters $\leq$ 19 mm were classified as resistant), or based on the US Food and Drug Administration (FDA) breakpoints for the VITEK methodology (MIC $\leq$ 1 μg/mL were classified as susceptible and MIC $>$ 8 μg/mL were classified as resistant) [26]. Carbapenem-resistant Enterobacterales isolates were screened to detect carbapenemase production using the modified carbapenem inactivation method [25].

Screening of drug resistance genetic elements

Carbapenem-resistant Enterobacterales isolates were screened for the presence of carbapenemase genes including class A carbapenemases such as KPC [27] and GES [28], metallo-β-lactamases such as VIM, IMP, NDM; and OXA-type carbapenemases such as OXA-48 like [27]. A selection of carbapenem-resistant strains was screened for the presence of OXA-23-like, OXA-24/40-like, OXA-51-like, and OXA-58-like genes in *A. baumannii* [29] and KPC, GES, VIM, IMP and NDM genes for *P. aeruginosa*.

Drug resistance definitions

Multidrug resistance (MDR) was defined as non-susceptibility to $\geq$ one agent in $\geq$ three antimicrobial categories [7] both in Gram-negative and Gram-positive microorganisms, except for *Staphylococcus* species other than *S. aureus*. MRSA isolates were defined as MDR isolates due to oxacillin and cefoxitin resistance predicting non-susceptibility to all categories of β-lactam (except anti-MRSA cephalosporins) included in MDR classification. Extensively drug resistance (XDR) was defined as non-susceptibility to $\geq$ one agent in all but $\leq$ two antimicrobial categories [7].

Carbapenem resistance (CR) was defined as non-susceptibility in *P. aeruginosa* and *A. baumannii* complex or resistance in *E. coli*, *K. pneumoniae*, and *E. cloacae* complex to $\geq$ one carbapenem (imipenem, ertapenem only in Enterobacterales, or meropenem). Extended-spectrum cephalosporin-resistance (ESCR) was defined as resistance to $\geq$ one extended-spectrum cephalosporin (ceftazidime, cefotaxime, cefepime or ceftriaxone in Enterobacterales and ceftazidime and cefepime for *P. aeruginosa* and *A. baumannii*). Fluoroquinolone resistance (FQR) was defined as resistance to $\geq$ one fluoroquinolone (ciprofloxacin or levofloxacin) in Gram-negative pathogens.

Difficult-to-treat resistance (DTR) was defined as intermediate or resistant to all reported agents in β-lactam (ceftazidime, cefotaxime, cefepime, ceftriaxone or piperacillin/tazobactam [except cefotaxime and ceftriaxone in *P. aeruginosa*] and ampicillin/sulbactam [*A. baumannii* only]); carbapenem (ertapenem, imipenem, or meropenem [ertapenem Enterobacterales only]), and fluoroquinolone (ciprofloxacin or levofloxacin) categories in Gram-negative pathogens according to the IDSA [9].

Statistical analysis

The significant differences between resistance percentages from each year were evaluated using one-way ANOVAs and Tukey test. The tests were analysed using SPSS® version 20.0 software (IBM Corporation, NY, United States) and the graphs were created using GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA) version 8.0. A $P \leq 0.05$ was considered as statistically significant.

Results

Clinical isolates included in the study

During the six-year period, a total of 10,455 clinical isolates were first included in the study. Antimicrobial susceptibility testing was performed on 3,239 strains (Table SI), of which 21.2% isolates were identified as *P. aeruginosa*, 19.7% isolates as *A. baumannii* complex, 16.7% as *S. aureus*, 15.1% as *K. pneumoniae*, 10.7% as *E. coli*, 5.5% as *E. cloacae* complex, 5.8% as *Enterococcus faecalis*, 1.5% as *Enterococcus faecium*, and 3.8% were *Staphylococcus* spp. (*S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. lugdunensis*, *S. caprae*, *S. pasteuri*, *S. simulans*, *S. urealyticus*, *S. capitis*, *S. caprae* and *S. xylosus*).

Most of the samples were obtained from low respiratory tract (48.5%), skin and soft tissue (23.5%), intraabdominal (7.3%), urinary tract (6.6%), and osteoarticular (5.3%) infections. Most of the isolates were collected from the following wards: internal medicine (33.4%), intensive care unit (30.4%), surgery rooms (15.6%), and COVID-19 unit (15.4%).

Susceptibility profile of ESKAPE pathogens

The antimicrobial susceptibility profile is shown in Table I for Gram-positive microorganisms and in Table II for Gram-negative microorganisms. The comparison of the resistance rates per year is shown in Figure 1.

