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Do bacteria isolated from ICU patients 'ESKAPE' antibiotic treatment? *In vitro* susceptibility of the *Enterobacteriaceae* family to tigecycline

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Abstract

Background: *Enterobacteriaceae* are currently causing the majority of healthcare-associated infections (HAI) and simultaneously expressing increasing levels of antibiotic resistance. The purpose of this study is to assess the *in vitro* sensitivity of MDR strains from the family *Enterobacteriaceae* to tigecycline in relation to their origin from patients hospitalized in intensive care units (ICUs) and non-ICUs.

Methods: The study involved 156 clinically significant strains of the *Enterobacteriaceae* family isolated from patients with complicated intraabdominal infections (cIAIs) and/or complicated skin and skin structure infections (cSSSIs) hospitalized in ICUs and other surgical departments. Tigecycline MICs were determined by Etest.

Results: The highest percentage of tigecycline non-susceptible (intermediate + resistant strains) *in vitro* strains among the *Enterobacteriaceae* species were observed for *Serratia* spp. 77.3%, followed by *Citrobacter* spp. (76.9%) and *Enterobacter* spp. (70%); whereas *K. pneumoniae* and *E. coli* showed 73–73.8% tigecycline susceptibility rates.

Conclusion: Tigecycline demonstrates a high level of antimicrobial *in vitro* activity when tested against *E. coli* and *K. pneumoniae*, even those with the ESBL-phenotype. Tigecycline retained activity against merely 22–30% of *Enterobacter*, *Citrobacter* and *Serratia* genera.

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Key words: intensive care unit; *Enterobacteriaceae*, infections; ESBL; AmpC; MBL; *in vitro* activity

The acronym ESKAPE was proposed to highlight the fact that some bacterial species (***E****nterococcus faecium,* ***S****taphylococcus aureus, Klebsiella pneumoniae,* ***A****cinetobacter baumannii,* ***P****seudomonas aeruginosa* and *Enterobacter species)* effectively "escape" the effects of antibacterial drugs [1]. All ESKAPE pathogens are currently causing the majority of healthcare-associated infections (HAI) while simultaneously expressing increasing levels of antibiotic resistance [2]. Therefore, nowadays we are witnessing a remarkable change, which consists of replacing susceptible microbiota with hospital strains in the majority of those considered multidrug resistant (MDR) [1, 3]. This seems to be not only a serious epidemiological and therapeutic dilemma nowadays but also poses a real threat of having no antimicrobial treatment for "ESKAPE" extensively resistant pathogens (XDR) in the nearest future [1, 4, 5].

Tigecycline is an antimicrobial drug belonging to glycylcyclines, registered by the European Medicines Agency (EMA) for the treatment of adults with complicated intra-abdominal infections (cIAIs) and complicated skin and skin structure infections (cSSSIs), except for diabetic foot infections [6]. According to the European Conference on Infections in Leukemia (ECIL), tigecycline could be used as a salvage therapy in leukemic and hematopoietic stem cell transplant recipients [7].

The purpose of this study is to assess the *in vitro* sensitivity of MDR strains of the *Enterobacteriaceae* family to tigecycline in relation to their origin from patients hospitalized in intensive care units (ICUs) and non-ICUs. Our study may contribute to the evaluation of the changing trends in *Enterobacteriaceae* drug resistance to antibiotics relevant in the treatment of cIAIs and cSSSIs.

METHODS

The study was approved by the Jagiellonian University Medical College Bioethical Committee (No. KBET/19/ /B /2013).

BACTERIAL ISOLATES

The study involved 156 clinically significant non-duplicate strains of the *Enterobacteriaceae* family isolated from patients with cIAIs and/or cSSSIs hospitalized in intensive care units (ICUs) and other surgical (non-ICU) departments in specialist hospitals in the area of Cracow during the period 2009–2013. The clinical materials were as follows: surgical wound exudates — 119 samples; peritoneal fluid — 25 samples; blood — 6 samples; and surgical biopsy — 6 samples.

SPECIES IDENTIFICATION

Species identification was carried out with API 20 E strips (bioMérieux) according to manufacturer's guidelines.

SUSCEPTIBILITY TO TIGECYCLINE TESTING

Susceptibility to tigecycline was determined by Etest (bioMérieux) according to the manufacturer's procedure on freshly prepared Mueller Hinton II Agar (Becton Dickinson). Plates were inoculated with 0.5 McF bacterial suspension. Culture plates were incubated in ambient air at 35 ± 1^0C for 18–20 h. *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 strains were used as quality control. Results were expressed as an MIC range, as well as MIC_{50} and MIC_{90} values in mg L^{-1} units.

