Published in "European Journal of Clinical Microbiology & Infectious Diseases
doi: 10.1007/s10096-017-3070-0, 2017" which should be cited to refer to this work.

Antimicrobial activity of octenidine against multidrug-resistant Gram-negative pathogens

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Abstract Multidrug-resistant (MR) Gram-negative (GN) pathogens pose a major and growing threat for healthcare systems, as therapy of infections is often limited due to the lack of available systemic antibiotics. Well-tolerated antiseptics, such as octenidine dihydrochloride (OCT), may be a very useful tool in infection control to reduce the dissemination of MRGN. This study aimed to investigate the bactericidal activity of OCT against international epidemic clones of MRGN. A set of five different species (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*) was studied to prove OCT efficacy without organic load, under "clean conditions" (0.3 g/L albumin) and under "dirty conditions" (3 g/L albumin + 3 mL/L defibrinated sheep blood), according to an official test norm (EN13727). We used five clonally unrelated isolates per species, including a susceptible wild-type strain, and four MRGN isolates, corresponding to either the 3MRGN or 4MRGN definition of multidrug resistance. A contact time of 1 min was fully effective for all isolates by using different OCT concentrations (0.01% and 0.05%), with a bacterial reduction factor of >5 $\log_{10}$ systematically observed. Growth kinetics were determined with two different wild-type strains (*A. baumannii* and *K. pneumoniae*), proving a time-dependent efficacy of OCT. These results highlight that OCT may be extremely useful to eradicate emerging highly resistant Gram-negative pathogens associated with nosocomial infections.

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Introduction

Multidrug-resistant (MR) Gram-negative (GN) bacteria pose a major and growing threat for healthcare systems, given the paucity of available and efficient antibiotics drastically complicating the treatment of infections [1, 2]. MR Enterobacteriaceae, *Pseudomonas*, and *Acinetobacter* strains have emerged as particularly serious concerns [3, 4].

Antiseptic molecules may help to reduce the dissemination of MR bacteria, especially in high-risk areas, such as intensive care units, when used in patient decolonization procedures [5, 6]. However, reduced susceptibility of some MRGN isolates to some frequently used biocides (i.e., chlorhexidine) has been reported [7–9]. A significant bactericidal activity of octenidine dihydrochloride (OCT) was demonstrated against mupirocin-resistant methicillin-resistant *Staphylococcus aureus* (MRSA) strains [10]. However, information about its efficacy against MRGN remains poorly investigated [11, 12]. In the present study, we aimed to assess the in vitro bactericidal activity of OCT against the most relevant GN species responsible for hospital-acquired infections, including isolates exhibiting MR phenotypes.

Table 1 Bacterial isolates tested, their resistance mechanisms, and susceptibility profile

