Alternative therapies for infectious diseases

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EDITORIAL

Antimicrobial resistance: a global threat
Increasing resistance means:

- Higher antibiotic doses are required
- More resistance
- More side effects
- More cost
A lack of antibiotics…

Declining Antibacterial Approvals (Past 25 Years)

1983-1987: 16
1988-1992: 14
1993-1997: 12
1998-2002: 10
2003-2007: 8
2008-2009: 6

Total # New Antibacterial Agents

Bad Bugs Need Drugs

10x '20
Ten new ANTIBIOTICS by 2020

Spellberg, CID 2004, Modified
Alternative approaches

- **Naturally-occurring antimicrobial agents**
  Phytomedicines (plant-based remedies in the form of teas, extracts and oils) are a multimillion dollar industry worldwide.
  - Medicinal plants
  - Essential oils
  - Garlic
  - Honey

- **Bacteriophage therapy**
  Bacterial viruses are making a comeback.

- **Probiotic therapy**
  Probiotic therapy uses a live microbial food supplement to beneficially affect the host.
Probiotic therapy

- Uses supposedly non-pathogens
- Both bacteria and yeasts have been used

- Vaginal candidiasis
- Bacterial vaginosis
- Urinary tract infections
- Diarrhoeal diseases
How do probiotics work?

- Antagonism through production of inhibitory substances
- Competitive inhibition for sites/nutrients
- Inhibition of toxins
- Immunomodulation of the host

**Lactobacillus GG**

- *Lactobacillus casei var rhamnosus*
- Extensively studied
- Reduces traveller’s diarrhoea
- Reduces rotavirus diarrhoea
- Reduces the severity of diarrhoea in childcare centres
- Has lots of non-specific effects
- Available commercially
Urogenital tract infections

Lactobacilli

- Depleted lactobacilli may lead to:
  - Increased UTI
  - Bacterial vaginosis
  - *Candida* vaginitis
  - Post-antibiotic infections

- Lactobacilli susceptible to nonoxynol-9
Urogenital tract infections

Lactobacilli

Possible mechanisms of control

- Production of antimicrobial substances (e.g. bacteriocins)
- Lactic acid production
- Hydrogen peroxide production
- Formation of a barrier population
Urogenital tract infections

Lactobacilli (*L. casei, L. crispatus*)

- Characteristics required as a probiotic
  - Ease of cultivation and non-pathogenicity
  - Adhesion
  - Population stability

- Preparations currently available
- Delivery systems need work
Clostridium difficile

- Clostridium difficile – an anaerobic Gram +ve bacillus
- C. difficile disease gained prominence because of renewed interest in anaerobic bacteria in the 1960s and 70s
- Specific anti-anaerobe drugs had been developed, e.g. clindamycin
- Clindamycin-associated diarrhoea became a real problem in some hospitals in the USA
- Outbreaks of pseudomembranous colitis
- Cause elucidated in 1978
Probiotic treatment of CDI

- Various probiotic treatment regimens
  - Oral *Saccharomyces*
  - Oral *Lactobacillus GG*
  - Rectal administration of faecal enemas
  - Non-toxigenic *C. difficile*
  - *Enterococcus SF68*
## Effect of LGG yoghurt on *Clostridium difficile*-associated diarrhoea

<table>
<thead>
<tr>
<th></th>
<th>Diarrhoea</th>
<th>No diarrhoea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yoghurt</td>
<td>1 (No <em>C. difficile</em>)</td>
<td>15</td>
</tr>
<tr>
<td>No yoghurt</td>
<td>7 (2 <em>C. difficile</em>)</td>
<td>10</td>
</tr>
</tbody>
</table>

Fisher’s exact test, P = 0.02
## Effect of LGG yoghurt on *Clostridium difficile*-associated diarrhoea

<table>
<thead>
<tr>
<th>Month</th>
<th>Specimens</th>
<th>No.positive</th>
<th>% positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>November</td>
<td>77</td>
<td>10</td>
<td>12.9</td>
</tr>
<tr>
<td>December</td>
<td>127</td>
<td>15</td>
<td>11.8*</td>
</tr>
<tr>
<td>January</td>
<td>133</td>
<td>7</td>
<td>5.2*</td>
</tr>
<tr>
<td>February</td>
<td>59</td>
<td>4</td>
<td>6.7</td>
</tr>
</tbody>
</table>

*P < 0.05
Saccharomyces boulardii

- A nonpathogenic yeast that:
  - Survives gastric acid
  - Multiplies to high numbers
  - But need to keep taking
  - Not inhibited by antibiotics
  - Does not affect normal flora
  - Prevents CDI in animals