EVALUATION OF RESISTANCE PATTERNS

The presence of ESBL and AmpC phenotypes in the examined isolates was confirmed by a double-disk susceptibility test (DDST) with ceftazidime (Oxoid) and cefotaxime (Oxoid) as indicators and amoxicillin (Oxoid), and clavulanic acid (Oxoid) as inhibitors of ESBL. The MBL mechanism of resistance was detected by DDST with an EDTA disk, a disk containing a metallo-β-lactamase inhibitor and disks of ceftazidime (Oxoid) and imipenem (Oxoid) in accordance with the recommendations of the Polish National Reference Centre for Antimicrobial Susceptibility Testing (KORLD), based on EUCAST guidelines [8].

STATISTICAL ANALYSIS

A statistical analysis was performed using R Language and Environment for Statistical Computing software [9]. Comparisons were made using Pearson's Chi-squared test with Yates' continuity correction, a *post hoc* test after Kruskal--Wallis, with Pairwise comparisons conducted using Wilcoxon's rank sum test. The significance level for all statistical tests was set at $P \leq 0.05$.

RESULTS

Out of the 156 strains tested, 139 (89.10%) had resistance phenotypes while 21 (13.46%) were considered generally susceptible to the antibiotic being tested. The main resistance phenotype was ESBL produced by 99 (63.46%) strains, among which 44 (44.4%) were *Klebsiella* spp. strains, 18 (18.2%) — *Serratia* spp., 17 (17.7%) — *E. coli*, 11 (11.1%) — *Enterobacter* spp., 6 (6.1%) — *Citrobacter* spp. ESBL-positive strains with nearly the same frequency came from 49 (49.5%) ICU and 50 (50.5%) non-ICU patients.

We found that 81 (50.6%) strains were inhibited by tigecycline at ≤ 1 mg L^{-1} (more detailed data are shown in Table 1 and Fig. 1).

Among the *Enterobacteriaceae* species and subsets tested, MIC_{50} values varied from 1 mg L^{-1} for all *Klebsiella* spp., *E. coli* without any resistant phenotype and *E. coli* ESBL-phenotype, *Enterobacter* spp. with AmpC phenotype and *Serratia* spp. without any resistant phenotype to 2 mg L^{-1} for *Enterobacter* spp. ESBL+MBL phenotype. However, MIC_{90} values were 3 mg L^{-1} for all tested species.

The highest percentage of tigecycline non-susceptible (intermediate + resistant strains) in vitro strains among the *Enterobacteriaceae* species was observed for *Serratia* spp. (77.3%), followed by *Citrobacter* spp. (76.9%) and *Enterobacter* spp. (70%); whereas *K. pneumoniae* and *E. coli* showed 73–73.8% tigecycline susceptibility rates at EUCAST breakpoints (Table 1, Fig. 1).

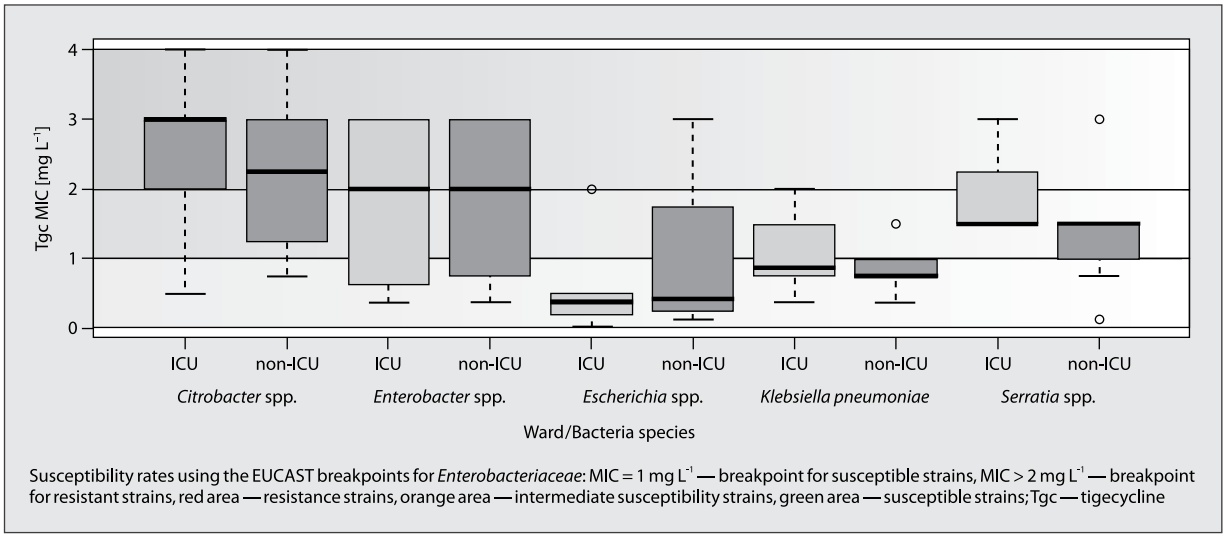
Moreover, the highest percentage of tigecycline non-susceptible *in vitro* strains among those considered resistance-phenotype strains was observed at 93.7% (62, 14, 11.4, and 6.3% for ESBL phenotype, ESBL+MBL phenotype, AmpC phenotype and ESBL+AmpC phenotype, respectively), whereas only non-resistant phenotype strains showed 6.3% tigecycline susceptibility rates. A comparison of the incidence of strains with the ESBL+ phenotype among strains sensitive and resistant to tigecycline demonstrated a statistically significant difference *Citrobacter* > *Enterobacter* > *Serratia* > **Klebsiella* > **E. coli* ($P = 0.02498$).