Species	Resistance mechanism*	Susceptibility to antibiotics																		Chlorhexidine MIC (mg/L)
		AMX	PPT	AMC	CEF	CTX	FOX	CAZ	FEP	ETP	IPM	MEM	ATM	TGC	SXT	CIP	GMI	TMN	AKN	
E. coli	WT	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0.5
E. coli	CTX-M-1	R	S	S	R	R	S	S	R	S	S	S	S	S	R	S	S	S	S	2.5
E. coli	CTX-M-15	R	I	R	R	R	S	R	R	S	S	S	R	S	R	S	S	R	S	1.2
E. coli	NDM-1	R	R	R	R	R	R	R	R	R	I	R	R	S	R	R	S	S	S	5
E. coli	VIM-15	R	R	R	R	R	R	R	R	R	R	I	I	S	R	S	R	R	I	2.5
K. pneumoniae	WT	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	160
K. pneumoniae	CTX-M-15	R	S	S	R	R	S	R	R	S	S	S	R	S	S	S	R	R	S	40
K. pneumoniae	GES-1	R	R	R	R	S	S	S	S	S	S	S	S	S	R	S	S	S	S	80
K. pneumoniae	KPC-2	R	R	R	R	R	R	R	R	R	I	I	R	S	R	R	S	R	R	40
K. pneumoniae	OXA-48	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	I	80
E. cloacae	WT	R	S	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	160
E. cloacae	CTX-M-15	R	S	R	R	R	R	R	R	S	S	S	R	S	R	R	R	R	S	40
E. cloacae	VEB-1	R	I	R	R	R	R	R	S	R	S	S	R	S	S	S	S	R	S	80
E. cloacae	KPC-2	R	R	R	R	R	R	R	R	R	I	I	R	S	R	R	S	R	S	160
E. cloacae	NDM-1	R	R	R	R	R	R	R	R	R	I	R	S	S	R	R	R	R	R	80
A. baumannii	WT	R	S	R	R	R	R	S	S	R	S	S	R	S	S	S	S	S	S	80
A. baumannii	PER-1	R	R	R	R	R	R	R	R	R	S	S	R	S	R	R	R	S	R	80
A. baumannii	VEB-1	R	R	R	R	R	R	R	R	R	S	S	R	S	R	R	R	R	R	80
A. baumannii	NDM-1	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	80
A. baumannii	OXA-23	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	S	S	S	80
P. aeruginosa	WT	R	R	R	R	R	R	S	S	R	S	S	I	R	R	S	S	S	S	10
P. aeruginosa	PER-1	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R	R	R	40
P. aeruginosa	VEB-1	R	R	R	R	R	R	R	R	R	S	I	R	R	R	R	R	R	R	40
P. aeruginosa	OprD	R	R	R	R	R	R	S	S	R	R	I	I	R	R	R	R	R	S	40
P. aeruginosa	VIM-2	R	R	R	R	R	R	S	S	R	R	R	I	R	R	R	S	S	I	40

*WT, wild-type; CTX-M-1, CTX-M-15, GES-1, VEB-1, and PER-1 are extended-spectrum β-lactamases; NDM-1, KPC-2, OXA-48, OXA-23, and VIM-2 are carbapenemases; OprD, deficient porin of *P. aeruginosa*

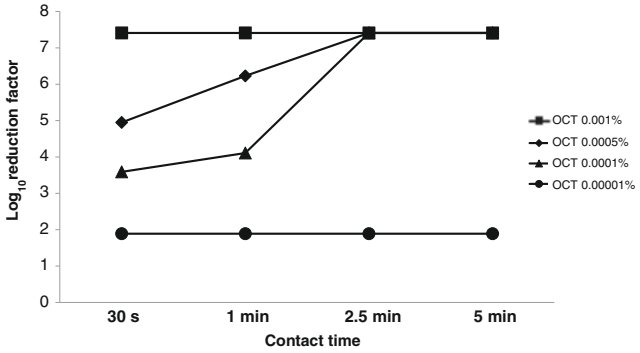

Fig. 1 Time-dependent efficacy of different concentrations of octenidine against *Acinetobacter baumannii* wild type. These data are representative of the efficacy observed for the other strains tested

Methods

We selected bacterial isolates from five different clinically relevant GN species (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*) from the Culture Collection of the Emerging Antibiotic Resistance Unit (University of Fribourg, Switzerland). Five clonally unrelated isolates were chosen for each species, including, in each case, a single susceptible wild-type strain, and four MR isolates previously characterized for their sequence type and resistance mechanisms, including the production of extended-spectrum β-lactamases (ESBLs) and carbapenemases (Table 1). Susceptibility to antibiotics was determined by disk diffusion, according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints [13]. All the clinical isolates corresponded to either 3MRGN (resistance to three out of the following antibiotic substances: penicillins, cephalosporins, quinolones, and carbapenems) or 4MRGN (resistance to all four pre-cited classes), according to the Robert Koch Institute definition of multidrug resistance [14].

Evaluation of the minimum inhibitory concentrations (MICs) of chlorhexidine digluconate (CHG; Sigma-Aldrich, St. Louis, MO, USA) were carried out, following the standards of the Clinical and Laboratory Standards Institute (CLSI) [15]. The range of concentrations tested was 0.3–312.8 mg/L, as used previously [10].