Among Gram-positive pathogens, *S. aureus* showed high resistance ($>$80%) to penicillin, moderate resistance ($>$23–40%) to oxacillin, erythromycin, clindamycin, ciprofloxacin, and levofloxacin, and low resistance ($<$10%) to daptomycin, tetracycline, doxycycline and trimethoprim/sulfamethoxazole. Resistance trends increased for oxacillin ($P<0.0001$) whilst resistance to daptomycin decreased over the years ($P<0.0001$, Figure 1B). *Staphylococcus* spp. isolates showed high resistance to oxacillin and low resistance to doxycycline and daptomycin. No resistance was detected to linezolid or vancomycin.

E. faecalis and *E. faecium* showed high resistance ($>$50%) to tetracycline, penicillin, erythromycin, ciprofloxacin and levofloxacin, and low resistance to vancomycin, ampicillin, linezolid and daptomycin. Of vancomycin-resistant *Enterococcus* spp. isolates, 71.9% were *E. faecium* and 28.1% were *E. faecalis*.

Table I
Antimicrobial susceptibility of Gram-positive microorganisms included in the study. Two different methodologies were used to perform antimicrobial susceptibility testing, disk diffusion and VITEK®

	S. aureus n=540; No. (%)				Other *Staphylococcus* spp. n=122; No. (%)				*Enterococcus* spp. n=237; No. (%)			
Methodology												
Disk diffusion	162 (30.0)				8 (6.6)				32 (13.5)			
VITEK®	378 (70.0)				114 (93.4)				205 (86.5)			
Antibiotic	*n*	S	I	R	*n*	S	I	R	*n*	S	I	R
Ampicillin	NA	NA	NA	NA	NA	NA	NA	NA	198	174 (87.9)	0 (0.0)	24 (12.1)
Penicillin	161	21 (13.0)	0 (0.0)	140 (87.0)	-	-	-	-	33	12 (36.4)	0 (0.0)	21 (63.6)
Oxacillin	505	304 (60.2)	0 (0.0)	201 (39.8)	112	14 (12.5)	0 (0.0)	98 (87.5)	NA	NA	NA	NA
Erythromycin	501	319 (63.7)	11 (2.2)	171 (34.1)	107	35 (32.7)	1 (0.9)	71 (66.4)	24	7 (29.2)	5 (20.8)	12 (50.0)
Clindamycin	344	235 (68.3)	0 (0.0)	109 (31.7)	95	40 (42.1)	1 (1.1)	54 (56.8)	NA	NA	NA	NA
Levofloxacin	489	371 (75.9)	2 (0.5)	116 (23.7)	105	55 (52.4)	1 (0.9)	49 (46.7)	34	15 (44.1)	1 (2.9)	18 (53.0)
Ciprofloxacin	355	264 (74.4)	3 (0.8)	88 (24.8)	97	51 (52.6)	2 (2.1)	44 (45.3)	36	13 (36.1)	3 (8.3)	20 (55.6)
Tetracycline	350	338 (96.6)	1 (0.3)	11 (3.1)	95	81 (85.3)	1 (1.0)	13 (13.7)	62	20 (32.3)	1 (1.5)	41 (66.1)
Doxycycline	497	490 (98.6)	3 (0.6)	4 (0.8)	105	92 (87.6)	6 (5.7)	7 (6.7)	-	-	-	-
Trimethoprim/ sulfamethoxazole	516	510 (98.8)	1 (0.2)	5 (1.0)	102	71 (69.6)	3 (2.9)	28 (27.5)	NA	NA	NA	NA
Daptomycin	495	450 (90.9)	8 (1.6)	37 (7.5)	95	90 (94.7)	0 (0.0)	5 (5.3)	159	107 (67.3)	48 (30.2)	4 (2.5)
Linezolid	508	508 (100.0)	0 (0.0)	0 (0.0)	108	107 (99.1)	0 (0.0)	1 (0.9)	228	222 (97.3)	2 (0.9)	4 (1.8)
Vancomycin	426	426 (100.0)	0 (0.0)	0 (0.0)	103	103 (100.0)	0 (0.0)	0 (0.0)	230	196 (85.2)	2 (0.9)	32 (13.9)

S: susceptible; I: intermediate; R: resistant; NA: not applicable; (-): not measured.

