Journal of Cranio-Maxillo-Facial Surgery 41 (2013) 321–326

Contents lists available at SciVerse ScienceDirect

Journal of Cranio-Maxillo-Facial Surgery

journal homepage: www.jcmfs.com

The ongoing battle against multi-resistant strains: *In-vitro* inhibition of hospital-acquired MRSA, VRE, *Pseudomonas*, ESBL *E. coli* and *Klebsiella* species in the presence of plant-derived antiseptic oils

Patrick H. Warnke [a,b,*], Alexander J.S. Lott [b], Eugene Sherry [c], Joerg Wiltfang [d], Rainer Podschun [e]

[a] *Dept. of Oral and Maxillofacial Surgery, The Royal Brisbane and Women's Hospital, Brisbane, QLD, Australia*
[b] *Clem Jones Research Centre for Stem Cells and Tissue Regenerative Therapies, Bond University, Gold Coast, QLD, Australia*
[c] *Orthopaedic Surgery Services, The Sydney Private Hospital, Sydney, NSW, Australia*
[d] *Dept. of Oral and Maxillofacial Surgery, Christian-Albrechts-University of Kiel, Kiel, Germany*
[e] *Institute for Infection Medicine, Christian-Albrechts-University of Kiel, Kiel, Germany*

ARTICLE INFO

Article history:
Paper received 22 March 2012
Accepted 26 October 2012

Keywords:
Essential oils
MRSA
VRE
ESBL
E. Coli
Klebsiella
Pseudomonas
Tea Tree oil
Eucalyptus oil
Lemongrass oil
Multi-resistant strains
Disinfection
Antimicrobial

ABSTRACT

The fight against hospital-acquired infections involving antibiotic-resistant microorganisms has become of critical concern to surgeons worldwide.

In addition to the development of new effective antibiotic chemotherapy, exploration of 'forgotten' topical antibacterial agents from the pre-antibiotic era has recently gained new attention. We report the promising efficacy of plant-derived antiseptic oils used in traditional aboriginal and south-east Asian treatments such as Lemongrass, Eucalyptus and Tea Tree Oil in the inhibition of clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), multi-resistant *Pseudomonas aeruginosa*, ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* in the in-vitro setting.

Large consistent zones of inhibition were observed for all three plant-derived oils tested in an agar diffusion test. The commonly used antibacterial agents chlorhexidine 0.1%, and ethanol (70%), and standard olive oil consistently demonstrated notably lower or no efficacy in regard to growth inhibition of strains. Notably, Lemongrass oil proved to be particularly active against gram-positive bacteria, while Tea Tree oil showed superior inhibition of gram-negative microorganisms.

As proven *in vitro*, plant-derived antiseptic oils may represent a promising and affordable topical agent to support surgical treatment against multi-resistant and hospital-acquired infections.

1. Introduction

The fight against hospital-acquired infections involving antibiotic-resistant microorganisms has become of critical concern to surgeons and clinicians worldwide. In maxillofacial surgery particularly units with critical care patients are increasingly affected by this fact. Worse, the World Health Organization has stated that international medical community is gradually losing this battle, and the world may even be on the brink of a 'post-antibiotic era'.

Since its emergence in 1988, the incidence of nosocomial infection with vancomycin-resistant *Enterococcus* (VRE) has increased remarkably throughout the developed world and has affected surgical treatment outcomes. *Enterococcus*, once considered a pathogen of minimal clinical importance on a surgical ward, has thus now emerged as a rapidly growing cause of morbidity in the immunosuppressed patient, causing urinary tract infection, endocarditis, bacteraemia and sepsis (Courvalin, 2006; Willems and Bonten, 2007). Vancomycin resistance is growing, and VRE is becoming endemic in an increasing number of intensive care facilities worldwide (McGeer and Low, 2000). This rapid spread, combined with the limited therapeutic options available to the clinician in treating VRE, is fast becoming a cause of major concern.

In response, a number of pharmacological approaches have been attempted, with mixed success. Many surgeons and physicians have encouraged the withholding of overpowering broad-spectrum

* Corresponding author. Clem Jones Research Centre for Stem Cells and Tissue Regenerative Therapies, Bond University, 4229 Gold Coast, QLD, Australia. Tel.: +61 7 5595 1220; fax: +61 7 5595 4538.
E-mail addresses: pwarnke@hotmail.com, pwarnke@bond.edu.au (P.H. Warnke).

1010-5182/$ – see front matter © 2012 European Association for Cranio-Maxillo-Facial Surgery. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jcms.2012.10.012

antibiotics as a first-line treatment as long as possible in order to delay the development of multi-resistance (Warnke et al., 2008). The novel antibiotic 'linezolid', introduced in 2000, was hoped to act as a solid bulwark against the steady spread of VRE. However, despite its early promise, resistant clinical isolates were discovered as early as 2002 and the incidence of infection due to resistant strains has been increasing across western Europe (Auckland et al., 2002).