- Is it effective in preventing human CDI?
Human studies with *S. boulardii*

- 11 of 13 patients with recurrent CDI cured (Surawicz *et al.* 1989)
- *S. boulardii* plus antibiotics resulted in a significantly reduced risk of recurrence of CDI versus antibiotics and placebo (McFarland *et al.* 1994)
- No help with preventing primary infections
- But may help prevent recurrences
Experiences with *S. boulardii*

- So far we’ve treated 50 patients with recurrent CDI
- All elderly
- Given vancomycin for 7 days plus lyophilised *S. boulardii* (500 mg bd) concurrently and then continuing for another 3 weeks
- 49 or the 50 patients cured
- 1 patient non-compliant!
Two recent systematic reviews:

- Pillai A, Nelson RL. Probiotics for treatment of *Clostridium difficile*-associated colitis in adults. (Cochrane Collaboration 2009)
Probiotics for CDI

“There is insufficient evidence to recommend probiotic therapy as an adjunct to antibiotic therapy for *C. difficile* colitis. There is no evidence to support the use of probiotics alone in the treatment of *C. difficile* colitis.”

The ultimate probiotic

- Faecal replacement (enema) therapy
- Lots of anecdotes, little hard data
- Safety issues, ethical issues
- Equipment required: blender, naso-gastric tube
- Low numbers reported – success rate >90%
Naturally-occurring antimicrobials

- garlic
- qinghaosu
- cranberries
- honey
- tea tree oil

- “Dysentery bush” (Grewia retusifolia)
- “Jelly leaf” (Sida rhombifolia)
- “Quinine tree” (Alstonia constricta)
- “Caustic bush” (Sarcostemma australe)
Medicinal plants

- Antimicrobial activity of plant extracts
  - many applications:
    - raw and processed food preservation
    - pharmaceuticals
    - alternative medicines

- Over 2700 plants active against *S. aureus* and MRSA (Mahady GB *Curr Pharm Design* 2005; 11: 2405-27)
  - eg berberine is a naturally occurring isoquinolone alkaloid present in a number of plants eg *Coptis chinensis* and *Berberis vulgaris*
  - *S. aureus* MIC of 25 µg/mL
Medicinal plants

- Extracts of *Hypericum perforatum*, commonly known as St John’s Wort, are also active against MRSA.
- Historically, St John’s Wort has been used to treat skin and wound infections.
- Active component appears to be hyperforin, a phloroglucin.
- More work is required on safety, particularly in relation to interactions with conventional medication.
Garlic (*Allium sativum*)

- First recorded use in 3000BC by the Sumerians – widely cultivated then
- Used by Egyptian pyramid builders
- The Romans extolled the virtues of garlic as did the Greeks including Hippocrates
- 1st evidence as antimicrobial from plague in France in 1721 – macerated garlic and wine
- Juice used by French and English in WW I to treat infected wounds

(Harris *et al.* *Appl Microbiol Biotech* 2001; 57: 282-6)
Antimicrobial properties attributed to allicin which is produced from alliin (alliinase)

Di-allyl tri- & tetra-sulphides very potent and μg amounts effective in vitro

Active against many Gram +ve (incl. MRSA) & -ve bacteria, and fungi including dermatophytes

Mode of action still being debated
Garlic anti-MRSA activity in vivo
(Tsao et al. J Antimicrob Chemother 2003; 52: 974-80)

- Previously shown anti-MRSA activity of serum from humans who had eaten garlic
- Infected BALB/cA mice with MRSA and treated with garlic extract, DAS & DADS p.o. (vanc)
- DAS & DADS at high conc. killed mice
- All 3 inhibited growth of MRSA in a dose dependant manner
- All 3 suppressed infection induced elevation of fibrinolectin and IL-6
- Significant antioxidant protection
Honey

- Long recorded history of use
- Antibacterial activity against a range of organisms: *Staph aureus* (incl. MRSA), *E.coli*, *Pseudomonas*, enterococci and *H.pylori*
- Activity attributed to high osmolarity, low pH, presence of $\text{H}_2\text{O}_2$ but there is something else (UMF)
- Renewed interest in wound care
Topical honey for diabetic foot ulcers


• 79 yr old man with type 2 diabetes mellitus
• 14 months of care (US$390,000)
• MRSA, VRE, Pseudomonas
Qinghaosu