Among Gram-negative microorganisms, *A. baumannii* complex isolates showed high resistance (>67%) to levofloxacin, ceftazidime, piperacillin/tazobactam, meropenem, imipenem, cefepime, ciprofloxacin, and gentamicin, moderate resistance (>31−50%) to ampicillin/sulbactam, tigecycline, and doxycycline, and low resistance to colistin (0.5%). Resistance trends to ampicillin/sulbactam and ciprofloxacin (P<0.001 each) increased whilst resistance to tigecycline (P<0.001) decreased over the years (Figure 1C).

P. aeruginosa isolates showed moderate resistance (>21−35%) to imipenem, meropenem, ceftazidime, and piperacillin/tazobactam, and low resistance (<20%) to ciprofloxacin, cefepime, levofloxacin, ceftazidime/avibactam, amikacin, tobramycin, and gentamicin. Colistin resistance was not detected. Over the years, higher resistance trends were observed to cefepime (P=0.004), ceftazidime (P=0.009), and piperacillin/tazobactam (P<0.0001) while susceptibility to amikacin, ciprofloxacin, and imipenem increased over the years (P<0.0001 each, Figure 1D).

K. pneumoniae isolates showed high resistance (>50%) to cefotaxime, ceftriaxone, trimethoprim/sulfamethoxazole, cefepime, and ceftazidime, and moderate resistance (35−49%) to ampicillin/sulbactam, ciprofloxacin, gentamicin, and low resistance (<20%) to levofloxacin, cefoxitin, ceftazidime/avibactam, piperacillin/tazobactam, amikacin, imipenem, meropenem and ertapenem. Colistin resistance was not detected. Over the years, higher resistance trends were observed to piperacillin/tazobactam, ceftazidime, cefepime, meropenem and ertapenem (P<0.0001 each, Figure 1E).

E. coli isolates showed high resistance (>60%) to ampicillin, cefotaxime, levofloxacin, ciprofloxacin, ceftriaxone, ampicillin/sulbactam, cefepime, ceftazidime, and trimethoprim/sulfamethoxazole, moderate resistance (28−38%) to gentamicin and cefoxitin, and low resistance (<5%) to piperacillin/tazobactam, amikacin, ertapenem, and meropenem. Imipenem or colistin resistance was not detected. Resistance trends showed a tendency to increase over the years for cefepime, ertapenem (P<0.0001 each) and trimethoprim/sulfamethoxazole (P=0.0002, Figure 1F).

E. cloacae complex isolates showed moderate resistance (21−53%) to cefotaxime, trimethoprim/sulfamethoxazole, ceftazidime, ciprofloxacin, gentamicin, and cefepime, and low resistance (<20%) to piperacillin/tazobactam, levofloxacin, ertapenem, imipenem, meropenem, amikacin, and colistin.

Screening of drug resistance associated genes

Regarding carbapenem resistance in Enterobacterales, 56.5% (n=13) were carbapenemase-producing Enterobacterales isolates. Three *K. pneumoniae* isolates harboured NDM; one *K. pneumoniae* isolate also harboured the OXA-48 like gene and two *K. pneumoniae* only harboured OXA-48 like. All carbapenemase-producing *E. coli* (n=5) harboured NDM, and one isolate also harboured VIM. Three *E. cloacae* isolates harboured NDM, and one isolate did not carry any of the analysed genes.

In carbapenem-resistant *A. baumannii* complex isolates (32.0%), OXA-51-like (100%) and OXA-24-like (99.4%) were detected. No OXA-23-like and OXA-58-like genes were detected. In carbapenem-resistant *P. aeruginosa* strains (15.1%), imipenem (14.3%), VIM (2.6%), and GES (2.6%) genes were detected.

Drug resistance classification

A comparison was performed of drug resistance classification in ESKAPE isolates according to Magiorakos *et al.* [7], CDC and IDSA definitions [9], shown in Table III.

MDR, FQR, and ESCR rates were <66% in *E. coli* (10/17 antimicrobial categories tested), 35−55% in *K. pneumoniae* (9/16

Table II
Antimicrobial susceptibility of Gram-negative microorganisms included in the study. Two different methodologies were used to perform antimicrobial susceptibility testing, disk diffusion and VITEK®