On the basis of the MIC values obtained for individual *Enterobacteriaceae* species, we have found that MIC median values vary between different species ($P = 1.702e\text{-}08$) (Table 2).

Table 1. Comparison of *in vitro* activity of tigecycline against species belonging to the *Enterobacteriaceae* family

Organism		n	MIC (mg L^{-1})			S %
			MIC range	MIC50	MIC90	
all *K. pneumoniae*		44	0.38–2.0	1.0	3.0	73.8
	ESBL phenotype	44	0.38–2.0	1.0	3.0	73.8
all *E. coli*		37	0.032–3.0	1.0	3.0	73
	without lactamases	16	0.125–3.0	1.0	3.0	75
	ESBL phenotype	17	0.032–3.0	1.0	3.0	64.7
	AmpC phenotype	4	0.19–3.0	1.5	3.0	75
all *Enterobacter* spp.		40	0.38–3.0	1.5	3.0	30
	ESBL phenotype	11	0.38–3.0	1.5	3.0	9.1
	ESBL + AmpC phenotype	8	0.38–3.0	1.5	3.0	37.5
	ESBL + MBL phenotype	10	2.0–3.0	2.0	3.0	0
	AmpC phenotype	11	0.38–3.0	1.0	3.0	72.73
all *Serratia* spp.		22	0.125–3.0	1.5	3.0	22.7
	without lactamases	3	0.75–1.5	1.0	3.0	66.67
	ESBL phenotype	18	0.75–3.0	1.5	3.0	16.67
	AmpC phenotype	1	3.0	–	–	0
all *Citrobacter* spp.		13	0.5–4.0	1.5	3.0	23.1
	without lactamases	1	0.75	–	–	100
	ESBL phenotype	6	0.5–3.0	1.5	3.0	16.67
	ESBL + MBL phenotype	1	3.0	–	–	0
	AmpC phenotype	5	1.0–4.0	1.5	3.0	20

MIC — minimum inhibitory concentration; MIC50/90 — MICs at which 50% and 90% of the isolates were inhibited, respectively; MIC values are given in mg L^{-1}; %S/ %R — susceptible and resistant strains respectively, according to EUCAST breakpoints



Susceptibility rates using the EUCAST breakpoints for *Enterobacteriaceae*: MIC = 1 mg L^{-1} — breakpoint for susceptible strains, MIC > 2 mg L^{-1} — breakpoint for resistant strains, red area — resistance strains, orange area — intermediate susceptibility strains, green area — susceptible strains; Tgc — tigecycline

Figure 1. Comparison of MIC distribution of tigecycline against the most numerous species of the *Enterobacteriaceae* family from ICU and non-ICU patients

DISCUSSION

It has been proposed to change the acronym ESKAPE to ESCAPE (*Enterococcus faecium, Staphylococcus aureus, Clostridium difficile, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacteriaceae*) to highlight the fact that, among others, pathogens belonging to the *Enterobacteriaceae* family can express increasing levels of antibiotic resistance. This is becoming an important clinical problem

Table 2. *Post hoc* test after Kruskal-Wallis with Pairwise comparisons using Wilcoxon rank sum test

	***Citrobacter* spp.**	***Enterobacter* spp.**	***E. coli***	***K. pneumoniae***
Enterobacter spp.	0.4472	–	–	–
E. coli	0.0011*	1.1e-05*	–	–
K. pneumoniae	0.0021*	0.0026*	0.0126*	–
Serratia spp.	0.3471	0.4472	0.0060*	0.0011*

* statistically significant values

associated with, on the one hand, reduced therapeutic possibilities, and on the other, an increase in morbidity, mortality, healthcare costs including long-term hospitalizations, particularly in ICUs [4, 10, 11]. In our discussion, due to the broad scope of the topic, we refer to data concerning the epidemiological situation in Poland.