The jersh leaves
Qinghaosu

- Extract of *Artemisia annua* first described in China for malaria in 1596
- Derivatives made chemically of parent compound artemisinin
- An oral form (artesunate) gives cure rates of around 90%
- When combined with mefloquine cure rates increase to 100% (Looareesuwan *et al.* *Lancet* 1992; 339: 821-824)
- “that parenteral artesunate should replace quinine as the treatment of choice for severe falciparum malaria worldwide” *Lancet* 2010; 376: 1647–1657.
Cranberries (Vaccinium spp.)
Cranberries

- American folk remedy for UTI
- In vitro studies show that CJ diminishes expression of fimbriae and binding of *E. coli* to cells *(Zafriri et al. AAC 1989; 33: 92-98)*
- A prospective, randomised, placebo-controlled trial showed a 50% reduction in incidence of bacteriuria *(Avorn et al. JAMA 1994 271: 751-754)*
Tea tree oil
Tea tree (*Melaleuca alternifolia*) oil

- pale yellow, viscous fluid
- approximately 100 components
- Mainly terpenes, sesquiterpenes and related alcohols
- compositional levels may vary
- partly regulated by the international standard for ‘tea tree’ oil (ISO 4730)
- 7 components - 80-90% of the whole oil
Components of tea tree oil

- terpinen-4-ol
- 1,8-cineole
- δ-terpineol
- α-terpinene
- δ-terpinene
- terpinolene
- ρ-cymene
- linalool
Can tea tree (*Melaleuca alternifolia*) oil prevent MRSA?

“The experimental evidence supporting the use of tea tree oil as a prophylactic for MRSA is compelling,…….”

### MIC/MBC (%) of TTO against skin organisms

<table>
<thead>
<tr>
<th>Organism (n)</th>
<th>MIC 90</th>
<th>MBC 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corynebacterium spp. (10)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Micococcus spp. (11)</td>
<td>0.5</td>
<td>6</td>
</tr>
<tr>
<td>CNS (60)</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td><em>E.coli</em> (113)</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td><em>K.pneumoniae</em> (14)</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td><em>S.marcescens</em> (11)</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td><em>S.aureus</em> (163)</td>
<td>0.5</td>
<td>2</td>
</tr>
</tbody>
</table>

Potential for resistance to develop

- Low - due to nature, degree and multiplicity of effects
- TTO multicomponent
- Likely to be several different mechanisms
- Gross effects, many non-specific affecting membrane
- Strong argument for use against multi-resistant organisms

Formulation issues

- Formulation of TTO into products requires careful consideration
- Many excipients/surfactants inactivate TTO
- Must test final product
- Things to avoid: SLS, sorbelene, plus many others
In vivo pilot study

- 30 patients – 15 in each group, random allocation
- 4% tto nasal ointment & 5% tto body wash for minimum of 3 d
- 2% mupirocin & Triclosan body wash
- Swabbed at 2 and 4 days post-treatment
- ITT analysis
In vivo pilot study

- control regime clearance 2/15 (13%)
- tto clearance 5/15 (33%)
- 95% CI 0.49 to -0.12, p=0.235

Decolonisation study

Standard
- 2% mupirocin tds 5 days ant. nares
- 4% chlorhexidine once a day/5 days
- 1% silver sulfadiazine once a day/5 days

Tea tree
- 10% tea tree cream tds 5 days ant. nares
- 5% tea tree body wash once a day/5 days
- 10% tea tree cream once a day/5 days

## Presence or absence of MRSA after 14 days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MRSA negative</th>
<th>MRSA positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>56</td>
<td>58</td>
<td>114</td>
</tr>
<tr>
<td>Tea tree</td>
<td>46</td>
<td>64</td>
<td>110</td>
</tr>
<tr>
<td>Total</td>
<td>102</td>
<td>122</td>
<td>224</td>
</tr>
</tbody>
</table>
### MRSA carriage and clearance at different sites

<table>
<thead>
<tr>
<th></th>
<th>Std total</th>
<th>Std cl (%)</th>
<th>TT total</th>
<th>TT cl (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nose</td>
<td>74</td>
<td>58 (78)</td>
<td>76</td>
<td>36 (47)</td>
</tr>
<tr>
<td>Throat</td>
<td>34</td>
<td>16 (47)</td>
<td>36</td>
<td>10 (28)</td>
</tr>
<tr>
<td>Axilla</td>
<td>4</td>
<td>2 (50)</td>
<td>14</td>
<td>8 (57)</td>
</tr>
<tr>
<td>Groin</td>
<td>14</td>
<td>4 (29)</td>
<td>10</td>
<td>8 (80)</td>
</tr>
<tr>
<td>Wound</td>
<td>26</td>
<td>8 (31)</td>
<td>34</td>
<td>16 (47)</td>
</tr>
</tbody>
</table>
Germ tube formation