N	*E. coli* No. (%)				*K. pneumoniae* No. (%)				*E. cloacae* complex No. (%)				*A. baumannii* complex No. (%)				*P. aeruginosa* No. (%)			
	348				**489**				**177**				**638**				**688**			
Methodology																				
Disk diffusion	59 (17.0)				153 (31.3)				55 (31.1)				299 (46.9)				187 (27.2)			
VITEK®	289 (83.0)				336 (68.7)				122 (68.9)				339 (53.1)				501 (72.8)			
Antibiotic	n	S	I	R	n	S	I	R	n	S	I	R	n	S	I	R	n	S	I	R
Ampicillin	162	21 (13.0)	1 (0.6)	140 (86.4)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ampicillin-sulbactam	275	91 (33.1)	19 (6.9)	165 (60.0)	199	84 (42.2)	18 (9.0)	97 (48.7)	NA	NA	NA	NA	616	231 (37.5)	78 (12.7)	307 (49.8)	NA	NA	NA	NA
Piperacillin/tazobactam	77	68 (88.3)	5 (6.5)	4 (5.2)	193	137 (71.0)	26 (13.5)	30 (15.5)	66	52 (78.8)	1 (1.5)	13 (19.7)	591	62 (10.5)	9 (1.5)	520 (88.0)	641	430 (67.1)	71 (11.1)	140 (21.8)
Ceftazidime/avibactam	-	-	-	-	12	9 (75.0)	1 (8.3)	2 (16.1)	-	-	-	-	NA	NA	NA	NA	91	78 (85.7)	1 (1.1)	12 (13.2)
Cefepime	334	121 (36.2)	1 (0.3)	212 (63.5)	459	202 (44.0)	11 (2.4)	246 (53.6)	166	123 (74.1)	8 (4.8)	35 (21.1)	611	69 (11.3)	15 (2.5)	527 (86.3)	671	489 (72.9)	61 (9.1)	121 (18.0)
Cefotaxime	125	35 (28.0)	1 (0.8)	89 (71.2)	149	56 (37.6)	1 (0.7)	92 (61.7)	49	23 (46.9)	0 (0.0)	26 (53.1)	-	-	-	-	NA	NA	NA	NA
Cefoxitin	99	68 (68.7)	3 (3.0)	28 (28.3)	135	108 (80.0)	2 (1.5)	25 (18.5)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ceftazidime	314	112 (35.7)	4 (1.3)	198 (63.0)	468	206 (44.0)	17 (3.6)	245 (52.4)	157	87 (55.4)	4 (2.5)	66 (42.0)	612	60 (9.8)	12 (2.0)	540 (88.2)	676	485 (71.7)	37 (5.5)	154 (22.8)
Ceftriaxone	269	86 (32.0)	1 (0.4)	182 (67.6)	302	116 (38.4)	0 (0.0)	186 (61.6)	-	-	-	-	-	-	-	-	NA	NA	NA	NA
Ertapenem	278	268 (96.4)	1 (0.4)	9 (3.2)	319	315 (98.7)	1 (0.3)	3 (0.9)	108	99 (91.7)	3 (2.8)	6 (5.6)	NA	NA	NA	NA	NA	NA	NA	NA
Imipenem	70	70 (100.0)	0(0.0)	0 (0.0)	143	141 (98.6)	0 (0.0)	2 (1.4)	60	56 (93.3)	1 (1.7)	3 (5.0)	575	81 (14.1)	6 (1.0)	488 (84.9)	621	416 (67.0)	3 (0.5)	202 (32.5)
Meropenem	347	341 (98.3)	1 (0.3)	5 (1.4)	481	476 (99.0)	0 (0.0)	5 (1.0)	172	167 (97.1)	0 (0.0)	5 (2.9)	630	89 (14.1)	3 (0.5)	538 (85.4)	678	464 (68.4)	46 (6.8)	168 (24.8)
Amikacin	279	265 (95.0)	4 (1.4)	10 (3.6)	323	308 (95.4)	0 (0.0)	15 (4.6)	107	104 (97.2)	1 (0.9)	2 (1.9)	-	-	-	-	616	543 (88.1)	7 (1.1)	66 (10.7)
Gentamicin	338	209 (61.8)	0 (0.0)	129 (38.2)	468	303 (64.7)	2 (0.4)	163 (34.8)	174	130 (74.7)	0 (0.0)	44 (25.3)	603	100 (16.6)	99 (16.4)	404 (67.0)	181	152 (84.0)	14 (7.7)	15 (8.3)
Tobramycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	113	86 (76.1)	15 (13.3)	12 (10.6)

(*continued on next page*)

Table II (*continued*)