Equally notable is methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA drastically increases patient morbidity, and often requires multiple admissions, thereby exposing other vulnerable patients (MacKinnon and Allen, 2000). In many surgical units and hospital wards, this has created a requirement for isolation and sterilization of facilities and instruments used for these patients during their hospital stay. Some hospitals may refuse admission to patients carrying multi-resistant pathogens thereby compromising the affected patient's ability to access appropriate health care. Furthermore, infection with MRSA has been shown to significantly lower both the quality of life and self-esteem of patients (Theaker et al., 2001).

Among the gram-negative pathogens, strains of multi-resistant *Pseudomonas aeruginosa* and extended-spectrum-beta-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* has become a worldwide serious problem in the hospital setting, limiting the therapeutic options and surgical treatment dramatically (Paterson, 2001). Multi-resistant invasive oral infection is rare, but if it occurs is has been shown to be of particular consequence, requiring prompt diagnosis, extensive and often aggressive surgical debridement and long-term antibiotic therapy (Ghanem et al., 2011; Lorenzini et al., 2011).

Carriage of highly resistant bacterial strains thus has clear financial, epidemiological and even psychological implications. In our maxillofacial surgery units we found that complex elective surgery for patients in urgent need of treatment, who were carriers of multi-resistant strains, had to be postponed until the contamination was cleared. The multifaceted consequences for the patients, their relatives or employers together with the effects on clinical efficiency and patient waiting lists are often enormous.

There is mounting international evidence supporting the use of plant-derived antiseptic oils against pathogenic microorganisms (Shapiro et al., 1994; Larrondo et al., 1995; Maudsley and Kerr, 1999; Warnke et al., 2004; Fisher and Phillips, 2009; Warnke et al., 2009). Further, both clinical and *in vitro* studies have demonstrated potent bactericidal properties of essential oils, including efficacy against antibiotic-resistant strains (Harkenthal et al., 1999; Peana et al., 1999; Sherry et al., 2001; Halcon and Milkus, 2004; Fisher and Phillips, 2009; Warnke et al., 2009).

We have previously reported on significant clinical utility of plant-derived essential oils. This included significant reduction in malodour caused by tumour ulceration and promotion of ulcer healing and slight re-epithelisation in maxillofacial and head and neck cancer patients (Warnke et al., 2004, 2005, 2006), as well as promotion of healing in MRSA osteomyelitis *via* injection of essential oils (Sherry et al., 2001). In 2009, we tested a large number of essential oils for antimicrobial properties *in vitro* and proved the significant efficacy of a small subset against clinical MRSA isolates from surgical wards and critical *Candida kruzei* strains (Warnke et al., 2009).

After those preliminary findings our aim was now to evaluate the antibacterial efficacy of three selected essential oils with proven antimicrobial properties (Warnke et al., 2009) on a wider range of clinical isolates of typically hospital-acquired pathogens frequently found on surgical wards: methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), multi-resistant *P. aeruginosa*, ESBL-producing *E. coli* and *K. pneumoniae*.

2. Material and methods

2.1. Test group – essential oils

The following oils were selected for analysis: Lemongrass oil, Eucalyptus oil, and Tea Tree oil. The oils were provided by Felton Grimwade & Bosisto's Pty Ltd, FGB, Melbourne, Australia. All oils were non-diluted and not chemically altered by any solvent or processing.

2.2. Control group – oils and antiseptics

The antibacterial effects of essential oils were compared with those of undiluted household olive oil as well as the commonly used clinical antiseptics ethanol (70%) and chlorhexidine (0.1%).

2.3. Bacterial strains

Clinical isolates of 5 different bacterial taxa were tested: methicillin-resistant *S. aureus* (MRSA) (n = 9), vancomycin-resistant *Enterococcus* sp. (VRE) (n = 10) multi-resistant *P. aeruginosa* (n = 15), ESBL-producing *E. coli* (n = 5) and ESBL-producing *K. pneumoniae* (n = 5). The strains tested are listed in Table 1.

Table 1
Bacterial strains tested. (*) Mucoid-derived strain

Microorganism	Strain
VRE	ATCC 51299
	VA 21691/06
	VA 21692/06
	VA 23031/06
	W 356
	W 354
	VA 19370/06
	VA 19763/06
	VA 20497/06
	VA 21258/06
MRSA	VA 11799/08
	VA 117697/08
	BK 6520/06
	ATCC 43300
	ATCC 33593
	VA 14965/08
	VA 14903/08
	VA 14633/08
	VA 14977/08
MR *P. aeruginosa*	CF 640
	CF 629
	CF 599
	CF 597
	CF 573
	CF 571
	CF 528
	CF 526
	CF 502*
	CF 567*
	CF 646*
	CF 644*
	CF 643*
	CF 602*
	CF 601*
ESBL *E. coli*	VA 11616/08
	VA 11546/06
	VA 11098/08
	VA 11700/08
	VA 12186/08
ESBL *Klebsiella pneumoniae*	ESBL 299
	ESBL 258
	ESBL 302
	ESBL 301
	ESBL 259