- Germ tubes may be important in the pathogenesis of candidal disease
  - adherence and penetration of epithelial cells
- Tea tree oil is potentially useful in the treatment of superficial candida infections

Aim: To determine whether tea tree oil inhibits germ tube formation in *C. albicans*
Germ tube formation by *C. albicans* 10231 in the presence of tea tree oil

Susceptibility of fluconazole susceptible *C. albicans* to tea tree oil gel
Susceptibility of fluconazole resistant *C. albicans* to tea tree oil gel
Cold sore pilot study

- Anecdotal and in vitro evidence suggest TTO may be useful in treating cold sores.
- Pilot study Sept 1999 - March 2000:
  - Randomised
  - Single-blind
  - Placebo-controlled
  - 20 patients

Cold sore pilot study

- patient presents when cold sore first develops
- apply ointment 5 times daily
  - 6% tea tree oil gel
  - placebo gel
- keep diary
- seen daily until completely healed (approx 1-2 weeks)
- specimen taken daily (except Sun)
Cold sore pilot study results

<table>
<thead>
<tr>
<th>Time (days) to:</th>
<th>TTO (n=9)</th>
<th>placebo (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>crust</td>
<td>3.7</td>
<td>4.6</td>
</tr>
<tr>
<td>re-epithelialisation</td>
<td>9.9</td>
<td>12.0</td>
</tr>
<tr>
<td>PCR negative</td>
<td>6.5</td>
<td>7.9</td>
</tr>
</tbody>
</table>

no significant differences between treatments due to small sample size

Other essential oils

- Wide range of other plant essential oils have antibacterial activity
- Of 52 tested by Hammer et al. (J Appl Microbiol 1999) only 6 had no activity
- Most active: lemon grass, oregano and bay
- Some highly specifically active against *Staph aureus* eg *Vetiveria zizanioides* MIC 0.008%
Manuka oil (NZ tea tree) vs Melaleuca oil from Australia
Bacteriophage therapy
D'Herelle/Twort
Advantages of phage therapy

- Highly specific
- No “collateral damage”
- No overgrowth
- Effective against multidrug resistant bacteria
- Cost of development relatively cheap
- Resistance of bacteria can be overcome
- No deleterious effects on eukaryotic cells

(Matusazki et al. J Infect Chemother 2005; 11; 211-9)
Bacteriophage therapy

- Ignored apart from Soviet bloc countries
- Reports in 1970s/80s of treating SA infections
- In UK, phage used to treat diarrhoeal disease in animals due to *E.coli*
- Phages for SA, VRE and *Vibrio* infections successful in mouse model
Experimental protection of mice

- ΦMR11 had broad host range
- No genes for known toxins or antibiotic resistance
- IP injection of $8 \times 10^8$ cells of *S. aureus* caused bacteraemia and death
- IP injection of ΦMR11 (MOI >0.1) suppressed *S. aureus* induced lethality
- ΦMR11 alone had no adverse effects

(Matsuzaki *et al.* *Clin Infect Dis* 2003; 187: 613-24)
Bacteriophage therapy - issues

- Bioavailability
- Some MRSA less susceptible to phage
- Safety concerns
  - Phage antibody
  - Toxins in preparations
  - Lysogenic conversion
- Development of resistance
Bacteriophage therapy

- Phages applied either topically, s/c, or via irrigation or drains
Anticancer effects

Cancer Chemother Pharmacol
DOI 10.1007/s00280-010-1267-3

Inhibition of established subcutaneous murine tumour growth with topical *Melaleuca alternifolia* (tea tree) oil

Sara J. Greay · Demelza J. Ireland · Haydn T. Kissick · Peter J. Heenan · Christine F. Carson · Thomas V. Riley · Manfred W. Beilharz
Subcutaneous tumour model

Tumour

20um

Tumour

L

W

METIQUE

Tea Tree Oil

20 ml
Antitumour effect of 10% TTO/DMSO in s.c tumour models

Greay et al. 2010 Cancer Chemo Pharmacol
Why haven’t these treatment options been widely explored further?
Often no obvious protection for a pharmaceutical company – no patent!
Many companies that produce these products don’t understand healthcare
Many trying to take advantage of interest
Too many unsubstantiated claims
No good regulatory processes in place
Poor quality products
Clinical trials expensive
Safety issues
Conclusions

- “Natural” & alternative therapies are viewed favourably by patients
- Less side effects than antibiotics
- Some problems relating to quality
- Lack of good data
- Worthwhile exploring further as adjunctive or replacement therapy
- Government involvement necessary