N	*E. coli* No. (%)			*K. pneumoniae* No. (%)			*E. cloacae* complex No. (%)			*A. baumannii* complex No. (%)			*P. aeruginosa* No. (%)		
	348			**489**			**177**			**638**			**688**		
Ciprofloxacin	274	70 (25.5)	22 (8.0)	182 (66.4)	309	129 (41.7)	37 (12.0)	143 (46.3)	112	76 (67.9)	4 (3.6)	32 (28.6)	312	50 (16.0)	1 (0.3)	261 (83.7)	468	357 (76.3)	26 (5.6)	85 (18.2)
Levofloxacin	53	16 (30.2)	0 (0.0)	37 (69.8)	151	118 (78.1)	4 (2.6)	29 (19.2)	57	50 (87.7)	1 (1.8)	6 (10.5)	291	23 (7.9)	0 (0.0)	268 (92.1)	177	138 (78.0)	7 (4.0)	32 (18.0)
Doxycycline	-	-	-	-	-	-	-	-	-	-	-	-	360	240 (66.7)	3 (0.8)	117 (32.5)	NA	NA	NA	NA
Tigecycline	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	500	200 (40.0)	145 (29.0)	155 (31.0)	NA	NA	NA	NA
Trimethoprim/ sulfamethoxazole	317	126 (39.7)	0 (0.0)	191 (60.3)	417	194 (46.5)	3 (0.7)	159 (52.8)	154	98 (63.6)	0 (0.0)	56 (36.4)	-	-	-	-	NA	NA	NA	NA
Colistin	282	0 (0.0)	282 (100.0)	0 (0.0)	385	0 (0.0)	385 (100.0)	0 (0.0)	131	0 (0.0)	130 (99.2)	1 (0.8)	600	0 (0.0)	597 (99.5)	3 (0.5)	625	0 (0.0)	625 (100.0)	0 (0.0)

S: susceptible; I: intermediate; R: resistant; NA: not applicable; (-): not measured.

antimicrobial categories), and 21–45% in *E. cloacae* (7/13 antimicrobial categories). CR and DTR were <5% in Enterobacterales.

Regarding *A. baumannii* complex and *P. aeruginosa*, a MDR possible XDR category was also included [7] in which isolates presented an incomplete testing for the XDR classification but were non-susceptible for 6/9 and 7/8 antimicrobial categories, respectively.

A. baumannii (8/9 antimicrobial categories) were classified as 85.7% CR, 87.8% ESCR, 82.8% FQR, 54.2% MDR, 59.7% DTR, 18.2% MDR possible XDR (non-susceptible to six antimicrobial categories), and 14.4% XDR. DTR rates per year were 49.2% (2018), 56.0% (2019), 38.0% (2020), 64.7% (2021), 56.0% (2022), and 62.9% (2023).

P. aeruginosa (6/8 antimicrobial categories) were 33.3% CR, 25.0% ESCR, 16.9% FQR, 17.4% MDR, 8.9% DTR, 6.0% MDR-possible XDR (non-susceptible to five antimicrobial categories). DTR rates were 12.3% (2018), 5.2% (2019), 4.1% (2020), 11.3% (2021), 13.5% (2022), and 5.6% (2023).

In *S. aureus* (9/17 antimicrobial categories) 37.8% were MDR. In *Enterococcus* spp. (7/11 antimicrobial categories) 20.7% were MDR. MDR-possible XDR or XDR classification was not performed due to low number of antimicrobial agents tested. None of the isolates were able to be categorised as pan-drug-resistant (resistant to all antibiotic) because not all the required antimicrobial agents were tested [7].

After examining the resistance trends exclusively in the COVID-19 area –a separate building in the hospital with 85 beds–, CR, MDR, FQR, and ESCR rates were similar ($P>0.05$) compared to resistance values shown by all isolates. However, MDR-possible XDR rates were lower in *A. baumannii* and *P. aeruginosa* (0.0% and 2.7%, respectively) in the COVID-19 area, compared to resistance values shown by the remaining wards (21.1% and 6.7%, respectively, $P=0.030$). XDR rates in *A. baumannii* were also lower in the COVID-19 area compared to all wards (5.6% vs. 15.8%, $P<0.008$).

Discussion

The emergence of AMR in ESKAPE pathogens in HAI is a global threat to worldwide human health, as it causes treatment failure and the reduction of available treatment options for severe infections, thereby increasing mortality rates [4]. Our study included the assessment of AMR rates of ESKAPE pathogens in a tertiary-care hospital from Monterrey, Mexico in over six years. Our results indicate high drug resistance rates mainly in Gram-negative pathogens but also in Gram-positive microorganisms (87.5% of meticillin-resistance in *Staphylococcus* spp., 39.8% of MRSA and 13.9% of VRE), which are higher compared to other studies from 2014–2019 in an Emergency department in Hungary [16] and bloodstream isolates from paediatric patients in Spain between 2013 and 2021 [18].