The efficacy of OCT was determined in accordance with BS EN 13727:2012+A1 [16], (a) without organic load, (b) under "clean conditions" (0.3 g/L BSA, Sigma-Aldrich, St. Louis, MO, USA), and (c) under "dirty conditions" (3 g/L BSA + 3 mL/L defibrinated sheep blood, Oxoid, Pratteln, Switzerland). Samples were incubated for different contact times (30 s, 1, 2.5, or 5 min) with OCT (Schülke & Mayr GmbH, Germany) diluted to the final test concentrations ranging from 0.00001% to 0.01%. After the given contact times, the activity of OCT was neutralized using a combination of 0.1% tryptone, 0.85% NaCl, 3% Tween 80, 0.3% lecithin, 3% saponin, and 0.1% histidine, without interfering with bacterial growth. Subsequently, serial dilutions of the final mixture were spread onto neutralizing agar plates and incubated for 24 h and 48 h at 37 °C. Colonies were counted and the reduction factor (RF) was determined as the difference between the $\log_{10}$ number of cells in the test solution at the beginning of the contact time and the $\log_{10}$ number of recovered colonies in the test solution. A 5 $\log_{10}$ reduction within ≤5 min was considered effective according to the test norm EN13727.

Results and discussion

Although all *E. coli* isolates had low MICs (ranging from 1 to 4 mg/L), all other isolates had MICs of CHG ranging from 32 to 128 mg/L, thus showing poor efficacy of the molecule against most MRGN isolates (Table 1). Noteworthy, to date, there is no breakpoint consensus to define biocide-reduced susceptibility, including for CHG. Based on the epidemiological cutoff (ECOFF) proposed by Morrissey et al. [17], we might consider most of our isolates as non-susceptible to CHG.

A reduction of >5 $\log_{10}$ was obtained for all wild-type strains after 30-s contact with OCT at a concentration of 0.01% (=100 ppm), 0.001% (=10 ppm), and 0.0005% (=5 ppm). OCT at a concentration of 0.0001% (=1 ppm) showed a time-dependent activity, achieving a reduction of >5 $\log_{10}$ after a contact time of 2.5 min. Finally, a

Table 2 Log_{10} reduction factor obtained with octenidine 0.01% at a contact time of 1 min for all test isolates, with or without organic load

Test isolates	Log_{10} reduction factor		
	Without organic load	With albumin 0.3 g/L, "clean conditions"	With albumin 3 g/L + erythrocytes 3 mL/L, "dirty conditions"
E. coli (all strains)	>5	>5	>5
K. pneumoniae (all strains)	>5	>5	>5
E. cloacae (all strains)	>5	>5	>5
A. baumannii (all strains)	>5	>5	>5
P. aeruginosa (all strains)	>5	>5	>5

concentration of 0.00001% (=0.01 ppm) was not effective for any given time lapse (Fig. 1).

All strains showed sensitivity against 0.01% OCT at a contact time of only 1 min, even in the presence of 0.3 g/L BSA ("clean conditions") or of 3 g/L BSA + 3 mL/L erythrocytes ("dirty conditions"), resulting in a reduction factor of >5 $\log_{10}$ (Table 2).

Conclusions

The present study showed that octenidine dihydrochloride (OCT) is highly effective against multidrug-resistant (MR) Gram-negative (GN) pathogens within a very short period of time, under either non-organic or organic conditions, independent of increased minimum inhibitory concentrations (MICs) towards chlorhexidine digluconate (CHG) or of the overall susceptibility to antibiotics. Among the tested isolates, some produced the most threatening resistance mechanisms that may be encountered worldwide in MRGN pathogens, namely carbapenemases.

The results obtained in this study encourage considering OCT, which is well tolerated and without resistances reported so far [18], as an alternative antiseptic for controlling the spread of MR bacteria, either being Gram-positive or Gram-negative. Further studies are required to better evaluate the impact of OCT in clinical practice for preventing infections caused by MRGN, such as that recently performed by Gastmeier et al. [19], who showed no significant impact on the prevention of MRGN acquisition in intensive care units.

Acknowledgments This study was partially supported by Schülke & Mayr GmbH and the University of Fribourg. Active compounds were provided by Schülke & Mayr GmbH, Norderstedt, Germany.

Compliance with ethical standards

Ethical approval Not applicable.

Conflict of interest L.P. received honoraria from Schülke & Mayr GmbH as a speaker for presenting data resulting from this study in international conferences.

Informed consent Not applicable.

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