The ICU is an environment in which there are interactions between the patient vs. the unit vs. bacterial pathogens. Patients admitted for treatment in the ICU are in a serious condition, immunosuppressed, usually with several underlying conditions, previously treated in other departments, and they undergo emergency intra-abdominal surgery. The patient's condition influences the length of the ICU stay and the type and number of procedures to which the patient is subjected (intubation, mechanical ventilation, vascular access, parenteral nutrition and other invasive procedures) [10, 12, 13]. An important link in the interaction between the patient and the ICU environment is the bacterial flora present in the unit, which has a high resistance to antibiotics (MDR strains) and its ability to quickly colonize the patient, environment, and staff [11, 13, 14]. After Rutkowska *et al.* [11] observed that after aproximately a week from the start of hospitalization in ICU, 96% of patients demonstrated a change in their microbiota, which demonstrated a change in their microbiota, which was replaced by pathogens characteristic of a given hospital department. Gram-negative bacilli are predominant in Polish ICUs [11, 15], which was also confirmed by our studies [unpublished results]. In our research, the genus *Enterobacter* was most frequently isolated during the ICU stay, followed by *K. pneumoniae* and *E. coli*, thus supporting other reports [11, 14, 16]. Our results confirm that the majority of *Enterobacteriaceae* strains including those from beta-lactamases such as ESBL phenotype situation in Polish hospitals is in line with the global trend of most reported infections being MDR-HAI [10]. In Poland, among patients admitted to ICUs, HAI infections make up 25% of all infections (45–60%) diagnosed in the course of patients' hospital stay in these units [11]. CSSSIs in Poland are decreasing (6.3% in 2012, 6.9% in 2013, 4.5% in 2014, 1.8% in 2015, of all infections) [17]. ICU treatment requires up to three times more frequent application of antibiotics than in other departments (136 DDD vs. 43 DDD per 100 person days) [14]. Often, antibiotic therapy necessitates a wide range of antibiotics, which is aimed at covering the spectrum of MDR pathogens. Due to the limited options for treating infections with MDR strains, the possibility of applying tigecycline is crucial. Tigecycline seems to be used in infections caused by many MDR strains, for example ESBL-positive phenotype strains [3, 6, 14]. In our study, tigecycline demonstrated the highest *in vitro* sensitivity to *K. pneumoniae,* even to ESBL+ and *E. coli* strains, a phenomenon which is confirmed by other authors [16, 18–20]. For *E. cloacae*, other authors have shown the high *in vitro* activity of tigecycline [19], which was an opposite result to the one in our study.

In TEST (Tigecycline Evaluation and Surveillance Trial) study for Europe, which was carried out in the period 2004–2014, 10 medical centres out of 226 were from Poland. For *K. pneumoniae*, *E. coli*, *Enterobacter* spp. and *S. marcescens*, the MIC_{90} values obtained were lower than in our investigation [21]. When interpreting the above results, it should be noted that the level of ESBL+ strains and sensitivity to tigecycline varied significantly between countries and microbes. TEST results demonstrated differences as regards bacterial drug resistance dividing Europe into areas of high and low drug susceptibility. Poland was listed among the countries with increased resistance to several classes of antibiotics (amoxicillin, cefepime, ceftriaxone), including thearpeutic treatments used to treat infections caused by ESBL+ strains (piperacillin-tazobactam, amikacin, levofloxacin) [21].

The similar MIC values obtained in our study for ICU and non-ICU strains may be caused by the fact that patients are admitted to the ICU from other hospital departments, among others, following exacerbation of the disease or post-operative complications. This means that they were in a hospital environment beforehand for varying durations, and were treated numerous times using various antimicrobial drugs, which favoured the selection of MDR strains.

Seeing that patterns of resistance change over time and between countries, we are convinced that local data, such as our hospital-based study, are necessary to guide clinicians in selecting appropriate antimicrobial therapy and in the choice of antibiotics for hospital formularies.

CONCLUSIONS

1. Tigecycline demonstrates a high level of antimicrobial *in vitro* activity when tested against *E. coli* and *K. pneu-*

moniae, even those with the ESBL phenotype. However, we found that MIC_{90} was evaluated higher than in other trials coming from Poland.

2. Tigecycline retained activity against merely 22–30% of *Enterobacter*, *Citrobacter* and *Serratia* which accounted for a large group of pathogens associated with cSSSI and cIAI occurring in ICU and non-ICU patients in the Małopolska region.

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