Although the prevalence of MRSA decreased in our hospital compared to 62% in intensive care unit patients during 2011–2012 [2], meticillin resistance trends increased over the six-year period. Another study from several nosocomial centres in Mexico over a 10-year period also showed MRSA decreasing trends (44.5%–26.2%) [3]. Instead, VRE rates in our hospital were higher than other reports from Mexico (*E. faecium*, 72% vs. <40% and *E. faecalis*, 28% vs. <5%) [3].

Carbapenem resistance was higher in *A. baumannii* (85.7%), followed by *P. aeruginosa* (33.3%), and lastly Enterobacterales

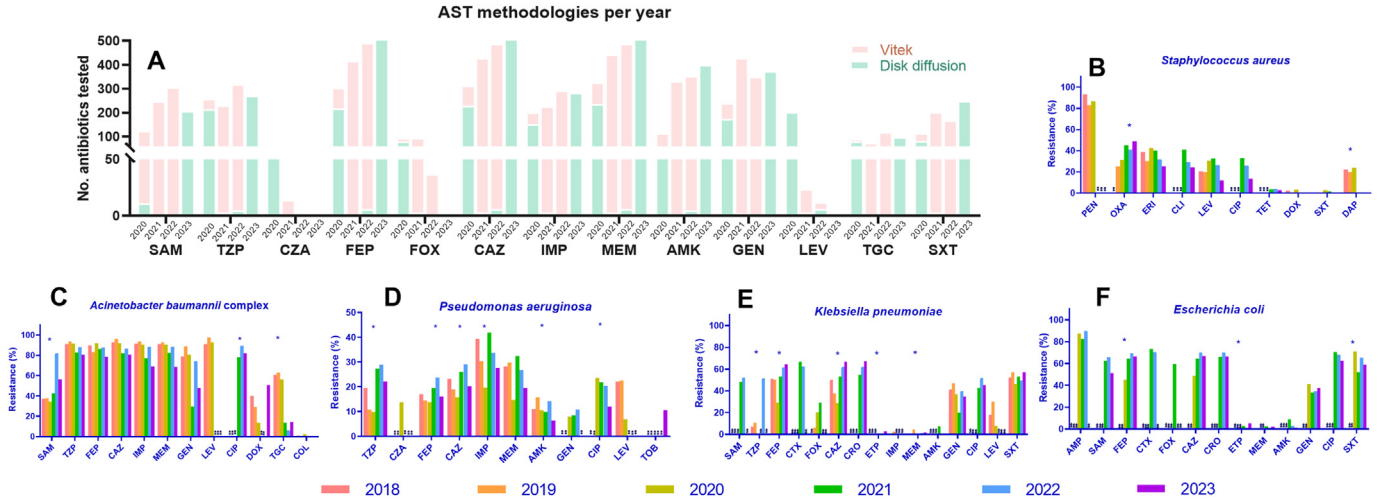

Figure 1. Resistance trends of Gram-positive and Gram-negative pathogens over 5 years. (**A**) AST assays were performed by two methodologies, Vitek® and disk diffusion, which are show per year. During 2018 and 2019, only disk diffusion was performed. For cefotaxime, ceftriaxone and ampicillin, only Vitek® was performed. For ciprofloxacin, doxycycline, and tobramycin, only disk diffusion was performed. Resistance rates are shown per year for *Staphylococcus aureus* (**B**), *Acinetobacter baumannii* complex (**C**), *Pseudomonas aeruginosa* (**D**), *Klebsiella pneumoniae* (**E**), and *Escherichia coli* (**F**). Isolates < 30 were not included in the analysis (⁂). Total susceptible values are not shown (e.g., LZD and VAN for *S. aureus* and COL for *P. aeruginosa*, *K. pneumoniae* and *E.* coli). AMK: amikacin; AMP: ampicillin; CAZ: ceftazidime; CIP: ciprofloxacin; CLI: clindamycin; COL: colistin; CRO: ceftriaxone; CTX: cefotaxime; CZA: ceftazidime-avibactam; DAP: daptomycin; DOX: doxycycline; ERY: erythromycin; ETP: ertapenem; FEP: cefepime; FOX: cefoxitin; GEN: gentamicin; IMP: imipenem; LEV: levofloxacin; MEM: meropenem; OXA: oxacillin; PEN: penicillin; SAM: ampicillin-sulbactam; SXT: trimethoprim/sulfamethoxazole; TET: tetracycline; TGC: tigecycline; TOB: tobramycin, and TZP: piperacillin-tazobactam. * $P \leq 0.05$.

Table III
Antimicrobial resistance classification for each microorganism according to several criteria. Three different criteria were used to classify drug resistance: Magiorakos *et al.*, CDC and IDSA

Microorganism	Magiorakos *et al.* [7] No. (%)				CDC No. (%)			IDSA No. (%)
	MDR	MDR possible XDR	XDR	Non-MDR	CR	ESCR	FQR	DTR
E. coli (*n*= 348)	227 (65.2)	-	-	121 (34.8)	8 (2.3)	226 (64.9)	219 (62.9)	6 (1.7)
K. pneumoniae (*n*= 489)	231 (47.2)	-	-	258 (52.8)	6 (1.2)	265 (54.2)	172 (35.2)	4 (0.8)
E. cloacae complex (*n*= 177)	47 (26.6)	-	-	130 (73.4)	9 (5.1)	80 (45.2)	38 (21.5)	4 (2.3)
A. baumannii complex (*n*= 638)	346 (54.2)	116 (18.2)	92 (14.4)	84 (13.2)	547 (85.7)	560 (87.8)	528 (82.8)	381 (59.7)
P. aeruginosa[a] (*n*= 688)	120 (17.4)	41 (6.0)	-	527 (76.6)	229 (33.3)	172 (25.0)	116 (16.9)	61 (8.9)
S. aureus (*n*= 540)	204 (37.8)	-	-	336 (62.2)	NA	NA	NA	NA
Enterococcus spp.[b] (*n*= 203)	42 (20.7)	-	-	161 (79.3)	NA	NA	NA	NA

MDR: multidrug resistance; XDR: extensively drug resistance; CR: carbapenem resistance; ESCR: extended-spectrum cephalosporin resistance; FQR: fluoroquinolone resistance; DTR: difficult-to-treat resistance; NA: not applicable; CDC: Centers for Disease Control and Prevention; IDSA: Infectious Diseases Society of America, (-): not measured.

[a] Eight isolates were unable to be classified according to IDSA criteria.

[b] Thirty-four isolates were unable to be classified.

(<5%). Since 2011–2012, CR *A. baumannii* increased (75.3% in intensive care unit patients vs. 85.7%) in our hospital [2], in which the most frequently gene detected was OXA-24/40-like (99.4%), higher than other studies (60.5–76%) [30,31]. In *A. baumannii*, tigecycline resistance decreased over the six-year period studied. This behaviour could be related to the AST methodology and breakpoints used specifically for this drug. In our study, high resistance percentage values (>60%) were observed after using disk diffusion as AST methodology, which has been previously reported to have a categorical agreement of 53% for Enterobacterales [32]. Disk diffusion methodology shows good correlation to broth microdilution -considered the gold standard-when the breakpoint of ≤ 12 mm for resistance was used [32]. However, in our study, we used the breakpoint of the BSCA (≤19 mm), which could have caused the report of false resistant isolates. Most recently, a categorical agreement of 83.9–85.1% and 14.8–16.1% of minor errors were reported after comparing VITEK to broth microdilution, which could cause the report of high MIC values, ultimately reporting false resistant isolates [33,34]. These previous reports highlight the importance of contrasting the results obtained routinely in our isolates by VITEK to broth microdilution. Incidentally, another AST methodology with fewer minor errors and more accessible to the

routine workflow of a clinical laboratory could be used, given the importance of this specific drug in *A. baumannii*.

The most frequently gene detected in CR *P. aeruginosa* was IMP, similar to previous reports [31], although different (23.2% of VIM) from another study [30]. Resistance trends were higher in *P. aeruginosa* to several antimicrobials, including β-lactams, fluoroquinolones, and carbapenems. In Enterobacterales, resistance to β-lactams increased over the years. In carbapenem-resistant Enterobacterales isolates, the most frequent gene was NDM, similar to previous reports [30,31,35]. KPC was not detected.

DTR infections have extended hospital stays and led to poor patient outcomes [10], including higher mortality rates (up to 50%) [15]. These infections are associated to previous antibiotic use, healthcare exposure, intensive care unit stay, and invasive mechanical ventilation use [15,35]. DTR in Gram-negative pathogens such as Enterobacterales, *P. aeruginosa* and *A. baumannii* is a cause of severe infections [10].

The prevalence of DTR in ESKAPE pathogens is different depending on the demographic region. Past reports of drug resistance rates of Gram-negative pathogens causing BSI showed high DTR rates in *A. baumannii* in China [13], India [14], United States [9], Korea [15], Hungary [16] and Italy [17]. In some regions, DTR was also high in *K. pneumoniae* in China [13], and India [14] and *P. aeruginosa* in Spain [18]. In most cases, DTR was lower in *P. aeruginosa* and Enterobacterales. In our study, DTR rates were higher in *A. baumannii* (59.7%), and lower in *P. aeruginosa* (8.9%) and Enterobacterales (<3%). DTR rates increased in *A. baumannii* from 2018 (49.2%) to 2023 (62.9%). According to our data, DTR seems to be higher in non-fermenting Gram-negative bacteria such as, *Acinetobacter* and *Pseudomonas*, compared with lactose fermenting Gram-negative, bacteria including, Enterobacterales, as previously noted [19].

Improved clinical outcomes have been reported after the administration of new antimicrobial agents rather than combinations of conventional antibiotics [36]. Treatment of severe infections due to DTR *A. baumannii* might require the replacement of polymyxin-based regimens by novel agents such as cefiderocol, eravacycline, and sulbactam-durlobactam, which may show activity against this pathogen [37]. Imipenem/relebactam could be used to treat DTR *P. aeruginosa* and DTR Enterobacterales, as susceptibility can be 62% and 82%, respectively [19,36]. Instead, ceftolozane/tazobactam could only be used to treat DTR *P. aeruginosa* and not DTR Enterobacterales, as susceptibility was 67.5% and 1.5%, respectively [19,36]. Furthermore, cefiderocol could be used to treat DTR in Enterobacterales, *P. aeruginosa* and DTR *A. baumannii* (>95% susceptibility) [38,39]. The optimisation of available novel antimicrobial agents is critical to resist AMR and improve patient outcomes [10].

Our hospital performs active surveillance of pathogens of epidemiological concern. However, our hospital does not yet report DTR resistance routinely. DTR surveillance in hospitals such as ours should be continuous updating local and regional DTR trends. The assessment of DTR can help in the surveillance of AMR among Gram-negative bacteria.

Among the limitations of this study, first is that the study relied on retrospective data collection, which can be subject to incomplete documentation, potentially affecting the reliability of the findings. Second, not all recommended antimicrobial agents were available to testing in our facility. Thus, we have yet to know the true scope of DTR rates in our hospital. In addition, two AST methodologies were used to determine AMR, which might affect final interpretation results. Most DTR reports focus on BSI and do not include specimens from other sites. Our study included specimens not only from BSI but also from other HAI such as LRTI. Thus, DTR comparison with previous reports might not be as reliable. Few studies reported risk factors for DTR, which include were infection source, such as, *A. baumannii*, intensive care unit stay, urban location, among others [9]. As we did not investigate specific patient data, we were unable to determine risk factors associated with the epidemiology of DTR ESKAPE pathogens in our hospital. In addition, whilst some differences were observed in the COVID-19 pandemic regarding drug resistance -lower frequency of resistant Gram-negative pathogens and higher frequency of resistant Gram-positive pathogens-, in our laboratory diagnostic services were almost exclusively directed towards SARS-CoV-2 during the pandemic, which might have delayed conventional microbiological investigations, although most patients had HAI. Lastly, the findings of the study might not be generalisable to other populations with different demographics, healthcare settings, or geographic regions. The sample characteristics of this study, along with the context in which the study was conducted should both be considered before applying the findings of this study to other populations.

Conclusions

The assessment of AMR rates of ESKAPE pathogens in a hospital from Mexico in over six years showed that MRSA prevalence seems to be decreasing in contrast to VRE. Both CR and DTR rates were high in *A. baumannii* and low in *P. aeruginosa* and Enterobacterales. DTR infections might require administration of new antimicrobial agents rather than combinations of conventional antibiotics. The assessment of DTR can help in the surveillance of AMR among Gram-negative bacteria.

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CRediT author statement

Adrián Camacho-Ortiz: Resources; Supervision; Validation; Visualisation; Writing - review & editing; **Samantha Flores-Treviño:** Conceptualisation; Investigation; Methodology; Software; Visualisation; Writing - original draft; Writing - review & editing; **Paola Bocanegra-Ibarias:** Conceptualisation; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualisation; Writing - original draft; Writing - review & editing.

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Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.infpip.2024.100426.

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